eCM XII: Surgical Treatment of Infection in the Presence of Fracture Fixation Implants

Charalampos Zalavras

Department of Orthopaedic Surgery, LAC+USC Medical Center, University of Southern California, Los Angeles, USA

Infection in the presence of fracture fixation implants is a challenging complication. Basic principles of infection control include debridement, fracture stabilization, local and systemic antibiotic therapy, soft tissue coverage and promotion of fracture healing.

Debridement is a critical factor for infection control. Non-viable tissue and foreign material promote biofilm development on their surface and lead to persistence of infection. Inadequate debridement is the key factor for failure of infection control, therefore all non-viable tissue and foreign material need to be removed for definitive control of infection. On the other hand, stability at the fracture site is a critical factor for bone healing and infection.

For this reason management of fracture fixation implants in the presence of infection appears to be controversial. The main issue to consider is whether the fracture fixation implants are providing stability at the fracture site and this can be determined by evaluating the status of the implants and the status of the fracture. Additional factors include the location of the fracture, the time post surgery, the pathogenicity of the organism, and the clinical course of the patient.

When existing implants are well fixed and provide stability in acute fractures complicated by infection we may temporarily retain them. A stable fracture may heal in the presence of infection and implants will have to be removed after the fracture has healed in order to definitively control the infection. Periarticular well-fixed fractures with acute infections can be treated this way because implant removal may jeopardize subsequent reduction and stabilization of the articular component. Alternatively, we may remove the existing implants and stabilize the bone with new ones.

Fracture fixation implants should definitively be removed if the fracture is healed (the implants are not needed anymore), if there is an infected established nonunion (the implants have failed to lead the bone to healing), or if the implants have become loose (they are not providing stability anymore).

Following implant removal radical debridement can be performed. Bleeding of bone and soft tissues is the main criterion of viability. Reaming of the medullary canal is required in infections following removal of intramedullary nails. Multiple specimens including fluid, soft tissue and bone should be sent for aerobic and anaerobic cultures with the addition of fungal and mycobacterial cultures in chronic infections.

Stabilization of the healing fracture or the nonunion with new implants will be necessary following removal of existing implants.

If there is a dead space after debridement, antibiotic-impregnated cement beads or spacers eliminate dead space and deliver local antibiotic therapy in high concentrations. Antibiotic-impregnated cement nails can be used for intramedullary infections. Systemic antibiotic therapy is administered as well based on culture and sensitivity results.

In the presence of soft tissue defects, soft tissue coverage is necessary. Local or free flaps may be used depending on the location and size of the defect.

Following control of the infection, autogenous bone grafting is required for nonunions and small bone defects. Complex reconstructive procedures such as distraction osteogenesis and vascularized bone grafts can provide a solution for large bone defects.

REFERENCES:

1. Berkes M et al. J Bone Joint Surg Am. 2010 Apr;92(4):823-8.
2. Patzakis et al. J Am Acad Orthop Surg. 2005
3. Rightmire E et al. Clin Orthop Relat Res. 2008 Feb;466(2):466-72.
4. Zalavras CG et al. Clin Orthop Relat Res. 2009 Jul;467(7):1715-20.

Sequential release kinetics of two substances from one-component polymeric coating on implants

[1]Strobel, C; [2]Bormann, N; [1]Kadow-Romacker, A; [3]Schmidmaier, G; [1,2]Wildemann, B

[1]Julius Wolff Institut, [2]Berlin Brandenburg Center for Regenerative Therapies, Charité - Universitätsmedizin Berlin,
[3]Department Orthopädie, Unfallchirurgie und Paraplegiologie, Universitätsklinikum Heidelberg

Introduction: Due to the disturbed vascularisation systemically applied substances might not reach the fracture side in an effective concentration. The use of scaffolds for drug delivery is possible in situations with larger bone defects, but the application of foreign material should be reduced to the minimum. Therefore, the present study aimed in the improvement of a local drug delivery system based on an implant coating. For early infection prophylaxis an immediate antibiotic release should occur followed by a more sustained release of an osteoinductive growth factor to stimulate bone healing.

Materials and Methods: As a carrier system Poly(D,L-lactide) (PDLLA) was chosen to coat titanium Kirschner-wires by a dipping technique. The release kinetics of incorporated factors was modified by changing the ratio of the PDLLA/Solvent/Drug (Tab. 1, Fig. 1). Ethyl acetate (EA) was used as solvent.

Tab. 1: Coating design: ratios of PDLLA, solvent and drug

	PDLLA/EA Substance
Layer 1	300mg/1.5ml 3% w/w BMP-2
Layer 2	100mg/1.5ml 10% w/w gentamicin

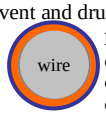


Fig. 1: 2-Layer coating scheme on the k-wire cross section

For control, single coatings as well as a substance free PDLLA were also analyzed.

The coatings were analysed by SEM.
Released BMP-2 was quantified by enzyme-linked immunosorbent assay and gentamicin by cloned enzyme donor immunoassay. In addition, the activity of the released growth factor and gentamicin was investigated *in vitro*. BMP-2 coated wires were placed via transwells into 24-well-plates seeded with the myoblast cell line C2C12 (culture medium: DMEM+1%FCS+1% Pen/Strep). After 3 days cell activity was measured with Alamar blue and BMP-2 induced osteogenic differentiation with Alkaline Phosphatase assay (ALP). Subsequently the transwells with the wires were placed onto freshly seeded cells and the procedure repeated weekly for 8 weeks in total. To detect gentamicin activity, the elution samples were applied on discs, which were placed on Mueller-Hinton-agar spiked with Staphyloccocus aureus. Agar plates were incubated over night and the zones of inhibition were documented.

Results: SEM pictures show a smooth homogenous coating before elution (Fig. 2a), whereas after elution all coatings were swollen and porous (Fig. 2b).

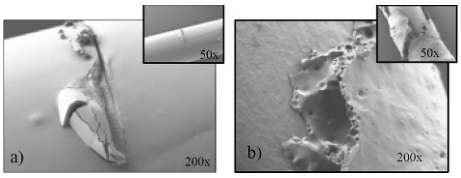


Fig. 2 SEM, artificial damage of the coating a) before elution b) after 10 weeks of cell culture

The release kinetics revealed an initial burst release of gentamicin and a slower and more sustained release of BMP-2 from the coated wires (Fig. 3). The different release kinetic is reflected by the *in vitro* activity of the two factors. Gentamicin showed an immediate inhibitory activity (Fig. 4) whereas BMP-2 stimulated the ALP-activity of C2C12 cells with a maximum after 2 weeks (Fig. 5).

Discussion:
The present study showed the realizations of different release kinetics from one simple polymer coating of implants by only changing the Polymer/Solvent/Drug ratio.
The initial release of gentamicin will prevent the colonisation of bacteria on the implant. The delayed release of BMP-2 might be beneficial to induce bone formation as shown in animal models, where the initial burst BMP release was less effective [1-3]. The next step is to use this combined coated wires to treat rats with an open osteotomy showing delayed healing [4]. This animal model shows an infection rate comparable to the clinical situation and is therefore suitable to prove the efficacy of this double coating.

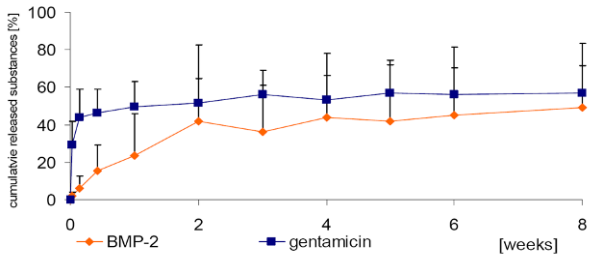


Fig. 3 Release kinetics of gentamicin and BMP-2 combined coated on one wire (n=6 per group)

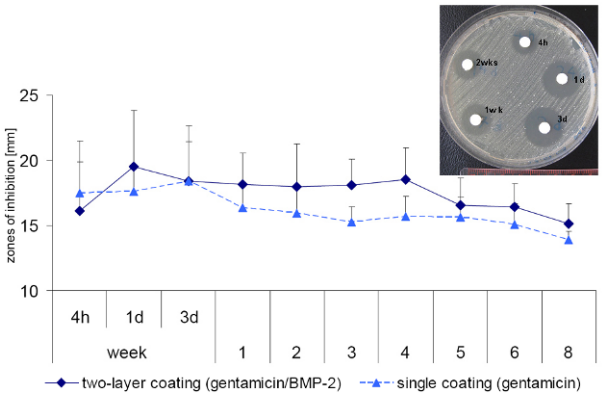


Fig. 4 Zone if Inhibition (n=6 per group)

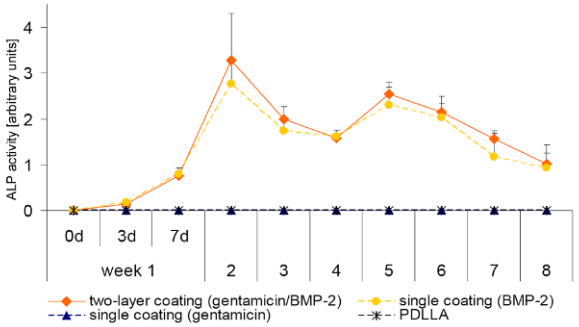


Fig. 5 ALP activity of C2C12 cells (n=6 per group). The ALP activity measured in optical density [OD] was normalized to 10^4 cells [arbitrary units]

References:
1. Betz OB, et al. Gene Ther. 2007, 14(13):1039-44;
2. Kempen DH, et al. Biomaterials. 2008, 29(22):3245-52;
3. Wu G. et al. Biomaterials. 2010, 31(29):7485-93;
4. Kratzel C, et al. BMC Musculoskelet Disord. 2008, 8;9:135.

Acknowledgement: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, SFB 760)

Daptomycin – Local application in implant-associated infection and complicated osteomyelitis

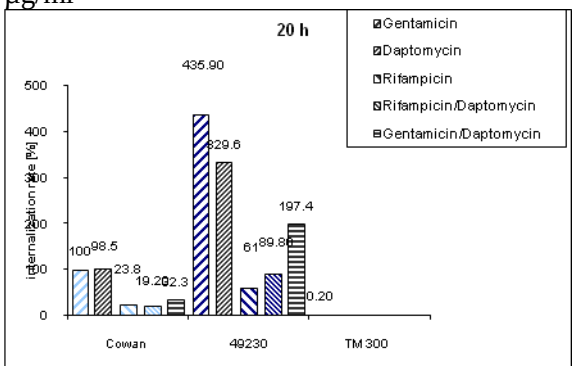
[11]Steffen B. Rosslenbroich MD, [1]Michael J. Raschke, MD, [1]Carolin Kreis, MD,
[1]Nancy Tholema-Hans, PhD, [2]Andreas Uekoetter, MD, [3]Rudolf Reichelt, PhD,
[1]Thomas F. Fuchs, MD

[1]Department of Trauma, Hand, and Reconstructive Surgery
[2]Institute of Medical Microbiology
[3]Institute of Medical Physics and Biophysics
University Hospital Muenster

INTRODUCTION: The constant rise of highly resistant gram-positive bacteria, mainly Staphylococcus species, causing inter alia implant associated infections and osteomyelitis creates a persistent urge to develop new antimicrobial agents. This study investigates the application of the lipopeptide antibiotic daptomycin in infections involving the human bone by taking special aspects for use in trauma and orthopedic surgery into consideration. These aspects include the potential use in polymethylmetacrylate (PMMA) for local application and its ability to show intracellular activity. Furthermore this study tries to set a algorithm for invitro-evaluation of new antimicrobial agents for use in musculoskeletal surgery.

METHODS: Compressive and tensile strength testing of two concentrations of daptomycin-laden PMMA was performed referring to the requirements stipulated by the ISO 5833[1] and compared to customary PMMA products.The micro-structure of the antibiotic-laden PMMA was evaluated by scanning electron microscopy. Intracellular activity of daptomycin was determined by a human osteoblast infection model comparing daptomycin, gentamicin, rifampicin and combinations[2]. Elution kinetics of the antibiotic-laden bone cement were measured by using a continuous flow chamber setup[3].

RESULTS: There was no significant negative effect of adding 1.225% and 7.5 % per weight of daptomycin to the PMMA in comparison to commercial PMMA products allowing values for compression strength and bending modulus clearly above required levels of 70 MPa and 1800 MPa. There was no significant difference in intracellular activity comparing gentamicin to daptomycin. A significant higher level of intracellular activity was reached applying rifampicin alone or in any agent combination. Elution of daptomycin from PMMA showed within the first hour initial peak values of 15-20 µg/ml



In-vitro infection model - Internalization rate for 20 h of 3 different *Staphylococcus* strains with application of different antibiotics. Invasiveness of Cowan with gentamicin set at 100%.

DISCUSSION & CONCLUSIONS: This study states that in terms of intracellular activity daptomycin shows no significant difference to commonly used antibiotics in osteoblasts. Adding daptomycin within the range of physiological application to PMMA shows no significant negative effects on its biomechanical properties. After loading the PMMA with daptomycin it is still able to elute and to remain antibacterial active. These in vitro results implicate that for complicated osteomyelitis and prosthetic joint infections due to pathogens, susceptible to daptomycin, this antibiotic substance is an applicable alternative to current used agents in orthopedic and trauma surgery

REFERENCES: [1] International-Organization-for-Standardization (2002).[2] Haslinger-Loeffler B et al.(2005) Cell Microbiol.[3] Perry et al. (2002) CORR 49-53.

Biomaterials-associated infections – *in vitro* and *in vivo* studies

H.J. Busscher and H.C. van der Mei

Department of Biomedical Engineering, The Kolff Institute, University Medical Center and University of Groningen, Groningen, The Netherlands

INTRODUCTION: Modern health care is greatly dependent on the use of biomaterials implants and devices for the restoration of function, after trauma, (oncological) intervention surgery or simply wear due to old age. Biomaterials implants surfaces in the human body are prone to infection, as can develop through three distinctly different routes. Peri-operative infection is the best documented route and usually causes early infection of an implant. Also immediate post-operative infection can be a cause of early failure. Late post-operative infections spreading from infections elsewhere in the body have also been described to be a cause for implant infection and failure. Since a biomaterial-associated infection (BAI) is difficult to treat with antibiotics due to the protection offered by the biofilm mode of growth and intra-cellular shelter, the fate of an infected implant often is removal, at great discomfort to the patient and costs to the healthcare system. Frequently even, the condition of a patient does not allow replacement surgery or removal of a device. BAI can be lethal when spreading through the body. Whereas the infection rate of primary implants may be considered low (4-6% on average depending on the implant type), infection rates in revision surgery are much higher around 15%.

PREVENTION STRATEGIES: Prevention strategies under investigation are numerous, but no generally effective way to prevent BAI has been found. Moreover, prevention of BAI of a primary implant may require different approaches than the prevention of BAI of secondary implants after treatment of BAI.

Prevention strategies based on biomaterials surface modification attempting to discourage microbial adhesion and biofilm formation have been forwarded in the literature, but none of them have clinically provided a breakthrough. The lack of a clinical breakthrough is partially due to the low incidences of BAI (though still being unacceptably high), requiring large numbers of patients to be enrolled in a study. Therefore, novel evaluation technologies are required that indicate whether new preventive strategies work under *in vivo* conditions.

BIO-OPTICAL IMAGING: Bioluminescence and Fluorescent Imaging are new evaluation technologies (BLI and FLI) that offer the opportunity to observe the *in vivo* course of BAI in small animals without the need to sacrifice animals at different time points after the onset of infection. BLI is highly dependent on the bacterial cell metabolism which makes BLI a strong reporter of viable bacterial presence. Fluorescent sources are generally more stable than bioluminescent ones and specifically targeted, which renders the combination of BLI and FLI a promising tool for imaging BAI.

THE RACE FOR THE SURFACE: In the concept of the race for the surface, successful implant coatings should favour tissue integration over microbial colonization. This suggests that new prevention strategies abandoning the concept of mono-functional fully non-adhesive, tissue-supporting, or immune-friendly coatings may have to be developed on the basis of multi-functional coatings better mimicking natural tissue. The efficacy of macrophages in removing adhering bacteria from a surface for instance, is much higher on cross-linked PEG coatings than on glass because bacteria do not switch on their natural defences on such highly hydrated coatings exerting only weak interaction forces, while macrophages are less immobilized for the same reasons. Polymer brush coatings, designed with an occasional RGD-group for instance, keep their non-adhesive functionality toward bacteria, while at the same time supporting tissue integration.

Societal pressure toward the development of effective coatings for biomedical implants and devices is huge and the coming decade will become the decade of effective antimicrobial coatings based on multi-functional coatings can be expected to yield clinical breakthroughs in the field.

REFERENCES: Busscher HJ, *et al.* Biomaterials, 2009, 30 4247-4248.

Antibiotics in Orthopaedic Use –Is There a Right Choice?

C Berberich, P Seidel,
Heraeus Medical GmbH, Philipp-Reis Straße 8/13, D-61273 Wehrheim, Germany
www.heraeus-medical.com

INTRODUCTION

Surgical site infections (SSI) in orthopaedics are a major source of postoperative morbidity. Although perioperative antibiotic prophylaxis is a common practice, orthopaedic infections are still high in numbers, due to the increasing use of osteosynthesis material and implants. Implants are avascular and can be easily colonized with biofilm-producing germs. For both, effective prophylaxis and treatment of orthopaedic infections, the right choice of the antibiotics used, the mode of application (only systemic or systemic & local), the timing, dosage and the duration of antibiotics are of extremely high importance. Their inappropriate use does not only lead to failures in prevention or treatment of infections, but may also promote microbial resistance development and may cause serious side effects for the patients.

GERMS & PATHOMECHANISMS OF ORTHOPAEDIC INFECTIONS

Staphylococci are among the most frequently found parthogens at infected biomaterial & implant surfaces. While *S. epidermidis* shows slight preference for polymeric surfaces, *S. aureus* is the more common germ isolated from metal surfaces and dead bone tissue. Both bacteria are perfect biofilm-producers and are protected from the immune mechanisms and action of antibiotics by their biofilm-associated mode of dormant life. Intracellular forms of such persister cells are a major cause for chronic orthopaedic infections and pose additional challenges to an effective antibiotic treatment. Other challenges include the trend to more polymicrobial infections and more difficult-to-treat germs, such as MRSA/MRSE.

SELECTION & USE OF ANTIBIOTICS

Prophylaxis: Broad-spectrum prophylactic antibiotics should help to eliminate the germs before they start to colonize the implant. For prophylactic purposes the recently published AAOS guidelines [1] recommend the use of cephalosporins, such as cefazolin or cefuroxim, administered within one hour prior to surgery. In cases of suspected beta-lactam allergy, clindamycin or vancomycin can be used. The latter one is also recommended in cases of MRSA colonisation. Due to extended infusion times, vancomycin should be started within two hours prior to incision. In cases of blood loss or long op duration, antibiotic administration must be repeated (e.g. cefazolin, every 2-5 hrs; vancomycin, every 6-12 hrs). There is no evidence of a benefit of continued antibiotic administration past 24 hrs of end of surgery [2]

Treatment: In cases of established infections, use of antibiotics is only considered as an adjuvant to surgical debridement. Typically, the choice of the appropriate antibiotic depends on the bacteria, its antibiotic sensitivity profile and the health state of the patient. A combination of rifampicin & a quinolone (or rifampicin & vancomycin in cases of MRSA) for at least 2 wks up to several months has shown good results [3]. In chronic infections with biofilm involvement, all foreign material must be removed and locally delivered antibiotics via e.g. PMMA as carrier (spacers, PMMA-chains) are of additional clinical benefit.

ROLE OF LOCAL ANTIBIOTICS

There is general consensus that PMMA chains or PMMA spacers loaded with specific antibiotics support the eradication of bone and joint infections, because of the high local concentrations achieved. The exact treatment time is, however, variable, ranging from few weeks up to several months. Only small amounts of these local antibiotics are systemically detectable and do not represent a major risk for side effects. Still a matter of debate is the benefit of antibiotic impregnated PMMA for infection prophylaxis. Although common practice in Europe, its routine use in e.g. primary arthroplasty is still discussed in other world regions. Meanwhile, evidence accumulates that joint infection rates are, indeed, lower, if antibiotic loaded bone cement with high initial release rates is routinely used in arthroplasty[4].

REFERENCES: [1]AAOS guidelines, 2004, statement 1027. [2]Prokuski. J. Am. Acad. Orthop. Surg. 2008; 16:283. [3]García-Lechuz & Bouza: Expert Opin. Pharmacother. 2009; 10:35. [4]Parvizi et al. Acta Orthopaed. 2008; 79:335.

Ag/SiOxCy plasma polymer coating for antimicrobial protection of fracture fixation devices

V.Alt[1,2], P. Khalilpour[3], M. Wagener[3], E. Domann[4] , R. Schnettler[1,2]

[1]Department of Trauma Surgery, University Hospital Giessen-Marburg, Giessen, Germany, [2] Laboratory of Experimental Surgery, Justus-Liebig-University Giessen, Germany, [3] Bio-Gate AG, Bremen, Germany, [4] Institute of Medical Microbiology, University Hospital Giessen-Marburg, Giessen, Germany

INTRODUCTION: Implant-related infections are often devastating situations in orthopaedic trauma surgery particularly if multi-resistant bacteria are involved. Protection of the implant surface by an antimicrobial coating exhibiting activity against multi-resistant bacterial strains is of high interest. Aim of this study was to investigate the antimicrobial effects of an Ag/SiO_xC_y plasma polymer coating for fracture fixation devices, such as nails, plates, and external fixators, including tests against methicillin-resistant Staphylococcus aureus (MRSA) and its biocompatibility.

METHODS: The antimicrobial activity of the coating deposited onto 12 x 3 mm stainless steel implants was tested *in vitro* against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and MRSA using different testing methods (ASTM E-2810, JIS Z 2801, proliferation assay). Additionally, the coated devices were implanted into the paravertebral muscle of rabbits and explanted after 2, 7, 14, and 28 days to test the remaining ex vivo antimicrobial activity. For biocompatibility assessment the Ag/SiO_xC_y plasma polymer coating was tested *in vitro* according to ISO 10993-5.

RESULTS: The Ag/SiO_xC_y coating exhibited excellent antimicrobial activity against all tested bacterial strains in all three *in vitro* tests. *Ex vivo* testing proved suppression of more than 99.9 % of bacterial proliferation by the coating compared to non-coated samples even after 28 days. ISO 10993-5 showed good biocompatibility of the coating without any indications of cytotoxic effects (Fig. 1).

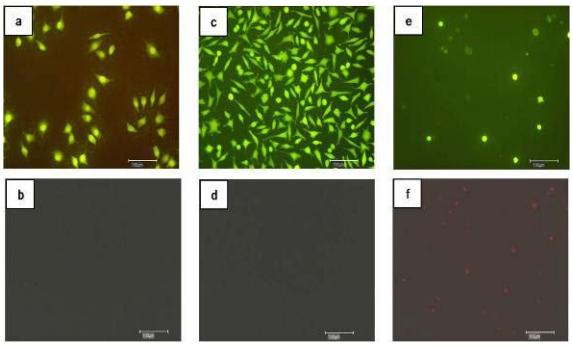


Fig. 1: Fluorescence microscopy after fluorescein diacetate (green staining of the cytoplasm of intact cells) and ethidium bromide (red staining of the DNA in the cell nuclei of damaged cells) of the Ag/SiOxCy plasma polymer coating (a,b), of the negative non-toxic control (polyvinyl chloride) (c,d) and the positive toxic control group (poly-tetrafluoroethylene) (e,f).

DISCUSSION & CONCLUSIONS: In summary, Ag/SiO_xC_y plasma polymer coating showed excellent antimicrobial activity including effectiveness against MRSA and good *in vitro* biocompatibility. Therefore it possesses high potential as a prophylactic agent in orthopaedic trauma surgery efficiently killing multi-resistant bacteria.

Controlled release of gentamicin for the prevention of *Staphylococcus aureus* biofilm formation on fracture fixation implants

R. Steck[1], D.J. McMillan[2], C. Lutton[1], N. Rosenzweig [1,2], K.S. Sriprakash[2], B. Goss[1], M.A. Schuetz[1,3],

[1]*Institute of Health and Biomedical Innovation, QUT, Brisbane*, [2] *Bacterial Pathogenesis Laboratory, QIMR, Brisbane,* [3]*Trauma Services, PA Hospital, Brisbane, Australia*

INTRODUCTION: Implant associated infections are a critical health concern following orthopaedic surgery. Sustained local delivery of antibiotics has been suggested as a means of preventing these infections. Poly(D,L-lactide) (PDLLA) is a biodegradable polymer that has been used to coat implants for the delivery of antibiotics and other bioactive molecules[1]. While effective, these studies show that antibiotics are released in a burst profile. Here we evaluated a method for controlled release of gentamicin from implant surfaces using the palmitate alkyl salt to decrease its solubility in aqueous solution.

METHODS: Steel Kirschner wires (K-wires, 1.8 mm diam.) were coated with Gentamicin-palmitate (GP)-PDLLA, gentamicin sulphate (GS)-PDLLA or vancomycin sulphate (VS)-PDLLA by a solvent casting method. Growth of *S. aureus* Xen29 biofilms after incubation overnight with approx. 10^5 CFU on the surface of the K-wires was monitored for 7 days using an IVIS *in vivo* bioluminescence imaging system (Caliper Life Sciences, USA). Elution of the antibiotics from coated K-wires was measured by placing coated K-wire segments in 10ml PBS at 37 ° C and collecting 500 ml aliquots in intervals up to 48 hours. Antibiotic concentration in the samples was determined by HPLC-tandem mass spectrometry with a detection limit of 0.5 mg/L.

RESULTS: In contrast to burst antibiotic release from the GS-PDLLA and VS-PDLLA groups within <8 hours, GP was released in a slower, sustained manner over 48 hours. However, the total amount of released antibiotics was smaller for the GP group (Fig 1). Colonisation of *S. aureus* Xen29 on gentamicin-coated K-wires was reduced by 90% when compared to the uncoated control group. Bioluminescence emitted by *S. aureus* Xen29 was reduced over seven days in all three antibiotic groups compared to the uncoated or PDLLA only coated control groups, demonstrating that growth and biofilm development over the longer term was impaired by antibiotic-PDLLA coating. However, no statistically significant differences were found between the antibiotic coating groups.

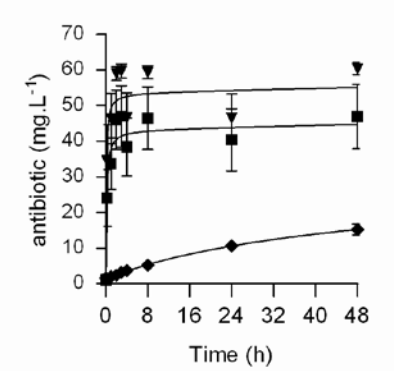


Fig. 1: Elution of GP (♦), GS(■), and VS(▼) from coated K-wires over 48 hours.

DISCUSSION & CONCLUSIONS: We demonstrated that the use of gentamicin palmitate does result in an extended delivery compared to both gentamicin sulphate and vancomycin, while the total amount of released gentamicin palmitate is lower. We assume that this was caused by a better miscibility of the GP in the coating solution, and therefore less total antibiotic adsorbed to the surface, available for short term release. Despite the different release profiles, all three antibiotics reduced the biofilm formation for at least seven days. In conclusion, these results indicate that using alkyl salts of antibiotics may be an effective strategy for controlling the release of antibiotics from implants.

REFERENCES: [1]Schmidmaier G., *Injury*, 2006, 37 S105-112.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Wesley Research Institute, Brisbane, Australia.

Clinical performance of Antibiotic Intramedullary nails

M.J. Raschke[1], C. Kreis[1], T. Fuchs[1]

[1] *Department of Trauma, Hand and Reconstructive Surgery, Muenster University Hospital*

INTRODUCTION: In musculoskeletal surgery there is a clear need for optimized implants in order to reduce the rate of complications.[1] They range from delayed healing and non-unions to extensive bone infections. Despite the use of systemic antibiotic therapy, deep wound infections and osteomyelitis remain serious complications that may lead to impaired healing, reduced limb function and life-threatening septic conditions. [2,3] The use of antibiotic-coated implants may reduce the rate of infection and facilitate fracture healing after surgical treatment.

METHODS: A key technology which allows biodegradable coating of medical devices was developed in which bioactive substances can be incorporated. Poly (D,L-lactide) (PDLLA) can be applied to metallic surfaces carrying incorporated active substances. Gentamicin loaded PDLLA coating was investigated in a rat infection model *in vivo* and *in vitro*. [4] In a prospective, non-randomized clinical study 21 patients with closed or open tibial fractures underwent surgical treatment with a biodegradable gentamicin-loaded coated nail (UTN - Protect, Synthes®) with a follow up of 6 month. Radiographic assessments of fracture healing and weight bearing capacity were determined at 5 weeks, 3 months and 6 months after surgery. Recently a clinical, randomized study is performed, where the surgical treatment with the gentamicin-coated ETN (Expert TN PROtect®, Synthes) is investigated. The nail was CE-certified in January 2011.

RESULTS: It was shown that the effectiveness of the PDLLA carrier is significantly superior to the collagen carrier. In vitro and in vivo elution tests proved a constant release of bioactive gentamicin after an initial peak over a period of 6 weeks. Results of the animal study showed a significant advantage of gentamicin coated implants compared to controls in preventing osteomyelitis. In analyses of the clinical study no disturbance of bone healing could be detected. Even in IIIb° open fractures infection could be prevented using the gentamicin coated UTN.

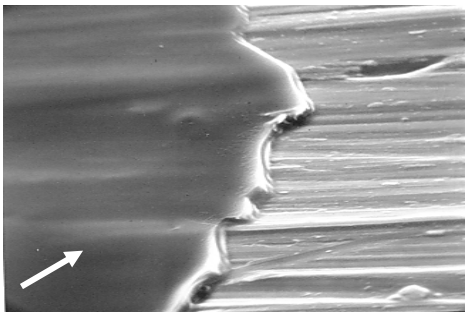


Fig. 1: SEM micrograph of PDLLA coating on titanium in a magnification of 2000times. The arrow marks the smooth even layer of the coating. On the right side the "rough" surface of the titanium gets obvious.

DISCUSSION & CONCLUSIONS: Antibiotic coated nails are of great value in the management of mayor lower limb trauma preventing life and limb threatening infections. The gentamicin-loaded coating of the UTN could be a viable alternative and might overcome the limitations of insufficient antibiotic delivery. The coating process can be seen as a key technology. A wide variety of implants made from different materials can be coated. The appropriate active substance can be delivered locally in a targeted and individualized manner according to the indication or the range of pathogens present.

REFERENCES: [1] Einhorn et al., Clin Orthop Relat Res, 1998 (355 Suppl): S2-3. [2] DT Tsukayama, Clin Orthop Realt Res, 1999: 22-29. [3] Young et al., Surg Clin North Am., 68: 167-180, 1988. [4] Lucke et al., Bone, 2003: 521-531.

ACKNOWLEDGEMENTS: This template was modified with kind permission from European cells and Materials Conferences.

Peri-implant tissue as a “safe heaven” for biofilm bacteria

S.A.J. ZAAT

Dept. Medical Microbiology, CINIMA (Center for Infection and Immunity Amsterdam), Academic Medical Center, Amsterdam, The Netherlands

Pathogenesis of biomaterial associated infection. One of the major causes of failure of inserted and implanted medical devices (“biomaterials”) is infection, mostly caused by commensal bacteria such as staphylococci. Bacteria are considered to adhere to its surface, but there is a second important effect of the biomaterial: as a foreign body it provokes a socalled foreign body response (FBR), a well-regulated inflammatory response. Initially, this response has the characteristics of an acute inflammation, but gradually changing into a more chronic inflammatory response, with novel tissue formation/encapsulation as the endpoint. In presence of a foreign body the clearance of bacteria from the peri-imlant tissue is less effective.

We extensively studied biomaterial-associated infection with *Staphylococcus epidermidis* in a mouse model. We analysed both the implanted material and the surrounding tissue, measured inflammatory mediators such as cytokines, and studied the histology. These studies showed that (i) bacteria were more often cultured from tissue than from the implants, and in higher numbers, (ii) some materials provoked a very strong inflammatory response in presence of bacteria, and others were anti-inflammatory, (iii) Bacteria were seen in large numbers in macrophages in the peri-implant tissue, at a distance of 10-20 cell layers away from the biomaterial-tissue interface, (iv) susceptibility to infection could be reduced by specific immunomodulation, (v) *S. epidermidis* in tissue resisted a regimen of rifampicin-vancomycin, and (vi) bacteria in tissue increased in numbers after several weeks, implying the tissue to be a reservoir for infection.

In a study on bacterial colonization of peri-biomaterial tissue in deceased Intensive Care patients, who did not have a documented infection, we also cultured staphylococci and enterococci from tisssue surrounding catheters, even from tissue not bordering the catheter. Bacteria were also detected within the tissue in immunohistology. So, also in humans tissue surrounding a foreign body is colonized by potentially infection-causing bacteria.

Biomaterial-associated tissue colonization and infection poses a number of novel research and clinical questions:

- How should biomaterials be developed with a low propensity to cause tissue infection?
- How should immunomodulatory strategies be developed to prevent/correct tissue infection?
- Can alternative whole animal models be developed in order to screen for optimal biomaterial characteristics in view of immune responses and infection susceptibility?

Influence of biomaterials on *Staphylococcus epidermidis* biofilm formation

L.G.Harris[1], R.Jeeves[1], H.Rohde[2], D.Mack[1]

[1]Medical Microbiology & Infectious Diseases, College of Medicine, Swansea University, UK. [2]Institut für Med. Mikrobiologie, Virologie & Hygiene, Universitätsklinikum Hamburg-Eppendorf, Universität Hamburg, Germany

INTRODUCTION: Implant related infections are frequently caused by *Staphylococcus epidermidis* and cost medical institutions millions of Euros each year. *S. epidermidis* infections are difficult to treat as they are capable of forming biofilms on the implants thus protecting the bacteria within the biofilm from phagocytosis and antibiotics[1]. However, the interactions between *S. epidermidis* and the biomaterial surface, and how the surface influences adhesion and biofilm formation have yet to be fully elucidated. This study investigated the hypothesis that the biomaterial surface influences *S. epidermidis* adhesion and accumulation mechanisms leading to differential expression of cell-wall adhesins and intercellular adhesive mechanisms involved in biofilm formation, therefore affecting *S. epidermidis* adhesion and biofilm accumulation. The adhesion and biofilm formation of three different *S. epidermidis* strains that use three independent mechanisms of biofilm accumulation mediated by polysaccharide intercellular adhesin (PIA, strain 1457)[2], accumulation associated protein (Aap, strain 5179-R1)[3], and extracellular matrix binding protein (Embp, strain 1585-RA)[4] to different medically relevant biomaterial surfaces were studied.

METHODS: To visualise and quantify *S. epidermidis* adherence and biofilm formation to titanium (Ti), stainless steel (SS), titanium-aluminium-vanadium (TAV) and polyurethane (PU), bacteria were cultured on the different surfaces in tryptone soya broth (TSB) at 37°C for 2h, 4h, and 24h, then stained with SYTO9, and visualised with a Zeiss confocal laser scanning microscope (CLSM). The density of adhering bacteria observed in each image were counted using AxioMeasure software. Statistical analysis was performed using a one-way ANOVA with Tukey pair wise posthoc test. Indirect PIA, Aap and Embp immunofluorescence assays were used to visualise and quantify intercellular adhesion.

RESULTS: CLSM results have shown that after 24h, all strains had developed mature multilayered biofilms almost indistinguishable from one another, regardless of the biomaterials colonised. At earlier time points adhesion differences in aggregate size of individual groups were observed (Fig. 1), and with the same strains the magnitude of adherent cell aggregates appeared to differ depending on the respective biomaterials.

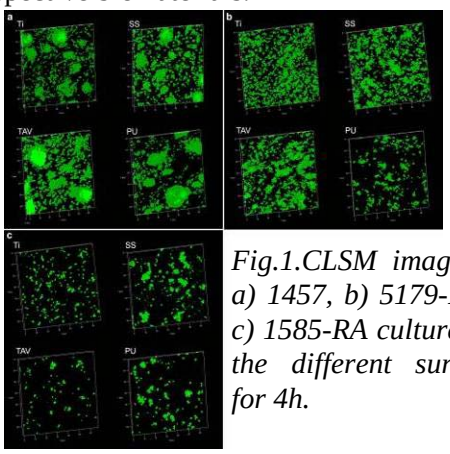


Fig.1.CLSM images of a) 1457, b) 5179-R1 & c) 1585-RA cultured on the different surfaces for 4h.

DISCUSSION & CONCLUSIONS: The three strains formed aggregates differently, as expected due to their different biofilm accumulation mechanisms. With the same strains the magnitude of adherent cell aggregates appeared to differ depending on the respective biomaterials, suggesting that the chemistry and/or topography of the biomaterial used may influence adhesion and the expression of intercellular adhesive mechanisms. Experiments are underway to quantify the observed differences in intercellular adhesion.

REFERENCES: [1]Mack D et al. Int J Artif Organs 2006; 29:343-59. [2]Mack D et al. J Bacteriol 1996; 178:175-83. [3]Rohde et al. Biomaterials 2007; 28:1711-20. [4]Christner M et al. Mol Microbiol 2010; 75 187-207.

ACKNOWLEDGEMENTS: Thanks to MRC for financial support, Smith & Nephew for the polyurethane surfaces, and to Paul Sheppard from Imaging Associates for assistance with the AxioMeasure software.

Effectivity of rifampicin-fosfomycin coating for cementless endoprostheses against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA)

V.Alt[1,2], K. Lips[2], K. Kirchhof[3], N. Düwelhenke[3], E. Domann[4] , R. Schnettler[1,2]

[1]*Department of Trauma Surgery, University Hospital Giessen-Marburg, Giessen, Germany,*
[2] *Laboratory of Experimental Surgery, Justus-Liebig-University Giessen, Germany,*
[3] *Biomet Deutschland GmbH ,Berlin, Germany,* [4] *Institute of Medical Microbiology, University Hospital Giessen-Marburg, Giessen, Germany*

INTRODUCTION: Implant-related infections are often devastating situations in orthopaedic trauma surgery particularly if multi-resistant bacteria are involved. The intention of the current work was to evaluate the antimicrobial effectivity of rifampicin-fosfomycin coated K-wires in a rabbit infection prophylaxis model against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

METHODS: In the first study 10^5 or 10^6 colony forming units (CFUs) of MSSA EDCC 5055 were injected into the intramedullary canal of the tibia of 6 animals containing a rifampicin-fosfomycin coated K-wire as well as of 6 animals with an uncoated K-wire implant as a control group. In the second study 105 or 106 CFUs of MRSA T6625930 were applied to the tibia of 6 animals with a rifampicin-fosfomycin coated K-wire and to 6 animals with an uncoated K-wire, respectively. After 4 weeks the animals were sacrificed and the operated tibiae were harvested. Clinical assessment, histology and microbiological investigations including standard agar plating of roll out K-wires and bone marrow biopsies of the tibiae followed by pulse-field gel electrophoresis were conducted for assessment of implant-related infection. Data were statistically evaluated using Fisher-Exact testing.

RESULTS: Both studies showed a statistically significant reduction of infection rates for rifampicin-fosfomycin coated implants compared to uncoated K-wires. In the first study with MSSA EDCC 5055 none of the 6 animals of the rifampicin-fosfomycin group showed clinical signs of infection or a positive agar plate testing. In only one of the 6 animals the histological evaluation revealed the presence of sporadic bacteria. In the control group an infection rate of 100% (5 of 5 animals were infected, preoperatively 1 animal exhibited pre-operative anatomical abnormalities of the lower limb and was excluded from the study) could be proven. Altogether, this corresponds to a statistically significant reduction of infection rate by the rifampicin-fosfomycin coating (p=0.015).
The second study using MRSA T6625930 also showed a statistically significant reduction of the infection rate in the rifampicin-fosfomycin coating group (p=0.015). None of the 6 animals showed clinical signs of infection or a positive agar plate testing. The histological evaluation revealed the presence of few bacteria in only one of the 6 animals. In contrast, the control group showed an infection rate of 100% (5 of 5 animals were infected, 1 animal died during surgery due to anaesthesia *complications)*

.**DISCUSSION & CONCLUSIONS:** In summary, the rifampicin-fosfomycin coating under investigation showed excellent antimicrobial activity both against MSSA and MRSA. If good biocompatibility and good new bone formation can be shown this coating is of high interest for clinical application in the future.

ACKNOWLEDGEMENTS: This work was funded by Biomet Deutschland GmbH, Berlin, Germany.

MSCRAMMs in Staphylococcal Bone Infections

M. Höök[1], E. Smeds[1], V. Ganesh[1], P. Francis[1], Y-P. Ko[1], C. Ross[1], M. Kang[1]

[1]Center for Infectious & Inflammatory Diseases, Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, Texas

INTRODUCTION: One can view the molecular processes that lead to bacterial infections as a war between the attacking pathogen and the defending host. The bacteria use panels of molecular weapons that we call virulence factors in this war. Recent studies have demonstrated that these virulence factors often are pathogen specific and can be host specific and disease specific. These virulence factors are interacting with and manipulating specific target molecules in the host. In our studies we are focusing on the molecular pathogenesis of staphylococci and have focused on a family of surface molecules that are covalently anchored to the cell wall of the bacteria and have similar structural organizations. These bacterial surface proteins often interact with components of the extra cellular matrix of the host and are called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules).

METHODS: We are using a comprehensive yet minimalistic strategy to first identify putative virulence factors. These putative virulence factors are then expressed as recombinant proteins and characterized. Potential host targets are identified by different screening methods and confirmed by biochemical and structural characterization. Subsequently the importance in staphylococcal virulence is determined in relevant models of infectious disease using genetically manipulated bacteria and mice. The lessons from these studies are then translated to the clinical situation.

RESULTS: This ambitious research plan has been carried out for the *S. aureus* clumping factor A (ClfA) which is a fibrinogen and complement binding MSCRAMM. Our data show that ClfA is a causative virulence factor in a mouse model of lethal sepsis and that the interaction of ClfA with fibrinogen is critical for this function. Two additional MSCRAMMs on *S. aureus*, the collagen binding MSCRAMM Cna and the bone sialoprotein binding protein Bbp, appear to play important roles in osteomyelitis. We will review our work in progress on these MSCRAMMs.

ACKNOWLEDGEMENTS: Our studies are supported by NIH grants AI20624 and AR44415.

Novel strategy of anti-infective implant coating in medicine

KD Kühn

Heraeus Medical GmbH, Philipp-Reis Straße 8/13, D-61273 Wehrheim

INTRODUCTION: Quality of life has been considerably improved by the increased use of medical implants. As a consequence of the growing use of implants, a parallel trend to more infections can be observed. Such implant-associated infections still represent a serious problem and an increasing risk, especially in arthroplasty, which is devastating for the patient and costly for any health care system (Kim 2008). Preventing the formation of a biofilm is the objective of this study. Uncemented prostheses can harbour bacteria, but are unable to eliminate them. Currently, a safe antibiotic protection from bacterial colonisation is not possible in such implants. **METHODS:** Gentamicinpalmitate (GP) was prepared according to Vogt et al (2005). Elution profiles were determined via fluorescent polarisation in the TDx (Figureott TDx system, Figureott Park, IL, USA). The determination of the antibacterial effectiveness was tested with the aid of proliferation assay according to ISO DIN EN 17025 (Kuehn and Bruenke 2010). Scanning electron microscopy (SEM) of the coated and uncoated implants was conducted with the TM-1000 (Hitachi) (Hemoteq AG, Aachen /Germany). Inhibiting areola testing was performed in Müller Hinton Agar plates. Biocompatibility tests were determined according to ISO 10993.

RESULTS: Evenly thin coatings could be placed on several implant surfaces. The GP proved to be a waxy solid matter, which adhered easily to the surfaces. Coating of approx. 50 µg to 250 µg of gentamicinbase (GB) per cm² surface were easily produced. For example, the release behaviour of the G from titanium surfaces of cementless endoprotheses, coated with GP is consistently analogous with the elution of the G from well known G containing bone cements. All coated cementless endoprotheses show a high initial release of the active ingredient within the first 24 to 48 hours, followed by a significantly reduced retarding elution phase in subsequent days. All uncoated and/or active ingredient free test implant references used in the in-vitro proliferation assay against *Staphylococcus epidermidis* DSM 18857 showed growth, and accordingly could not inhibit the spread of the test bacteria. In contrast to this, the antibacterial effect of all GP coated implants had a clearly detectable bactericidal effect in the test. In the proliferation assay the coated implants with approximately 250 µg GB per cm² and samples with approximately 100 µg GB per cm² showed no bacteria growth. Inhibition areolas of GP coated discs with the a.m. high and low concentrations were comparable (39 or 40 mm/24h). The reference PMMA (with gentamicinsulfate GS) moulds showed slightly reduced diameters (35 mm/24h) reaching 88 % of the GP coated discs (Fig. 8,9,). The GP showed a bactericidal effect against the deployed test germs and all required biocompatibility tests fulfilled the ISO 10993.

DISCUSSION & CONCLUSIONS:. The use of self-adhesive antibiotic fatty acid complexes represents a new option for the anti-infective coating of such implants. GP as well as other antibiotic/antiseptic complexes with fatty acids have already been successfully tested *in-vitro* with cementless titanium endoprotheses, bone substitutes, vertebral implants, vascular grafts, surgical sutures and dental titanium implants (Stemberger et al 2007, Matl et al., 2008, 2009, Kuehn 2010). The coating of medical implants made of these antibiotics/antiseptics fatty acids solely consist of the agent as well as an intrinsic fatty acid. Such anti-infective substances contain no polymer and no critical degradation products are produced. It can be hypothesized that these *in-vitro* results are comparable to the findings of antibiotic loaded cemented arthroplasty, and can therefore potentially provide a significant contribution to the reduction of infection in the use of titanium prostheses and other implants.

REFERENCES: Kim, S. 2008 Arthritis Rheum. 59(4), pp.481-488; Kuehn KD, Bruenke J.(2010 IJNBM, 3(1), 107-117; Kuehn KD. 2010 IJNBM, 3(1), 94-106; Matl, F.D. et al. 2008 Antimicrob Agents Chemother. 52 (6), pp. 1957-1963; Matl, F.D. et al. 2009 J Biomater Sci Polym Ed. 20(10), pp.1439-1449; Parvizi, J. et al. (2008). Acta Orthop. 79(3), pp.335-341; Stemberger A., et al 2007. In: Walenkamp, G., Local antibiotics in arthroplasty, Thieme Verlag, 13-21.; Vogt, S.et al . (2005) Mat.-wiss. U. Werkstofftech. 36, 12, 814-819;

PROTECTING POLYMER SURFACES AGAINST BACTERIAL ADHESION AND BIOFILM FORMATION

Hans J. Griesser[1], Krasimir Vasilev[2], Hardi Ys[1,3], Chi Ndi[1,4], Stefani S. Griesser[1], Marek Jasieniak[1], Htwe Mon[1,4], Susan Semple[5]

[1] Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[2] Mawson Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[3] University of Tadulako, Palu, Indonesia
[4] School of Pharmacy and Medical Sciences, University of South Australia
[5] Sansom Institute, University of South Australia, Adelaide, SA 5000, Australia

Introduction. Bacterial attachment and biofilm formation might be reduced by application of a thin coating that deters bacterial colonisation.[1,2] For biomedical devices a coating should also allow attachment of human tissue to facilitate wound healing, or for catheters and contact lenses be lubricious and not bio-adhesive. As requirements differ for antibacterial coatings for different implants and devices, we are studying several approaches for fabricating antibacterial coatings.[3] Passive, non-adhesive thin coatings are generated by the covalent grafting of hydrogel polymers. Alternative strategies comprise covalent immobilization of antibiotic molecules, or release of silver ions through a diffusion-regulating thin overlayer.[4]

Methods. Our strategies are based on plasma polymer thin film coatings, as this platform coating approach can be transferred to many polymeric, metallic, and ceramic materials. Plasma polymers with chemically reactive surface groups (e.g., amine, epoxide, aldehyde) enable covalent immobilisation of antibacterial compounds onto their surfaces. Alternatively, we load plasma polymer coatings with silver nanoparticles, from which Ag^+ ions outdiffuse.[4] A third approach comprises covalent grafting of end-functionalised PEG or pNIPAM polymer chains onto plasma polymers. Organic compounds investigated in this study were the commercially available antibiotic novobiocin[5] and novel antibiotics of the serrulatane class,[5] the latter are substituted diterpenes extracted from Australian plants used in traditional medicine.[6] Chemical compositions of coatings were checked by XPS and ToF-SIMS. Samples were tested for bacterial attachment and biofilm formation, and with mammalian cell lines. Serrulatanes were also tested in solution for bacterial inhibition and cytotoxicity. Aliquots taken from broth above samples were used to test for effects on bacteria in solution.

Results. As expected, protein-resistant coatings comprising covalently grafted PEG or pNIPAM completely resisted biofilm formation, while bacteria in solution remained viable. Surface-immobilised PEG, novobiocin, and serrulatanes reduced bacterial attachment by up to 99.8%. While biofilms formed on control surfaces within 48 hrs, these coatings prevented biofilm formation. Plasma polymer coatings loaded with Ag nanoparticles also were effective; Ag^+ delivery can be adjusted via the properties and thickness of the plasma polymer film and the silver loading [4] However, coated samples showed adverse effects on mammalian cell lines in many cases, with silver in particular. With organic antibiotics, the surface density appears important and an optimum must be found between deleterious cell effects and antibacterial effectiveness.[5]

Discussion and Conclusions. All strategies are effective in reducing or preventing biofilm formation, but adverse effects on mammalian cells need further investigation, and mechanisms need to be elucidated. With serrulatanes, this is now underway within a collaboration under the CCMX scheme.

References

1. H.J. Griesser, K. Vasilev, H. Ys and S.A. Al-Bataineh, in: *Surface Modification of Biomaterials*; R. Williams (Ed.), Woodhead Publishing, Cambridge, UK, 2011.
2. K. Vasilev, J. Cook and H.J. Griesser, *Expert Review of Medical Devices* **6**, 553 (2009).
3. H.J. Griesser, H. Ys, C.P. Ndi, L. Britcher, K. Vasilev, M. Jasieniak, S.S. Griesser, S.J. Semple, *Chemistry in Australia* **75** (10), 5, 2008.
4. K. Vasilev, V. Sah, K. Anselme, C. Ndi, M. Mateescu, B. Dollmann, P. Martinek, H. Ys, L. Ploux, H.J. Griesser, *NanoLetters* **10**, 202 (2010).
5. H. Ys, PhD Thesis, Univ. South Australia, 2010.
6. C.P. Ndi, S.J. Semple, H.J. Griesser, S.M. Pyke, M.D. Barton, *Phytochemistry*, **68**, 2684 (2007).

Acknowledgment. Partial support by CCMX MatLife, RMS Foundation and BASF.

Bone cell viability and antibacterial efficacy of porous TiO_2-Ag coatings

B.S. Necula[1], L.E. Fratila Apachitei[1], J.P.T.M. van Leeuwen[2], S.A.J. Zaat[3], I. Apachitei[1], J. Duszczyk[1]

[1]Delft University of Technology, Group of Biomaterials Technology, 2628 CD, Delft, NL
[2]Erasmus Medical Center, Department of Internal Medicine, 3015 GE, Rotterdam, NL
[3]Academic Medical Center, Department of Medical Microbiology and Center for Infection and Immunity Amsterdam (CINIMA), 1105 AZ Amsterdam, NL

INTRODUCTION: Despite advanced sterilization techniques, strict surgery rules and systemic antibiotic prophylaxis, indwelling medical devices represent a great risk for developing implant-related infections.
The aim of this work was the *in vitro* testing of bone cell viability and antibacterial efficacy of Ag-bearing coatings prepared by Plasma Electrolytic Oxidation (PEO) process on Ti6Al7Nb biomedical alloy.

METHODS: The porous coatings were produced in electrolytes based on Ca and P salts bearing two different concentrations of Ag nanoparticles (0.3 and 3.0 g/l) as bactericidal agent. The surface morphology of the coatings was characterized by Scanning Electron Microscopy. Simian Virus Human Fetal Osteoblast (SV-HFO) cells viability was quantitatively determined using the Alamar Blue assay after 2, 5 and 7 days. The *in vitro* antibacterial activity of the Ag-bearing coatings against methicillin-resistant *Staphylococcus aureus* (MRSA) was assessed using the direct contact assay, specifically developed to mimic the conditions of an infection of a primary total joint replacement [1].

RESULTS: The coatings produced by PEO under the selected conditions revealed a porous structure consisting of both amorphous and crystalline TiO_2 phases, and the presence of Ag nanoparticles on the surface and inside of the pores (Fig. 1).

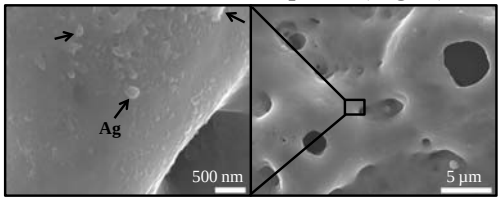


Fig. 1: Surface morphology of TiO_2-0.3Ag coatings

In vitro viability tests (Fig. 2) showed SV-HFO cells proliferation after 2, 5 and 7 days on TiO_2-0.3Ag and TiO_2 (no Ag) coatings without any significant differences between them. However, the TiO_2-3Ag surfaces inhibited cell proliferation at all time-points.

In vitro antibacterial testing (Fig. 3) proved excellent MRSA killing rates after 24 hours for both TiO_2-0.3Ag and TiO_2-3Ag coatings with efficiencies of 98.25% and >99.75%, respectively. On the TiO_2 control surface the number of CFU increased 1000-fold.

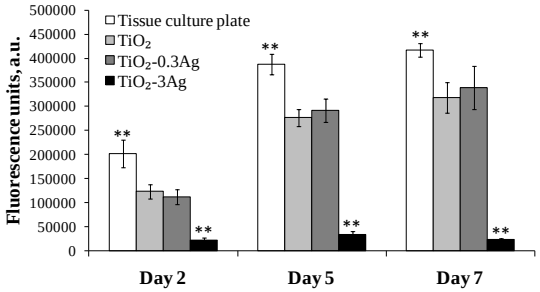


*Fig. 2: SV-HFO cell viability on TiO_2- Ag coatings (n=9, ** p< 0.001)*

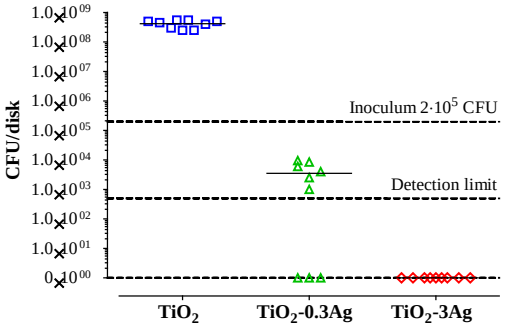


Fig. 3: Antibacterial effect of TiO_2-Ag coatings against MRSA (n=3).

DISCUSSION & CONCLUSIONS: From the 2 different coatings tested, the one produced with relatively lower concentration of Ag nanoparticles showed both bone cell viability and high bactericidal activity. The findings suggest that the PEO process may be a powerful surface modification technology for surface finishing of bone implants, allowing production of coatings with multiple biofunctionalities.

REFERENCES: [1]Necula et al., Acta Biomater 5 (2009), pp. 3573–3580

Antibacterial HA coatings with defined crystallinity to tune Ag release

S.Lischer[1], C.R. Bradbury[2], B.Wampfler[1], Q.Ren[1], Ph.Gruner[3], K.Maniura[1]

[1] Empa, Swiss Federal Laboratories for Materials Science & Technology, Lerchenfeldstr. 5, CH-9014 St. Gallen, [2] Empa, Feuerwerkerstr. 39, CH-3602 Thun, [3] Medicoat AG, Gewerbe Nord, CH-5506 Mägenwil

INTRODUCTION: The administration of antibiotics to prevent bacterial infections after e.g. an implant surgery is increasingly replaced by the use of silver-releasing biomaterials. To overcome the critical post-implantation period of biofilm formation or bacterial adhesion at the implant-tissue interphase, it is beneficial if implants possess antibacterial properties. Infections after total hip arthroplasties still occur in 1 to 2% of the cases[1,2]. Surface modification is a common feature in implant technology to improve the implant properties for tissue integration as well as antibacterial activity. Silver-containing hydroxyapatite (HA) coatings provide the possibility to control silver release off these coatings. They therefore show an antibacterial effect and even appear to enhance osseointegration.

METHODS: The vacuum plasma spraying method (VPS) was used by Medicoat AG to produce Ag-containing HA coatings. The coatings were characterised by X-ray diffraction to determine their composition and crystallinity. The adhesion of the coating to the substrate was analysed according to ISO 13779. The silver release was measured by ICP-OES. The cytotoxicity assay is based on the ISO 10993-5 and evaluates if the HA-Ag coatings release toxic substances. The extract of the coatings is added to primary human bone cells and 3T3 mouse fibroblasts at different concentrations and quantified by MTT and DNA assay. The antibacterial activity of the coatings was analysed by standard agar diffusion test, biofilm formation and proliferation test with two bacteria strains: *S. aureus* (Gram-positive) and *E.coli* (Gram-negative).

RESULTS: The VPS technology is a useful method to produce HA coatings with different concentrations of silver and a range of crystallinity. The mechanical properties and the composition of the coatings fulfilled the regulatory requirements. The HA and the HA-Ag coatings resisted a higher adhesion force than required by the ISO-Norm and XRD measurements showed that the coatings are conform to the standard. ICP-OES analysis showed a high silver release in the first 24h of exposition in TRIS-buffer and cell culture media, followed by a lower and continuously decreasing release over 4 days. The total amount of released silver is higher in the TRIS buffer compared to the cell culture media. The silver release data correspond with the results of the antibacterial tests. The higher the Ag release, the higher the inhibition of bacterial growth. It is also shown that HA-Ag coatings have different effects on gram-positive and gram-negative bacteria due to the different constitution of their cell membrane. The results of the cytotoxicity test showed that both HA coatings with and without silver are biocompatible to 3T3 mouse fibroblasts as well as primary human bone cells.

CONCLUSIONS: The antibacterial HA coating of an implant could help to reduce the incidence of bacterial infections in orthopaedic surgery and enhance at the same time the adherence of bone to the implant surface. In this project, silver-containing HA coatings could be produced in a reproducible way. The coatings exhibit antibacterial properties and are cytocompatible.

REFERENCES: [1]Anagnostakos, K et al., 2009, Classification of hip joint infections. Int. J. Med. Sci 6. [2]Fink, B et al., 2009, Revision of late periprosthetic infections of total hip endoprostheses: pros and cons of different concepts. Int. J. Med. Sci. 6

ACKNOWLEDGEMENTS: The Commission for Technology and Innovation "CTI Medtech" is acknowledged for financial support of the project under the contract 9071.1 PFLS-LS.

The PolyPid - A novel local delivery system: from the physic-chemical aspects to innovative and superior therapeutic potential

N. Emanuel, D. Segal, Y. Rosenfeld, O. Cohen and Y. Barenholz

http://www.polypid.com PolyPid Ltd, Israel

INTRODUCTION: PolyPid technology is a novel local drug delivery system (DDS) that was planned to control the release of many drugs and biological agents. It was developed in order to fulfill the major deficiencies of the two well established drug delivery systems, polymers and lipids. These deficiencies include a large burst release upon administration and lack of prolonged and controlled drug release necessary to achieve therapeutic efficacy. By synergistically "fusing" the right lipids with the right polymers PolyPid has succeeded to achieve a novel DDS with unmatched performance. This fusion is based on the self assembly of pharmaceutical known polymeric and lipids components into a highly organized nano-scale derived super-molecular structures. The lipids used are mainly synthetic phospholipids and cholesterol. The release rate of the drug can be pre-programmed by the selection of the exact polymer and lipid composition. The PolyPid DDS was characterized by many physical methods including differential scanning calorimetric (DSC), SEM and X-ray diffraction. It was demonstrated that each one of the major component significantly contributing to the final organized structure, and that the drug molecules are fully integrated in the final well organized structure. PolyPid DDS shows a direct correlation between the physic-chemical features of the system and its performance. PolyPid technology platform enables to entrap a large variety of either a single or combination of agent(s) and to release them at a pre-programmed zero-order kinetics rate for the desired time in the preset range of several days to several months. The drugs reservoir is fully protected not only against biological destruction but also against hydration, particularly important when long lasting activity of sensitive drugs is required. This biocompatible and biodegradable family of drug carriers can supply implantable solutions for prolonged and complicated medical protocols. The flexibility of PolyPid solutions allows it to serve as independent structures as well as coating material that can be used with numerous medical devices and implant with a large variety of active materials.

BonyPid™

The first PolyPid technology based family of products is BonyPid™ which is designed to serve in the orthopedic field. Bones are subject to severe morbidity, and bone infections following orthopedic grafting procedures are common and catastrophic to the patient. Systemic administration of antibiotics is not sufficiently effective due to low penetration to the diseased tissue and the high concentrations needed are not always safely tolerated. Currently available local delivery systems are not sufficiently effective due to high burst and short lasting effect. Consequently, prolonged and controlled local delivery of antibiotics to the bone tissue can play a major role in the treatment of acute and chronic (osteomyelitis) bone infections.

The BonyPid™ is based on the commonly used bio-degradable and biocompatible bone-void-filler particles. These particles are coated with a fine layer of the PolyPid based biodegradable formulation which include a potent antibiotic. Upon in-vivo hydration the entrapped anti-bacterial agent is released over a predefined period and at a pre-set zero order kinetic release rate that was pre-designed to achieve sufficient local drug concentrations. The coating surface is gradually disintegrated layer by layer thereby releasing the antibiotic into the surrounding tissue, while the bone-void-filler scaffold remains and supports bone recovery.

BonyPid™ has been tested pre-clinically in infected rabbit's tibia model. It was clearly demonstrated that the therapeutic efficacy of BonyPid™ is highly effective in both acute and chronic infection models as well as significantly advantageous over the non-formulated free drug.

Alternative approaches for the diagnosis and treatment of staphylococcal biofilm-associated infection

MS Smeltzer[1], KE Beenken[1], VP Zharov[2], EI Galanzha[2]

[1]Department of Microbiology and Immunology, [2] Department of Otolaryngology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

INTRODUCTION: Infections associated with indwelling medical devices are difficult to treat even when the offending bacteria are not resistant to the preferred antibiotics. A primary reason for this is formation of a biofilm, the presence of which confers a level of intrinsic resistance that significantly compromises conventional antimicrobial therapy. For this reason, the treatment of biofilm-associated infections often requires surgical intervention to debride infected tissues and/or remove infected biomedical implants. As part of a comprehensive approach to this problem, we explored the use of an antibiotic-independent nanotherapy approach to the treatment of staphylococcal biofilm-associated infection.

METHODS: We have demonstrated that mutation of the staphylococcal accessory regulator (*sarA*) limits biofilm formation to a therapeutically-relevant degree in all strains of *Staphylococcus aureus*. Studies examining the mechanistic basis for this deficiency have led us to conclude that the primary factor is the increased production of extracellular proteases in *sarA* mutants and the impact of these proteases on specific surface-associated proteins (1). We have taken advantage of antibodies directed against these proteins to deliver gold-plated carbon nanotubes (GNTs) to the surface of staphylococcal cells and to show that the laser-induced generation of photothermal (PT) and photoacoustic (PA) effects defined by these GNTs can be used to kill *S. aureus* (2). However, these experiments were limited to planktonically-grown bacteria. Here we extend this work to demonstrate that the same approach can be used to kill biofilm-associated and blood-borne staphylococci.

RESULTS: The production of protein A is a defining characteristic of *S. aureus*, and there are reports suggesting that protein A plays a functional role in biofilm formation (3). We took advantage of this to explore the use of GNT-conjugated protein A-specific antibody for the eradication of *S. aureus* cathter-associated biofilms. The results confirmed that protein A is on the surface of *S. aureus* cells within a biofilm and that the laser-induced generation of photothermal (PT) and photoacoustic (PA) effects can be used to eradicate these bacteria (Fig. 1). We also confirmed that the same approach can be used to eradicate bacteria within the bloodstream, a critical determinant of many secondary infections including those associated with bone and implanted orthopaedic devices.

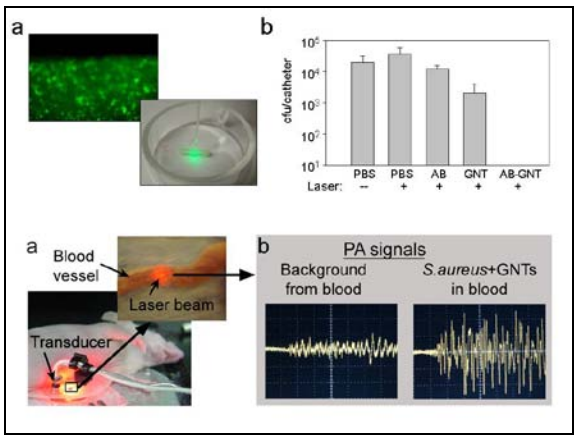


Fig. 1. Top panel (a) Immunofluorescence image illustrating localization of GNT-conjugated protein A antibodies to *S. aureus* cells within a catheter-associated biofilm (upper left) and application of laser energy to catheter-associated bacteria (lower right). (b) Cell counts of bacteria recovered from catheters as a function of antibody (AB)-conjugated GNTs and the application of laser energy. Bottom panel (a) Setup for the treatment of staphylococcal bacteremia in a murine model. (b) Photoacoustic signals as a function of localization of antibody-conjugated GNTs. Subsequent experiments confirmed the killing of blood-borne staphylococci using this method (data not shown).

DISCUSSION & CONCLUSIONS: The results we present suggest that our antibody-based nanotherapeutic approach could be used to overcome many of the problems that define staphylococcal biofilm-associated infections including those involving bone and implanted orthopaedic devices.

REFERENCES: [1] Beenken et al., PLoS ONE, 5:e10790 (2010), [2] Zharov et al., Biophysical J., 90:619-627 (2006), [3] Merino et al., J Bacteriol., 191:832-843 (2009).

Serrulatane EN4, a New Antimicrobial Compound Exerts Potent Acitvity against Adherent Biofilm-Forming Bacteria *In Vitro*

J. Nowakowska[1], H. J. Griesser[2], C. Acikgoz[3], M. Textor[3], R. Landmann[1], N. Khanna[1]

[1]*Infection Biology, Department of Biomedicine, University Hospital of Basel, CH,* [2] *Ian Wark Research Institute, University of South Australia,* [3]*Laboratory for Surface Science and Technology, Department of Materials, ETH Zurich, CH*

INTRODUCTION: Implant-associated infections are mainly caused by biofilm-forming microorganisms such as *Staphylococcus (S.) aureus* and *S. epidermidis*. Successful treatment requires the use of bactericidal drugs, which are active against adhering bacteria. Serrulatane EN4, belongs to the family of diterpenes isolated from the *Eremophila* plant species and was previously reported to exert antimicrobial activity [1]. Our goal is to elucidate the activity of free and surface-coupled serrulatanes against these adherent microorganisms.

METHODS: Serrulatane EN4 extract was kindly provided by H. Griesser. Methicillin resistant *S. aureus* (MRSA) strain WSPA A, its isogenic methicillin susceptible mutant ME 230, *S. aureus* SA113, *S.epidermidis* 1457 and PIA-mediated biofilm-deficient *S.epidermidis* 1457 *Δica* mutant were used. The minimal inhibitory and minimal bactericidal concentrations (MIC and MBC) in the logarithmic and stationary growth phase were evaluated by macrodilution method according to the CLSI guidelines. Antimicrobial susceptibility of adherent bacteria was measured after 24 hours exposure of surface-attached bacteria to serial dilutions of EN4. Time-kill studies were performed as previously published [2]. Cytotoxicity of EN4 on HL-60 human promyelocytic leukemia cell line was determined by Annexin V/propidium iodine flow cytometry analysis.

RESULTS: The EN4 MIC of *S. aureus* and *S. epidermidis* in logarithmic and stationary growth phase were 75.7 and 151.4µM and the MBC 151.4 and 302.8µM, respectively (Tab.1). EN4 was similarly effective against MRSA and elicited antimicrobial activity towards different Gram-positive, but not to Gram-negative bacteria. Additionally, EN4 was bactericidal against adherent bacteria independently of PIA-mediated biofilm (Fig.1). In time-kill studies, EN4 showed rapid and concentration-dependent killing of staphylococci. At concentrations above 154.1 µM, EN4 reduced bacterial counts by >3 $\log_{10}$ CFU/ml in 2 hours but did not cause HL-60 cytotoxicity until after 24 hours exposure.

Table 1. Susceptibility to EN4 of planktonic staphylococci.

Strain	Logarithmic growth phase		Stationary growth phase
	MIC(µM)	MBC(µM)	MBC(µM)
WSPA A	75.7	151.4	302.8
ME 230	75.7	151.4	302.8
SA 113	75.7	151.4	151.4
S.epidermidis 1457	75.7	302.8	302.8
S.epidermidis 1457 *Δica*	151.4	302.8	302.8

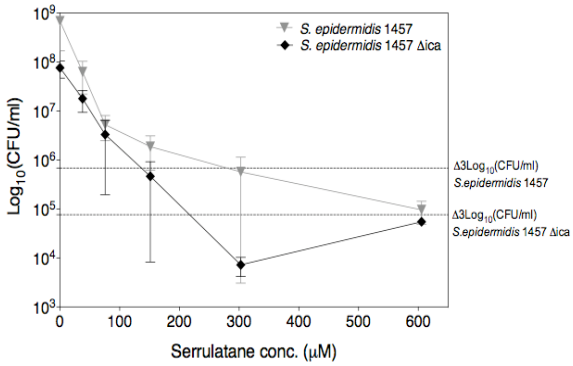


Figure 1. Activity of EN4 against adherent bacteria.

DISCUSSION & CONCLUSIONS: Free serrulatane EN4 shows potent and similar bactericidal effects against logarithmic, stationary growing and surface-adhering staphylococci independent of PIA-mediated biofilm and may therefore be a promising antimicrobial compound for the treatment of implant-associated infections. Antimicrobial and cytotoxic aspects of EN4 and other serrulatanes, both free and surface-coupled, are currently addressed *in vivo* and the exact mechanism of action is studied.

REFERENCES: [1] Ndi CP et al. J. Nat. Prod. 2007, 70 (9):1439-43. [2]Gordon O et al. Antimicrob Agents Chemother 2010, 54(10):4208-18.

ACKNOWLEDGEMENTS: CCMX, MatLife, BASF and RMS foundation are acknowledged for their financial support.

Characterization of a three-species biofilm *in vitro*

M. Astasov-Frauenhoffer[1], O. Braissant[2], I. Hauser-Gerspach[1], A. U. Daniels[2], R. Weiger[3], T. Waltimo[1]

[1]*Institute of Preventive Dentistry and Oral Microbiology, University of Basel, Basel, CH,* [2]*Laboratory of Biomechanics and Biocalorimetry, University of Basel, Basel, CH,* [3]*Clinic for Periodontology, Endodontology and Cariology, University of Basel, Basel, CH.*

INTRODUCTION: Microscopic analyses are invaluable tools, allowing versatile views of the structure of biofilms. Scanning electron microscopy (SEM) provides high resolution and magnification; whereas confocal laser scanning microscopy (CLSM) combined with fluorescence *in situ* hybridization (FISH) visualizes the spatial organization and enables the quantification of bacteria. Isothermal microcalorimetry (IMC) provides complementary monitoring of biofilm vitality by measuring aggregate metabolic activity of biofilm microorganisms.
The aim of this study was to establish an efficient system to characterize a three-species *in vitro* biofilm and to observe possible variability between samples.

METHODS: An anaerobic flow chamber model in which planktonic bacteria circulate in simulated body fluid (SBF) was developed. Polished protein-coated titanium disks served as substrates for bacterial adherence. *Streptococcus sanguinis* ATCC 20068 grown overnight in Schaedler broth at 37°C, *Fusobacterium nucleatum* ATCC 10953 and *Porphyromonas gingivalis* ATCC 20709 anaerobically grown in enriched thioglucolate at 37°C for 96 h were harvested, resuspended (10^7-10^9 CFU/mL) in SBF containing 0.2 % glucose and allowed to adhere to the substrates. Bacterial suspension was refreshed at 24 h intervals. After 72 h incubation, the test specimens were removed and prepared for: (i) SEM; (ii) FISH combined with CLSM; (iii) IMC metabolic monitoring on supplemented Columbia agar at 37°C for 480 h.

RESULTS: A continuous biofilm interspersed with exopolysaccharide matrix covering the entire substrate was observed by SEM (Fig. 1A). CLSM showed the bacterial proportions within the biofilm: *S. sanguinis* 41.31 ± 4.83 %, *F. nucleatum* 17.73 ± 2.12 % and *P. gingivalis* 40.96 ± 4.85 % (Fig. 1B). However, the total counts of bacteria per view, 1228.43 ± 451.72, 549.43 ± 232.48, 1297.71 ± 661.16, respectively, differed significantly between experiments ($p<0.05$). IMC revealed possible heterogeneous activity among the biofilm samples. IMC heatflow curve shapes were similar, supporting a consistent sequence of metabolic activity. However, maximum activity varied highly: mean maximum heat flow (Fig. 2A) and mean heat flow after 72 h (Fig. 2B) values were 35.81 ± 42.42 µW and 13.09 ± 21.96 µW. Conversely, differences in time to reach peak heat were small: 20.56 ± 4.48 h (Fig. 2C) also supporting a consistent metabolic sequence. All results are mean ± standard deviation.

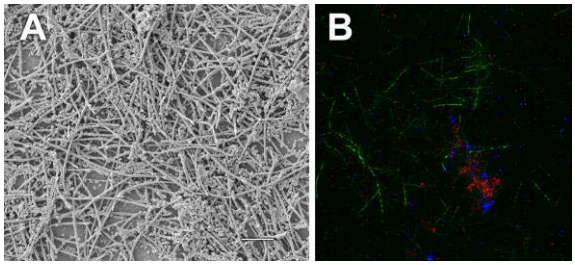


Fig.1. Microscopic analysis: (A)SEM of 72h biofilm (bar indicates 5 µm), (B)FISH analysis: S. sanguinis (blue), F. nucleatum (green) and for P. gingivalis (red). (63x oil) (n=3)

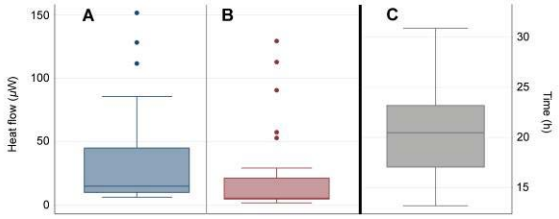


Fig.2. ICM results. (A)Maximum heat flow peak value (µW), (B)mean heat flow after 72h (µW), (C)time to peak heat flow (h). (n=24)

DISCUSSION & CONCLUSIONS: FISH combined with CLSM and SEM seem to be efficient tools for analyzing biofilms in terms of their three-dimensional structure. No changes in relative proportions of the species were detected by increase in bacterial load. However, IMC showed high variations in the amount of metabolic activity – plus persistent activity after 480 hours. Thus, IMC provides complementary insights into biofilm dynamics.

Mechanisms of *Staphylococcus epidermidis* biofilm formation in different types of biomaterial-related infections

D. Mack, L. G. Harris, R. Jeeves, B. Pascoe, A. P. Davis, T. S. Wilkinson

Medical Microbiology and Infectious Diseases, College of Medicine, Swansea University, UK

INTRODUCTION: *Staphylococcus epidermidis* is the prototype organism involved in medical biofilm disease resulting in infected implants like intravascular catheters or joint prostheses yearly affecting millions of patients worldwide. These infections persist despite antimicrobial treatment due to organization of *S. epidermidis* in surface adherent biofilms frequently requiring device removal. Biofilms are formed in two phases: initial attachment of bacteria is followed by accumulation of bacteria in multiple layers. Attachment is a multifactorial process involving a variety of specific protein and polysaccharide factors depending on surface properties.

RESULTS & DISCUSSION: We identified polysaccharide intercellular adhesin (PIA) as the central functional factor in biofilm accumulation. PIA is a homoglycan of β-1,6-linked N-acetylglucosamine residues of which 15-20% are deacetylated. PIA is synthesised by the gene products of the *icaADBC* locus. Epidemiological studies defined PIA as the main functional molecule involved in biofilm accumulation in *S. epidermidis*. Using isogenic biofilm-negative *icaA*-insertion mutants expression of PIA and biofilm formation were defined as essential virulence factors of *S. epidermidis* in foreign body infection models [1].

Interestingly, expression of PIA as a mechanism for biofilm accumulation is not unique to *S. epidermidis*. Numerous other staphylococcal species including *Staphylococcus aureus*, S. *caprae*, *S. lugdunensis*, and other coagulase-negative staphylococci possess the *icaADBC* locus and may synthesise PIA. Additionally, a number of Gram-negative human pathogens including *Escherichia coli*, *Aggregatibacter actinomycetemcomitans*, *Actinobacillus pleuropneumoniae*, *Yersinia pestis*, *Acinetobacter baumannii*, and *Bordetella spp.* synthesise PIA using enzymes encoded by orthologous gene loci referred to as *pgaABCD* or *hmsHFRS*, indicating that PIA is a general principle in biofilm formation in many eubacteria [1].

Study of the molecular epidemiology of *S. epidermidis* strains revealed that almost all isolates from port-catheter infections were *icaADBC*-positive and proficient for PIA-synthesis while the strains from prosthetic joint infections produced biofilms frequently in an *icaADBC*- and PIA-independent manner [2]. Consequently we discovered two additional mechanism of biofilm accumulation, which were completely polysaccharide-independent. The highly prevalent accumulation associated protein (Aap) is activated by proteolytic processing by staphylococcal or host proteases generating an intercellular adhesin mediating biofilm accumulation [3]. Apparently, *S. epidermidis* can use factors of innate immunity to generate phagocytosis resistant bacterial aggregates and biofilm leading to immune escape and persistence. The giant 1 MDa extracellular matrix binding protein Embp acts as an intercellular adhesin in some *S. epidermidis* strains independent of PIA and Aap [4].

The accessory gene regulator *agr* is the major quorum sensing system in staphylococci, which uses four different octapeptides, defining *agr*-groups, as quorum signals. RNAIII transcript is the major effector of *agr*-activation in the late exponential growth phase, which also encodes δ-toxin, a proinflammatory factor belonging to the phenol-soluble modulins produced by staphylococci. In chronic device related infection *S. epidermidis* strains of *agr*-group 1 are overrepresented compared to controls. Additionally, strains emerge during chronic infection, which no longer express δ-toxin investigated as a surrogate of *agr*-activation. Transcriptional analysis revealed that there is no correlation between δ-toxin expression and RNAIII upregulation indicating presence of post-transcriptional regulators controlling δ-toxin expression [5].

REFERENCES: [1] Mack D. et al., Top. Curr. Chem. 2009; 288:157-82. [2] Rohde H. et al. Biomaterials 2007 ; 28 :1711-20. [3] Rohde H. et al. Mol. Microbiol. 2005; 55:1883-95. [4] Christner M. et al. Mol. Microbiol. 2010; 75:187-207. [5] Harris L.G. et al. J. Hosp. Infect. 2010; 76 Suppl. 1:S21.

Novel vaccine strategies and improved diagnostics for musculoskeletal infections

M.E. Shirtliff [1,2], G.A. O'May [1], R.A. Brady [1], M.L. Prior [1], J.G. Leid [3]

[1]Department of Microbial Pathogenesis, Dental School, University of Maryland-Baltimore, Baltimore, Maryland, USA; [2]Department of Microbiology and Immunology, School of Medicine, University of Maryland-Baltimore, Baltimore, Maryland, USA; [3]Department of Microbial Pathogenesis, Dental School, University of Maryland-Baltimore, Baltimore, Maryland, USA [4]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

INTRODUCTION: *Staphylococcus aureus* is the major etiological agent in musculoskeletal infections due to the ability of this pathogen to develop from an acute phase to a persistent, chronic and recurrent biofilm infection. *S. aureus* biofilms were grown on pins and implanted into the tibia of mice resulting in a chronic and persistent biofilm implant infection. Cytokines, antibody subtypes and T-cell populations were measured. It was found that an early adaptive inflammatory immune response occurred due to Th1 and Th17 T-cell subtypes. By reducing the inflammatory cytokines, the host was able to spontaneously resolve the infection. Therefore, we sought to design a vaccine that promoted a Th2 response and an early antibody response that could prevent the fully mature biofilm phenotype from forming *in vivo*.

METHODS: Previously-identified biofilm up-regulated antigens in *S. aureus* were used in a biofilm-specific, quadrivalent vaccine and taking into account *in vivo* antigen expression and the heterogeneous nature of protein production within the biofilm. Vaccinated rabbits were challenged in a model of tibial osteomyelitis. Antibiotic treatment was also administered to eradicate the remaining non-attached, sensitive planktonic cells. Efficacy was evaluated by bacterial culture and radiographic scoring. In addition, these biofilm up-regulated antigens for a rapid lateral flow assay to test the ability to detect host antibodies against these antigens, thereby providing for a diagnostic system for hard-to-detect biofilm infections. Lastly, we labelled antibodies against biofilm up-regulated antigens to test for *in vivo* localization and detection of biofilm infections.

RESULTS: Vaccination coupled with vancomycin treatment effectively cleared an MRSA biofilm infection in a rabbit model, while antibiotics or vaccine alone failed (Fig. 1). In addition, antibodies against biofilm-specific antigens could be used as a rapid (<10 min.) diagnostic for *S. aureus* biofilm infections and labelled antibodies against these antigens localized at the site of infection (Fig. 2).

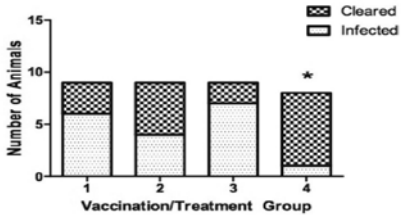


Fig. 1. Animals vaccinated with (1) PBS, (2) PBS + subsequent treatment with vancomycin, (3) the quadrivalent vaccine, or (4) the vaccine plus vancomycin

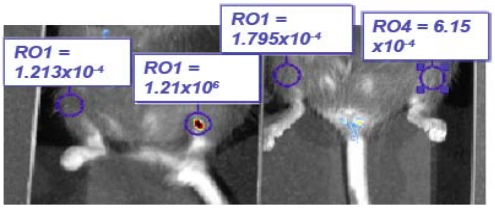


Fig. 2. Anatomical position, Xenogen image of labeled antibodies localized at a site of prosthetic implant infection in the left tibia (image on left) vs. a sterile inserted pin (image on right).

DISCUSSION & CONCLUSIONS: Taking the biofilm mode of growth into account allows for the development of novel prevention and diagnostic modalities in musculoskeletal infections.

REFERENCES: [1] Prabhakara R, et. al. Infect Immun. 2011 Apr;79(4):1789-96. [2] Brady RA, et. al. Infect Immun. 2011 Apr;79(4):1797-803. [3]Brady RA, et. al. Infect. Immun. 74(6):3415-3426, 2006.

ACKNOWLEDGEMENTS: These studies were funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health grant R01AI69568.

Development of a Passive Immunization for Methicillin-Resistant *Staphylococcus aureus* (MRSA) Osteomyelitis

SL. Kates[1], JJ. Varrone[1], JL. Daiss[1], EM. Schwarz[1]
[1]Department of Orthopaedics, University of Rochester Medical Center, Rochester, NY
http://www.urmc.rochester.edu/ortho/research/ourresearchteam.cfm

INTRODUCTION: *Staphylococcus aureus* (*S. aureus*) is the single leading cause of osteomyelitis, a bacterial infection of bone that is characterized by progressive inflammatory destruction (osteolysis). It is estimated that 20-50% of these cases are due to MRSA. Although the overall rate of MRSA infection in patients undergoing primary total joint replacement (TJR) surgery is very low (~1%), the management of these infections is very challenging and requires a two-stage surgical procedure. Moreover, the reinfection rate for revision TJR due to MRSA is very high (40-50%). Thus, investigations of non-traditional approaches, like passive immunization, to decrease the reinfection rate are warranted. To this end, the goal of this study is to evaluate the effects of monoclonal antibodies (mAbs) against the glucosaminidase (Gmd) subunit of *S. aureus* autolysin (Atl), which is known to be an immuno-dominant antigen in animal infection models. Since Atl is essential for cell wall digestion during binary fission, and Gmd mutants are unable to divide, we hypothesized that effective anti-Gmd mAbs will inhibit *S. aureus* proliferation and/or survival *in vitro* by inhibiting cell division and forcing the microbes to grow as fused bacteria.

METHODS: Antibodies: ELISA and dot-blot screening of 33 candidate hybridomas revealed five clones (1C11, 1E12, 2D11, 3A8, 3H6) that produced IgG1 mAb with high affinity binding to recombinant Gmd. These hybridomas were grown in DMEM media containing 10% FBS, and mAbs were purified from the culture supernatant using Protein G sepharose and then concentrated and dialyzed to make 1 mg/ml stocks.

Growth Assay: The *S. aureus* strain Xen29 was grown in LB media for 12 hours to achieve a mid-log growth suspension. 100 CFU Xen29 was placed in each treatment well of a 96-well plate, supplemented with 50 µg/ml of antibody, and readings were taken at 490 nm every two hours. The same growth assay was then slightly modified to study the dose-dependent effect of the five mAbs on *S. aureus* growth. MOPC-21 was used as an irrelevant isotype control in all growth assays.

Scanning Electron Microscopy: Xen29 *S. aureus* was grown for 12 hours in LB media to achieve a mid-log growth suspension. For the treatment group, 10,000 CFU of Xen29 was incubated with mAb 1C11 for 1 hour, whereas the negative control was not treated with mAb. Samples were then plated onto round glass coverslips, fixed, dehydrated, and coated with gold for visualization by SEM.

In vivo protection study: Mice (n=5) were passively immunized with placebo or anti-Gmd (50mg/kg) via i.p. injection on day -3, challenged with a Xen29 infected trans-tibia implant on day 0, and the infection was quantified by *in vivo* bioluminescence as described in Li et al *J. Ortho. Res.* 2008, 26:96-105.

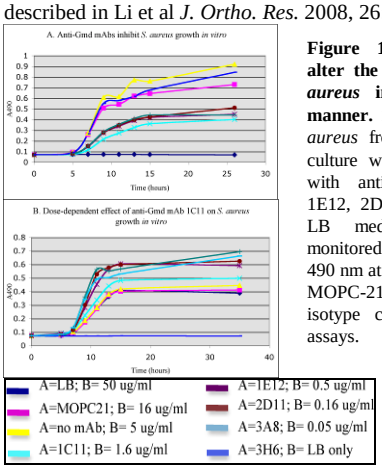


Figure 1. Anti-Gmd mAbs alter the growth of Xen29 *S. aureus* in a dose-dependent manner. 100 CFU of Xen29 *S. aureus* from a mid-log growth culture was incubated at 37°C with anti-Gmd mAbs 1C11, 1E12, 2D11, 3A8, and 3H6 in LB medium. Growth was monitored by light scattering at 490 nm at the indicated intervals. MOPC-21 was used as an isotype control in all growth assays.

RESULTS: Four of the five monoclonal antibodies against glucosaminidase inhibited growth of *S. aureus* in our *in vitro* growth assays, whereas the irrelevant IgG1 control MOPC-21 had no effect on *S. aureus* growth (Figure 1a). The effect was dose-dependent and consistent with a high affinity interaction between each antibody and Gmd (Figure 1b). Consistently, SEM revealed that *S. aureus* treated with 1C11 failed to complete binary fission and grew as chains of bacteria in large aggregates, in contrast to the single-cell suspension of the control culture (Figure 2).

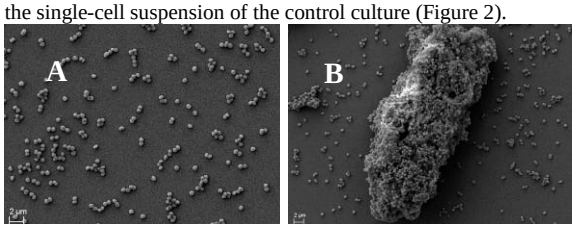


Figure 2. Anti-Gmd mAbs alter planktonic growth of *S. aureus in vitro*. Wild-type *S. aureus* grew as a single-cell suspension under normal growth conditions (A). In contrast, *S. aureus* treated with anti-Gmd mAb 1C11 did not undergo cell division and grew as chains that precipitated out of culture as large aggregates (B), as revealed by scanning electron microscopy.

The anti-Gmd mAb also protected mice from implant associated-osteomyelitis, as evidenced by a significant reduction in BLI (Fig. 3)

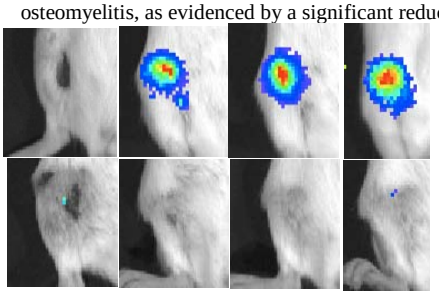


Figure 3. Anti-Gmd mAbs protect mice from implant-associated OM. Mice (n=5) were passively immunized with placebo (top) or anti-Gmd (bottom) prior to challenge and BLI was obtained on days 0,3,5 & 7.

DISCUSSION: Our pursuit of anti-Gmd mAbs as a passive-immunization for MRSA is multifold. First, we identified Gmd as a protective antigen in a murine osteomyelitis. Secondly, the DNA sequence of Gmd is 100% conserved in all known *S. aureus* strains, suggesting that mutation towards antigenic variation of this critical enzyme may not be possible. Lastly, we propose three distinct mechanisms of action for anti-Gmd mAbs that are synergistic with standard antibiotic chemotherapy: 1) opsonization to facilitate bacterial clearance by phagocytic cells; 2) complement mediated lysis of opsonized bacteria with exposed periplasm due to aborted cell wall metabolism; and 3) direct effects on bacterial viability and/or growth inhibition. Here we demonstrated that anti-Gmd mAbs significantly inhibit *S. aureus* growth as determined by light scattering. Results from our ongoing metabolic labeling experiments to confirm this will be discussed. Additionally, scanning electron microscopy of treated cultures revealed that *S. aureus* failed to complete cell division, which explains the sedimentation observed in our treated cultures. These data indicate that anti-Gmd mAbs are attractive candidates for the development of a passive vaccine, and may prevent reinfection in patients undergoing total joint replacement revision surgery due to MRSA-induced osteomyelitis.

ACKNOWLEDGEMENTS This work was supported by Codevax LLC, the University of Rochester Center for Musculoskeletal Research and a Kirchstein-NRSA T32 Training grant from the U.S. Department of Health and Human Services.

In Vitro Modelling of Interactions between Bacteria, Osteoblast-like cells and Macrophages in the Pathogenesis of Biomaterial-Associated Infections

R. Kuijer, G. Subbiahdoss, I.C. Saldarriaga Fernández, J.F. da Silva Domingues, H.C. van der Mei, H.J. Busscher

Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

INTRODUCTION: Biomaterial-associated infections constitute a major clinical problem that is difficult to treat and often necessitates implant removal. Pathogens can be introduced on an implant surface during surgery and will then compete with host cells which are trying to integrate the implant. The ultimate fate of an implant depends on the outcome of this 'race for the surface'. In this study we investigated the role of macrophages in our in vitro model for this so-called 'race for the surface'[1] using various bacterial strains with different virulence.

METHODS: Poly(methyl methacrylate) (PMMA) was used as substrate. PMMA plates were rinsed in 70% ethanol for 10 minutes and subsequently washed with ultrapure water. The contact angle was 73±3°. Human U2OS osteoblast-like cells were routinely cultured in DMEM-low glucose + 10% FBS and 0.2 mM ascorbic acid-2-phosphate. J774 murine macrophages were routinely cultured in DMEM-high glucose + 10% FBS (optimum culture medium). Macrophages were harvested by scraping. The bacterial strains, *S. epidermidis* ATCC 35983, *S. epidermidis* ATCC 35984, *S. epidermidis* 3399, *S. aureus* ATCC 12600, *S. aureus* A20734, *S.aureus* 7388, *P. aerigunosa* DN7348, *P. aeruginosa* PA01, *P. aeruginosa* ATCC 27853, were cultured using routine microbiological methods and before seeding suspended in sterile PBS at a concentration of 3 x 10^6 bacteria/ml. Growth and biofilm formation of all bacterial strains as well as U2OS and J774 cells in modified culture medium (98% DMEM+FBS and 2% TSB)[1] was confirmed.

The competitive assay was performed in a parallel plate flow chamber at 37°C. Bacterial deposition and U2OS cell adherence, spreading and growth were observed with a CCD camera mounted on a phase contrast microscope. Bacteria were allowed to adhere to a density of 10^3 bacteria/cm^2, after which the flow was switched to sterile PBS. Then a suspension of U2OS cells (6 x 10^5 cells/ml) with or without J774 cells (12 x 10^5 cells/ml) were seeded using optimum culture medium. Cells were allowed to adhere for 1.5 hour, after which flow was continued with modified culture medium at 0.14 s^{-1} for 48 hours under continuous monitoring. Then cells were fixated, stained with TRITC-phalloidin and analyzed using a CLSM.

RESULTS: After 1.5 h of U2OS cell adherence 2.5 x 10^4 cells/cm^2 were seeded independent of the absence or presence of bacteria or the strain involved. Macrophages did decrease biofilm formation of all bacterial strains by phagocytosis of bacteria. This effect was observed up to 20 h with *S. epidermidis*, and up to 14 h and 10 h with *S. aureus* and *P. aeruginosa*, respectively. At those moments macrophages burst and released ingested bacteria of which at least part seemed to be able to contribute to biofilm formation. U2OS cell death was noticed after 18-20 hours in the presence of *S. aureus* or *P. aerigunosa* independent of the presence or absence of macrophages. Of the three different *S. epidermidis* strains, the slime producing *S. epiderimidis* 3399 and *S. epidermidis* 35984 caused a stronger reduction in U2OS cells in 48 h than did the non-slime producing *S. epidermidis* ATCC 35983, in the absence of macrophages.

DISCUSSION: The introduction of J774 macrophages in the in vitro model for the study of the competition between mammalian cells and bacteria for a biomaterial surface, did reduce the number of adhering bacteria up to the moment the macrophages died. This had no measurable affect on the adherence, spreading and growth of U2OS osteoblast-like cells. In the presence of *S. epidermidis* strains, the ability of the bacteria to produce slime seems a predictor for U2OS cell survival.

REFERENCES: [1] Subbiahdoss et al. Acta Biomat 2009; 5:1399.

Molecular mechanisms of complement evasion: learning from staphylococci

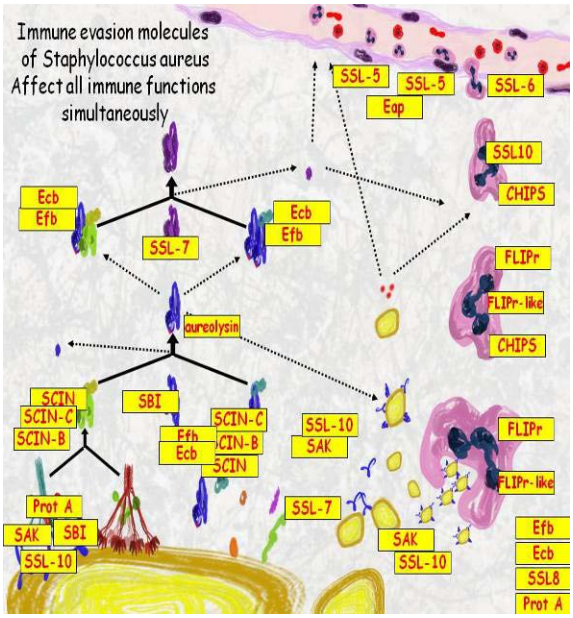
Jos A.G. van Strijp j.vanstrijp@umcutrecht.nl

University Medical Centre Utrecht, the Netherlands

INTRODUCTION: We show that every pathogenic bacterium scrutinized so far to possess a wide array of small, evolutionary conserved proteins which serve to evade the innate immune system. Many of these proteins are directed against some part of the complement system. The sole identification of novel proteins is the easy part. Figuring out the exact molecular mechanism of action is the most labor-intensive part of the process but also the most exciting.

In the beginning of this millennium we characterized an inhibitory activity in the supernate of growing staphylococci. This was identified as a protein that blocks the activation of neutrophils via the formyl peptide receptor (FPR) and C5aR. We dubbed it chemotaxis inhibitory protein of staphylococcus aureus or CHIPS. Next in line awaiting discovery was the Staphylococcal Complement INhibitor or SCIN. SCIN binds to both C3-convertases, and jams the complement system. In the wake of CHIPS and SCIN we went on to describe multiple other immune evasive molecules. These immune evasive molecules will have an interesting career ahead. In the first place the molecules explain why pathogenic bacteria are able to survive and replicate in the human body. Next, they lead researchers directly to key immunological functions. Third they are leads for anti-inflammatory therapy.

The group now consist of several subgroups. Kok van Kessel heads the group that focuses on evasion strategies for specific receptors on white blood cells that are involved in the process of phagocytosis. Carla de Haas has a group that works on the evasion of G-protein coupled receptors. Suzan Rooijakkers' group is specialized in identifying molecules that target the human complement system. Others look at intervention of various inflammatory events.



DISCUSSION Exciting new molecules have emerged from the group in the last year that will be published in the near future. Examples are: a unique broad Fc-receptor antagonist, evasion of Toll-like receptor signaling, bacterial transmigration inhibitors, a highly unexpected complement inhibition strategy and many more. We have shown the importance in vivo and are now exploring the possibility to include these molecules in bacterial vaccines.

REFERENCES: Rooijakkers et al, Trends Microbiol. 2005; Rooijakkers, van Strijp. Mol Immunol. 2007; Bestebroer et al. FEMS Microbiol Rev 2009; Laarman et al J Mol Med. 2010; Serruto et al Nature Rev Microbiol, 2010.

Treatment of infected non union of the long bones with calcium sulfate pellets impregnated with antibiotics.

S. Saghieh[1], A. Murtada[1], A.M. Sheikh Taha[1], K. Masrouha[1].

[1] Orthopedic Division, Surgery Department, American University of Beirut Medical Center, Beirut, Lebanon.

Introduction

Local delivery of antibiotics is an appealing treatment option for infected non union of long bones. The ultimate carrier is the one that can assure a reliable delivery of various types of antibiotics, be absorbable and promote at the same time bone healing.

In this retrospective study, we investigated the use of calcium sulfate granules impregnated with vancomycin and gentamycin in patients with infected non union of long bone.

Methods

We reviewed the treatment of 15 patients with infected non-union of long bones at the American University of Beirut – Medical Center.

Surgical procedure

A radical debridement of all necrotic and nonviable bone and soft tissue from the wound was accomplished, followed by thorough irrigation of the site with copious amounts of normal saline. The average size of the defects was 5.2 cm (0-16 cm) after debridement. Different strategies were used to close the defect including acute shortening with or without gradual bone lengthening, bone transport, bifocal treatment with or without a temporary cement spacer. External fixators were used in all but one patient. At the time of grafting, the average defect was 0.5 cm (range 0-2.5cm). The calcium sulfate powder was mixed with 1g of vancomycin powder with 240 mg of tobramycin in all cases. All patients underwent surgical debridement followed by application of calcium sulfate (Stimulan-Kit) bone graft substitute impregnated with antibiotics (1 gram of vancomycin and 240 mg of gentamycin) to fill in the residual gap resulting

Antibiotics

Adjuvant antibiotic therapy was administered based on the culture results and sensitivities of the various cultured organisms. Antibiotics were administered intravenously during the hospital stay (3 to 42 days) and shifted to oral antibiotics on discharge for a mean duration of 4 weeks (range 0 – 9 weeks). Only our first patient received 6 weeks of intravenous antibiotics.

Outcome measures

Eradication of infection was defined by a drop in ESR to normal level and resolution of draining sinus or other local signs of infection.

Bone healing was assessed on radiograms taken periodically.

SF-36™ Health survey was used to capture practical, reliable, and valid information about functional health and well-being of these patient's. Both the Physical Component Score (PCS) that combines physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH) and the Mental Component Score (MCS) that combines vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH) were measured.

Results

Infection

All draining sinuses that were present preoperatively (six cases) resolved and no recurrence was noticed on postoperative visits. ESR went back to normal in all patients.

Union/ Bone formation

All but one patient achieved union. The failed union was in a patient with a floating elbow. There was a succesfull union on the humerus side and failure of union on the ulna side. Two autograft procedures were performed with persistence of the non union.

SF 36

SF was measured in 12 patients. The average score of the Physical Component (PCS) was 45 and the average score of the Mental Component Score (MCS) 49.

Discussion

Surgical debridement, obliteration of dead space resulting from debridement and a long course of antibiotics remain the mainstay in the management of Osteomyelitis. Many studies have demonstrated that combining debridement with the use of antibiotic impregnated material achieve better eradication of infection and possibly decrease the duration of systemic antibiotics needed (1-6) . Infection was eradicated in all of our patients regardless of the duration and the way of administration of systemic antibiotics.

The bone graft proved to be biocompatible in that it did not inhibit the normal growth of bone. Moreover, its resorption at an average time of 3 months added to this property. Union was achieved in all but one patient in the study. This reflected the osteoconductive role of the bone graft substitute.

The measured SF-36 score delineates the complexity of the problem and the critical status of these patients who have endured multiple previous surgeries.

One drawback from this clinical trial was the lack of a control group with which to compare the data. Thus, this study represents an initial clinical experience only. Randomization of the patients with a control group that received standard therapy (i.e., a two-stage procedure of antibiotic cement bead insertion followed by bone grafting) could assist in eliminating several biases.

In summary, using bone graft substitute impregnated with antibiotics was very effective in treating severe bone infection and non-union. Because of its resorbability and osteoconductive properties, it promoted bone growth and subsequently bone union. It provided a matrix to deliver good concentrations of antibiotics locally and thus better eradication of infection.

References

1. Cho SH, Song HR, Koo KH, et al. Antibiotic-impregnated cement beads in the treatment of chronic osteomyelitis. *Bull Hosp Jt Dis* 1997;56:140–144.

2. Cornell CN, Tyndall D, Waller S, et al. Treatment of experimental osteomyelitis with antibiotic-impregnated bone graft substitute. *J Orthop Res* 1993;11:619–626.

3. Gerhart TN, Renshaw AA, Miller RL, et al. In vivo histologic and biomechanical characterization of a biodegradable particulate composite bone cement. *J Biomed Mater Res* 1989;23:1–6.

4. Patzakis MJ, Mazur K, Wilkins J, et al. Septopal beads and autogenous bone grafting for bone defects in patients with chronic osteomyelitis. *Clin Orthop* 1993;295:112–118.

5. Solberg BD, Gutow AP, Baumgaertner MR. Efficacy of gentamycin-impregnated resorbable hydroxyapatite cement in treating Osteomyelitis in a rat model. *J Orthop Trauma* 1999;13:102–106.

6. Walenkamp GHIM. How I do it: chronic osteomyelitis. *Acta Orthop Scand* 1997;68:497–506

The surgical ritual – The myths and the science

JA Fairclough

University Of Wales Institute Cardiff, UK , Cyncoed Rd, CARDIFF, South Glamorgan

The human body is a walking reservoir of bacteria. Our very existence depends upon the some of these very microbes which, when in the wrong place at the wrong time, may kill or produce serious illness. To the surgeon, every operation is a battle to prevent the microbes, which abound in our bodies and also in the external environment from colonizing wounds or invading other essential bodily systems and producing disease processes.

In early centuries death from infection was common even in the mid-19th century the role of bacteria as a cause of infection was not understood and physicians were ignorant of how infections transferred from one patient to another. It was only following the observations of the early medical scientists such as Semmelweis, Koch and Pasteur that the concept of germ theory and the introduction of basic sterile procedures was first acknowledged. It was only in late 1800s that surgeons accepted the role of disease transmission and scrubbed up and washed their hands between patients or before surgery, causing infections to be transferred from one patient to another. Over time, as the body of scientific knowledge increased, the role of bacteria in the development of wound infection and became recognized. `In an attempt to defeat infection patterns of behavior developed which have now become an established part of the "Surgical Ritual". Enter any western hospital and the casual observer can easily recognize the gleaming operating theatres, with shining clean surfaces where uniformed staff operate in sterile clothing; with gloved hands, often dressed as Spacemen under vast enclosures of filtered laminar controlled air. What the observer does not see are the complex series of protocols, which will be followed to attempt to reduce the incidence of infection.

Why then does infection still happen? Is the "Surgical Ritual" failing?

What is the scientific evidence for these rituals?

The factors involved in the production or elimination of infection are multiple and where infection rates are less than 1% for certain types of surgery the isolation of any single part of the surgical procedure is confounding if not scientifically impossible. The understanding of the complex microbiology of the skin microflora highlights that the majority of the organisms are commensal and reside in the hair follicles and sweat glands and cannot be removed entirely and indeed provide beneficial protection against colonization by other more pathogenic organisms.

In joint surgery the presence of any organism can cause infection of the arthroplasty requiring the removal of the prosthesis. The mechanism of the introduction of the organism into the joint is multifactorial involving the patient, the staff and the environment .Despite the difficulty in obtaining scientifically robust data as to the efficacy of the various parts of the surgical ritual, the ritual involving the preparation of the patient and the control of the environment has become an established, increasingly complex and expensive norm. Is it time to evaluate objectively which of the practices which have the object of reducing surgical infection are based on science and which on ritual?

Do you wash your hands after you blow your nose?

REFERENCES:

Graham, Fairclough JA. J Royal College of Surgeons of Edinburgh. 36(4):264-6, 1991. Fairclough JA. Johnson D. Mackie I. .Journal of International Medical Research 14(2):105-9, 1986. Moucha CS, Clyburn T, Evans RP, Prokuski L.J Bone Joint Surg Am. 2011 Feb;93(4):398-404. Cheng K, et al. Foot Ankle Int. 2009 Oct;30(10):992-7 Swenson BR et al. Infect Control Hosp Epidemiol. 2009 Oct;30(10):964-71. Reichman DE, Greenberg JA. Rev Obstet

166 CASES OF *MYCOBACTERIUM MARINUM* TENOSYNOVITIS OF THE HAND AND WRIST: CLINICAL FEATURES, MANAGEMENT AND RESULTS

J.P.Y. Cheung[1], Boris Fung[1], W.Y. Ip[1]

[1] *Department of Orthopaedics and Traumatology, University of Hong Kong Medical Centre, Queen Mary Hospital, Hong Kong*

INTRODUCTION: *Mycobacterium marinum* infections followed an unpredictable clinical course and delay in definitive diagnosis was frequently encountered. Chow et al. found that the average duration of infection before patients were seen by the Orthopaedics unit was 3.3-3.7 months.[1] The initial diagnosis was commonly found to be mistaken for conditions such as rheumatoid arthritis and trigger finger which may lead to inappropriate management like steroid injections. The objective of this study was to review our experience with treatment of *Mycobacterium marinum* tenosynovitis of the hand and wrist and to assess for any clinical parameters that were associated with poor functional outcome and also suggest a treatment algorithm for these infections.

METHODS: All patients with *Mycobacterium marinum* tenosynovitis of the hand and wrist from 1981 to September of 2009 were included in this retrospective study. Inclusion criteria included tendon sheath infections of the hand and wrist, exposure to a marine environment, history of trauma by marine life and positive histology of granulomata by biopsy or positive culture. Exclusion criteria included positive culture of organisms other than *Mycobacterium marinum*, cutaneous and lymphoid lesions. Analysis of clinical parameters was performed to identify any associations with functional outcome (Total Active Motion system).

RESULTS: 166 patients were studied but 156 patients completed the entire follow-up protocol. Most patients worked in the fishing industry (50.6%) and there was male predominance (65.1%). Subjects usually presented late with an average duration of 4.9 months (0.25-120). 37 (22.3%) patients had intralesional steroid injections prior to admission. 100 (64.1%) patients had an excellent (TAM >195˚, >75% return of motion) outcome, 28 (17.9%) patients had good (TAM 130–195˚, 50–75%) outcome, 22 (14.1%) patients had fair (TAM 65–130˚, 25–50%) outcome and 6 (3.8%) patients had poor (TAM <65˚, <25%) outcome. Thus, according to our criteria, 128 patients had satisfactory outcome (82.1%) and 28 patients had poor outcome (16.9%). Patients with poor functional outcome usually had intralesional steroid injections ($p<0.001$) and patients treated 2 months after injury had poor outcome ($p=0.004$). The management provided did not affect the outcome. All 32 patients treated conservatively had satisfactory outcome and 96 of 124 with operative treatment had satisfactory outcome ($p=0.001$). Student t-test showed that numbers of days of mobilization ($p=0.441$) and duration of antibiotics ($p=0.244$) was not associated with outcome.

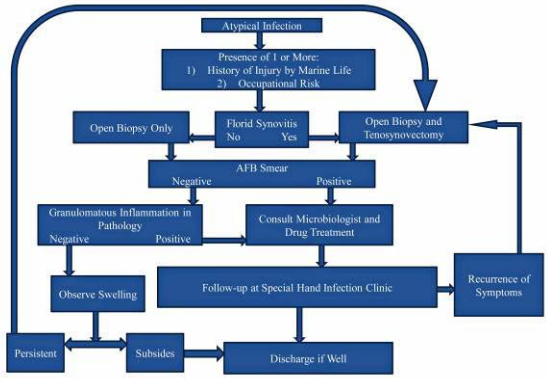


Fig. 1: Our treatment algorithm

DISCUSSION & CONCLUSIONS: There was no consensus on the optimal treatment option for *Mycobacterium marinum* tenosynovitis. This study found that only delayed treatment and steroid injections were associated with poor outcome. Conversely, antibiotic regimens and duration were not associated with poor functional outcome.

REFERENCES: [1] Chow SP, et al. Mycobacterium marinum infection of the hand and wrist. Results of conservative treatment in twenty-four cases. *J Bone Joint Surg Am* 1987;69:1161-8.

The Role of Occult Infection in the Aetiology of Aseptic Loosening of Joint Arthroplasty using Ultrasound Sonication and Conventional Sampling Techniques

J. Kempthorne, R. Ailabouni, S. Raniga, D. Hammer, D. Murdoch, G. Hooper

Department of Orthopaedics, Canterbury District Health Board, Christchurch, NZ
Department Clinical Microbiology, Canterbury Health Laboratories, Christchurch, NZ

INTRODUCTION: It has been suggested that occult infection could contribute to aseptic loosening of joint prosetheses requiring joint revision surgery[1.2]. It has also been suggested that sonication is a more sensitive tool for the detection of periprosthetic infection [3]. The aims of the study were to determine the incidence of occult or unrecognised infection in a sample of patients undergoing revision surgery for aseptic loosening. We also sought to examine the role of ultrasound sonication in aseptic loosening of implants.

METHODS: A prospective trial was conducted at two hospitals in Christchurch, New Zealand over a two-year period. At the time of revision surgery, an intra-operative swab, aspirate and several tissue samples were taken for conventional cultures. The extracted prosthesis was placed into a sterile leakproof plastic container and submerssed in saline, then sonicated in the lab for 10 minutes. The sonicate fluid underwent a prolonged routine cultures (14 days) to increase the rate of detection of slow growing organisms. All cases were patients undergoing revision surgery for aseptic loosening or prooven infection. The control group was comprised of patients having revision surgery for any other indication. These implants were subjected to the same protocol as the study group.

RESULTS: A total of 122 patients were included in the study; 54 in the Aseptic Loosening [AL] group, 15 Infections and 53 controls. There were significantly more smokers in the AL group than in the controls (p=0.04). The mean age for revision in the Infection group was less than the control group (p=0.005). There was no association between having abnormal pre-operative blood tests and returning a positive culture in either the aseptic loosening or control groups. There were 20 cases with positive intra-operative cultures in total; 8 in the aseptic loosening group, 2 in the controls and 10 the infection group (14.8%, 3.8% and 66.7% of the groups respectively). There were significantly more infections in the aseptic loosening group when compared to the aseptic controls (p=0.05). Conventionnal sampling techniques were positive in 18 of 20 cultures (90%). Sonication was only positive in 10 out of the 20 cultures (50%) (p = 0.0001). Sonication was concordant with the conventional sampling techniques in half of the positive cultures in the AL group and overall. There were only two cases of sonication cultures being positive when conventional samples were negative; one each in the aseptic loosening group and infection gorup. Sonication was positive in half the aseptic loosening culutres and 60% of the infection cultures only. The only bacteria to be isolated from sonicate cultures were Staphylococcus Aureus and Coagulase Negative Staphylococci. We only cultured staphylococcus species from the aseptic loosening and control groups.

DISCUSSION & CONCLUSIONS: There was a clinically significant rate of positive culture results in the aseptic loosening group of around 15%. Ultrasound sonication was less sensitive than conventional sampling techniques in our study, even in the infection group. Our findings suggests that unrecognised infection is potentially present in a significant proportion of aseptic loosening cases undergoing revision surgery but does not support the routine use of ultrasound sonication for its detection.

REFERENCES: [1] Tunney MM et al., J Bone Joint Surg. 1998; 80B: 568-572 37 S1-S2.[2] Tunney MM et al., J Clin Microbiol. 1999; 37: 3281-3290. [3] Trampuz A et al., N Eng J Med. 2007; 357: 654-663.

ACKNOWLEDGEMENTS: Canterbury Health Laboratories microbiology staff and surgical nursing staff in Christchurch.

Molecular imaging of joint prosthesis infection

A. Signore[1,2], M. Pacilio[1], D. Familiari[1]

[1]Nuclear Medicine Unit, Faculty of Medicine and Psychology, Sapienza University, Rome, Italy; [2] Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, The Netherlands

Infection associated with joint prosthesis, although uncommon, is an important complication, ranging between 1-2% for primary implants and 3-5% for revision implants. Distinguish infection from aseptic loosening of prosthesis is extremely important because treatments are different, but, unfortunately, can be difficult. Radionuclide imaging is, nowadays, the imaging modality of choice because is not limited by the presence of artifacts induced by metallic hardware such as plain radiography, CT or MRI that often detect osteomyelitis only in a late stage. ^{111}In-/^{99m}Tc-HMPAO-WBC are considered the techniques of choice because leucocytes do not accumulate in sites of mineral turnover. Images are analysed comparing intensity of uptake in the region of interest to the intensity of bone marrow and, when infection is present, uptake increases with time. Several authors have reported a good sensitivity and a reasonable specificity (>90%). Some studies reported an increased sensitivity, specificity and accuracy of imaging by combining ^{99m}Tc-WBC with ^{99m}Tc-sulfur colloid scan for bone marrow (100%, 91% and 95% respectively). Both radiopharmaceuticals accumulate in bone marrow, where ^{111}In-/^{99m}Tc-WBC also accumulate in inflammation sites but colloids do not. Only when WBC site of accumulation differs from ^{99m}Tc-sulfur colloid scan, osteomyelities is suspected. The main disadvantage of using radiolabelled WBC is the laborious preparation and the specialized equipment required and the handling of potentially blood infected blood. As an alternative to WBC, ^{18}F-FDG for PET/CT imaging has generated a considerable interest. However, an increased FDG uptake around prosthesis is very common and should not always be considered positive of infection. This phenomenon can cause a high number of false-positives. We recently performed a meta-analysis of all papers published in the field of infection with FDG and we found out that there is no scientific evidence yet that FDG can replace WBC for joint prosthesis. We recently investigated the role of FDG in the differentil diagnosis of soft tissue infection versus osteomyelitis in the diabetic foot and found also in this case that WBC scan has a superior diagnostic accuracy.

In addition to FDG and WBC, several radiolabelled anti-granulocyte antibodies (IgG, Fab' or IgM), have been developed. These accumulate in infection sites by extravasation facilitated by enhanced vascular permeability and bind to leukocytes.

Besilesomab (Scintimun®) is a murine immunoglobulin of IgG1 isotype that specifically binds to NCA-95, an epitope expressed at the cell membrane of granulocytes. A pilot study, in which we compared the distribution of ^{99m}Tc-Scintimun to ^{18}F-FDG and ^{99m}Tc-HMPAO-labelled leukocytes in patients with suspected hip prosthesis infection, is ongoing in our department. These 3 methods have never been compared before in the same patients. Preliminary data confirmed that ^{99m}Tc-HMPAO-WBC scan is the "gold standard" technique but Scintimun® seems to be highly accurate and could replace WBC scan. ^{18}F-FDG confirmed to have a great sensitivity but a low specificity and lead to many false-positives.

REFERENCES:

C. Love, S.E. Marwin, C. Palestro. Nuclear Medicine and the infected joint replacement. Seminar Nucl Med 39:66-78 (2009).

W. van der Bruggen et al. PET and SPECT in osteomyelitis and prosthetic bone and joint infections: a systematic review. Semin Nucl Med 40: 3-15 (2010).

S. Basu, A. Alavi. Positron Emission Tomography as a diagnostic tool in infection: present role and future possibilities. Semin Nucl Med 39:36-51 (2009).

D. Familiari, et al. Can sequential ^{18}F-FDG-PET/CT imaging replace WBC imaging in the diabetic foot? J Nucl Med (2011) in press.

A. Signore, V.E. Soroa, E. De Vries. Radiolabelled white blood cells or FDG for imaging of inflammation and infection? Q J Nucl Med Mol Imaging 53:1-3 (2009)

Non-invasive biomechanical assessment of implant fixation with *in-vivo* computed tomography: perspectives for infected implants.

V.A. Stadelmann [1], I Potapova[1]

[1]*AO Research Institute, AO Foundation, Davos, CH*

INTRODUCTION: The past years have seen a growing number of implant revisions and increasing associated hospitalization costs. The principal causes of revisions are periprosthetic bone loss and infections. Clinical data suggests that these start during the operation or early in the post-operative stage. Although the integration of an implant is obviously a dynamic process, no data exists quantifying the adaptation of the bone architecture around an implant in an infection situation and its impact on the fixation strength. The aim of this study is to demonstrate the potential of in vivo micro computed tomography (microCT) and image-based finite-element analysis (microFE) to evaluate the mechanical fixation of an implant and its evolution over time. The potential for a similar approach in infection is also discussed.

METHODS: In brief, titanium screws were inserted surgically in the tibias of twelve rats. Bone adaptation around the implant was assessed using in-vivo microCT at days 0, 3, 6, 9, 14, 20 and 27 at a resolution of 12 µm in 10 animals. Two rats were scanned only at days 0 and 27 and served as radiation controls. After the tibias were harvested each specimen was destructively pulled out to determine the ultimate force and stiffness of the screw-bone interface. microFE was applied to the microCT scans to estimate implant pullout stiffness. The image data for each specimen was prepared for linear elastic analysis using the voxel conversion approach [1]. The stiffness was evaluated by applying a 1% strain on the screw head and setting boundary conditions on the bone that reproduce the conditions of the biomechanical testing (Fig 1).

RESULTS: Eleven of the seventy-four scans (15%) were discarded due to the presence of artifacts. Contact bone volume fraction (c-BV/TV) increased from 43±7% to 55±8% between day 0 and day 27 ($p<0.05$). The microFE stiffness increased from 137±30 N/mm to 265±45 N/mm ($p<0.05$) and microFE failure load increased from 105±30 N to 180±20 ($p<0.05$). These increases were most prominent between day 0 and day 14: respectively 93%, 80% and 75% of the total increases in c-BV/TV, stiffness and failure load were reached after two weeks.

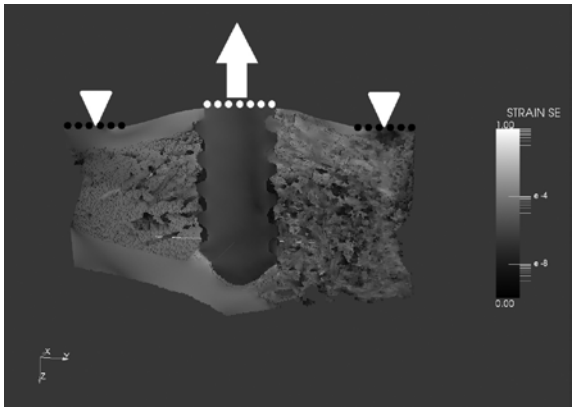


Fig. 1: Boundary conditions for the numerical models were applied to simulate uniaxial pullout of the screw.

DISCUSSION & CONCLUSIONS: This technique has clear limitations. The presence of a metallic implant induces artefacts in the micro-CT data. Radiations may impair the long-term health of the animals, but on this short-term study no difference was measured between the control and exposed animals, in terms of body weight, or contact bone volume. This preliminary study was performed to demonstrate the feasibility of the method. This approach gives new insights on the dynamic aspect of implant-bone interaction and not just the final state. It allows monitoring of the mechanical integration of an individual implant over time. Comparable data on bone integration in implant related osteomyelitis is unavailable to date, however, early identification of osteomyelitis adjacent to an implant could potentially improve diagnosis and thus also patient care. Together with the potential for specific bacterial labelling, this technique has the potential to provide new insights in the role of infections on the evolution of implant integration.

REFERENCES: [1] R. Müller and P. Rüegsegger, Med Eng Phys 17 (1995), pp. 126–133

From swab to SEM: Our protocol on tka infection suspicion

R.Ferrari [1], C.C.Castelli [1] A.Goglio [2], A.Grigis [2], A. Raglio [2], S.Buoro [3], G.Virotta [4] A.Remuzzi [5] L.Condorelli [5]

[1,2,3,4] *Ospedali Riuniti di Bergamo* [1] *Departments of Orthopaedics and Traumatology* [2] *Dep. of Microbiology,* [3] *Dep. of Clinical Biochemistry Lab.,* [4] *Dep. of Nuclear Medicine* [5] Mario Negri Institute
Bergamo, Italy

INTRODUCTION: The most feared complication after total knee arthroplasty is deep infection. Successful treatment needs early and good diagnosis preferably based on a microbial analysis, and adequate knowledge on the patient profile. There is no single test that will consistently predict infection. Pathogen detection techniques often are difficult to interpret. False positive results may be caused by contamination, and false negative results often are a consequence of presumptive antibiotics in spite of evidently running infection. The WBC count in the synovial fluid presume the probability of infection, but give no specific information regarding the underlying diagnosis. The purpose of this study was to attempt an algorithm for the diagnosis at the suspicion of TKA infection..

METHODS: We considered all patients with a symptomatic total knee replacement (ex: painful knee or elevated levels ESR & CRP or suggestive bone scan). Shut out the possibility of aseptic loosenig, all patients had undergone pre operative aspiration (culture & WBC count) and specimens (bone & synovial) from any area suspicious for infection, in operating room after sterile skin preparation and draping. No patients received preoperative (two weeks antibiotic wash out) or intraoperative antibiotics. Local anaesthetics were not used because of their bacteriostatic properties. We repeat the procedure until the bacteria detection: if the bacteria were identified , the patient is to undergo (after a proper systemic antibiotic therapy) a two stage revision arthroplasty.
If it is impossible to identify the bacteria, we perform a frozen section. If the results are more than 10 polymorphnuclear leukocytes/high power field we considered the TKA infected and we continue in two stage procedure. Samples obtained from explanted knee prostheses are send for SEM (scanning electron microscope) analysis & sonication procedure.

DISCUSSION & CONCLUSIONS: The diagnosis of infection is a serious problem: often vague symptom & modest inflammatory response with imaging tecnique available that are frequently in difficult to rule out the infection. The light distinction between pathogens & contaminants in the bacteriological cultures and a not standardized procedure sampling surgery biopsies fall through the attempt of a firm diagnosis. We think that our algorithm proposal, may be helpful in judging the probability of infection.

REFERENCES: [1]. Bauer Tw, wt Al. J Bone Joint Surg Am 2001;88:869–882. [2.] Zimmerli W, et Al.. Infection 2003 *31*, 99–108. [3]. Mason JB, et Al. *J Arthr*. 2003;18:1038–1043. [4] *J. Gallo,et Al*. B*iomed. Pap. 2004 148(2), 123 – 129.* [5.] A. Trampuz et Al. The American Journal of Medicine 2004 Vol. 117 556-562.

Comparison of Cultures with a New Multiplex PC/Mass Spec. for the Detection of Orthopaedic Biofilm Infections.

J.W. Costerton, N. Sotereanos, P.J. DeMeo (wcostert@wpahs.org, nsoterea@wpahs.org, pdemeo@wpahs.org)

Department of Orthopedic Surgery, Allegheny General Hospital, Pittsburgh, PA. USA

INTRODUCTION: Orthopedic surgeons have noted that routine cultures often fail to detect bacteria in pre-operative aspirates and in intra-operative specimens, even when clinical signs of infection are present. For this reason, therapy is often more empirical than is desirable. A new Multiplex PC/Mass Spec. technology[1] may be more sensitive and more accurate than cultures in the detection and identification of the biofilm bacteria that cause all device-related and other chronic bacterial infections[2].

METHODS: Forty consecutive patients scheduled for surgical revision of total joints were sampled, intra-operatively, and specimens were processed by routine culture and by the T-5000 Ibis multiplex detection system. Parallel samples were also examined by 454 pyro-sequencing, and by the use of appropriate fluorescence *in situ* hybridization (FISH) probes with confocal laser microscopy, to validate the detection and species identity provided by the culture and Ibis systems. Three different intra-operative specimens were taken from each patient so that the consistency of the identification of organisms present could be established.

RESULTS: We found that fulminate infections with *Staphylococcus aureus* were detected by routine cultures in half (4/8) of the cases in which this organism was found by the Ibis technology and by FISH staining. The detection rate of cultures fell below 20% in the detection of *Staphylococcus epidermidis*, and of other coaggulase negative Staphylococci, and in the detection of Streptococci and other less common orthopedic pathogens. In many instances in which large amounts of DNA from specific pathogens was detected by the Ibis system, cultures yielded negative reports. The Ibis data were highly concordant between multiple specimens from the same patient, and agreement with the FISH and pyro-sequencing data was remarkably close.

DISCUSSION & CONCLUSIONS The lack of sensitivity seen in many medical specialties (e.g. ENT[3], Urology), in the detection and recovery of biofilm bacteria by routine culture methods, is also seen in prosthetic joint infections (PJI). This failure of biofilm bacteria to propagate on culture media[4], can be overcome by several methods that do not depend on the ability of bacterial cells to grow on agar media. The multiplex PCR/Mass Spec system, 16 S-based pyro-sequencing, and the use of species-specific FISH probes all agreed well with each other and showed promise in the possible replacement of cultures in the diagnosis of PJI. Pyrosequencing and the use of FISH probes and confocal microscopy are too labour intensive to be used in routine diagnosis but we have found that the Ibis system, which is relatively economical and yields data on bacterial identity and antibiotic sensitivity in approximately 6 hours, is both accurate and practical.

REFERENCES: [1] Ecker D. et al., Nature Rev. Microbio., 2008, 6 : 553-558; [2] Costerton JW. et al., Science, 1999, 284 : 1318-22; [3] Post JC. et al., JAMA, 1995, 273 : 1598-1604 [4] Veeh R. et al., J. Infect. Dis., 2003, 188:519-30.

Molecular Engineering of an Orthopaedic Implant: From Bench to Bedside

Irving M. Shapiro

Department of Orthopaedic Surgery, Jefferson Medical College, Philadelphia PA 19107, USA

INTRODUCTION: Patients who receive implant devices can experience biomaterial-associated infections which can lead to destruction of local tissues, patient disability and morbidity, and on occasion, death. Currently, arthroplasty infection rates range from1%–5%; in trauma patients or at risk populations, the infection rate is further elevated. Moreover, since 73% of revision arthroplasties may be due to cryptic bacterial infection, the actual infection rate may be even higher than those reported earlier. In terms of patient numbers, 4.4 million people in the United States have received at least one internal fixation device, and 800,000 new hip arthroplasties are performed annually.
While the pathogenesis of the condition is complex, there is ample information that it is due to bacteria adhering to the implant surface and synthesizing a complex glycocalyx that protects the organisms from immune surveillance and antibiotic treatment.

METHODS: To address this problem, we are developing a new type of orthopaedic implant that besides providing secure fixation for osseointegration exhibits a bactericidal surface that prevents potential infection or eradicates established infection. To generate this surface, we employ silane chemistry to covalently bond the antibiotic vancomycin, via a membrane soluble linker, to a titanium surface using a solid state synthesis scheme that preserves the activity of the antibiotic and minimally changes the surface structure of the metal.

Results: The attachment remained stable under a number of conditions, including exposure to fluid environments, press-fit insertion into bone, and saturating levels of *S. aureus*. The activity of the tethered antibiotic was retained under all these conditions and was maintained in the face of repeated challenges with bacteria. Furthermore, the microbicidal activity of this surface was remarkably different from equivalent or even high solution concentrations of vancomycin. To examine the utility of this new system in vivo, we have developed an animal model in which *S. aureus* is directly perfused into bone. Microbiological counting, and microCT and histological analysis indicated that the tethered antibiotic inhibits implant colonization with *S aureus* and supports bone healing.

We predict that this smart bactericidal surface may serve as a starting point for the development of the next generation of bioactive implants.

Clinical Presentation and Bacteriological Analysis of Fight Bite Injuries in Tropical North Queensland

A.S. Miller[1,2], T. Ayres[1], P. Musio[1], J McBride[3] ,Wonnacott A[1]

[1]Cairns Base Hospital, Queensland Australia, [2] Royal Gwent Hospital Trauma and Orthopaedic Unit, Newport, Wales, UK [3] Australian Institute of Tropical Medicine, James Cook University, Queensland Australia

INTRODUCTION: Clenched fist injuries contaminated with oral flora (Fight bites/Tooth knuckle injuries) are clinically very important as they predispose to septic arthritis, extensor tendon sheath infections and carry a significant morbidity. Prompt empirical antibiotic treatment along with early surgical debridement and irrigation is essential in these high risk injuries. To date, there are no randomised studies to direct antibiotic therapy. The aim of this study was to identify common pathogens and update the empirical antibiotic usage.

METHODS: A retrospective review of all tooth knuckle injuries over the course of a calendar year. Data was collected from review of inpatient notes and extended microbiology reports. All patients included underwent intraoperative deep tissue aspiration, swabbing and tissue sampling before receiving tinadazole and cephalothin intravenously (as per local guidelines). Patients were excluded if they received any antimicrobial treatments prior to theatre. 25 patients were identified for review. Data including age, sex, location of lesion, time to presentation, organism/s isolated and sensitivities to commonly prescribed antibiotics was collected.

RESULTS: Eighteen male and seven female patients sustained fight bites during the time period. The average time to presentation was just over 2 days. There were a high number of associated medical comorbidities, in particular diabetes (12%), alcoholism (48%) and smoking (44%). The majority of injuries were in the patient's dominant hand (84%) in the 3rd finger (40%) overlying the metacarpophalangeal joint in extensor zone 5 (72%). Fifteen of the twenty five samples sent for microscopy grew a single predominant organism whilst the remaining ten (40%) were reported as containing mixed flora.

Table1. Frequency of Isolated Organism

Organism	Number
Streptococcus	18±
Eikenella Corrodens	8
Staphylococcus	7
Prevotella	2
Fusobacterium	2
Others*	3

*± predominantly pyogenes *Haemophilus, Corynebacterium and Veillonella*

Two patients re-presented with infections following a 14 day course of tinadazole and cephalothin. In both cases more than one aerobic bacterium was isolated. One patient received an amputation following septic arthritis and a further patient was considered for amputation due to loss of range of movement. Both grew Eikenella Corrodens.

DISCUSSION & CONCLUSIONS: Tooth Knuckle injuries carry a significant morbidity for patients. The patient demographics, delay in presentation and associated medical comorbidities undoubtedly contribute toward this[1]. However, polymicrobial septic arthritis and E. Corrodens infections are almost unique to this injury[2]. Appropriate early empirical antibiotic and surgical irrigation is essential in reducing complications. Streptococcal infections were found to be most common followed by E. Corrodens, Staphylococcus and anaerobes. Significant complications occurred in patients with multiple pathogens in particular those where E.Corrodens was cultured. Based on the organisms and sensitivities demonstrated here, Penicillin or first generation Cephalosporins alone may not be adequate. However, Penicillin based antibiotics with beta lactamase cover such as Co-Amoxiclav or fluoroquinalones such as Moxifloxacin have almost 100% sensitivity to organisms isolated here[2].

REFERENCES: 1 Perron AD. American Journal of Emergency Medicine 2002;20(2) 114-117 2 Talan DA. Clinical Infectious Disease 2003;37:1481-9

Detection of periprosthetic joint infections: Blood infection markers in patients undergoing revision arthroplasty

T. Randau[1], M. Wimmer[1], D. Kuberra[1], B. Reichert[1], B. Stoffel-Wagner[2], D.-C. Wirtz[1], S. Gravius[1]

[1] *Clinic for Orthopedics and Trauma Surgery, University of Bonn, Germany;* [2] *Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Germany*

INTRODUCTION: Differential diagnostics of patients presenting with persisting or recurrent pain after total joint arthroplasty present an ongoing clinical problem. Especially the differential diagnosis between aseptic loosening and periprosthetic joint infection (PJI) is crucial and misdiagnosis can lead to serious and permanent impairment. Current recommendations for diagnostics of PJI mainly depend on pathogen detection, histology and synovial fluid aspiration, but serum analysis for infection parameters is also widely used. In this study we evaluate the diagnostic value of C-reactive protein (CRP), Procalcitonin (PCT), blood leukocyte count (WBC) and Interleukin 6 (IL-6) in patients with revision joint arthroplasty.

METHODS: 103 Patients with revision arthroplasty of the hip or knee were recruited prospectively between 03/2010 and 03/2011. Along with clinical and radiological parameters, serum levels of CRP, PCT, WBC and IL-6 were determined preoperatively. Pateints were further diagnosed according to our algorithm for PJI to diagnose either aseptic loosening, mechanical instability or PJI, including synovial fluid analysis and histopathology and microbiological testing of periprosthetic tissues. The results of the blood levels were matched to the intra-operative findings, microbiological and histological analysis, and sensitivity, specificity and positive prediction values were determined for the different blood-based analysis.

RESULTS: Over the time of one year we were able to include 103 patients prospectively in this study (55 hip arthroplasty, 48 knee arthroplasty). Of these cases, 32 were diagnosed with PJI, 48 with aseptic loosening of at least one component of the arthroplasty and 23 with mechanical instability. Pathogens were detected in 25 of the 32 cases of infection. A pathologic leukocytosis above 10 G/L showed in only few patients. The sensitivity to indicate a PJI was therefore poor with <30%, while specificity reached around 80%. A similar effect was seen for PCT, which was even lower in its sensitivity and did cross the reference of >0.04 µg/l in four cases, two of them with SIRS/sepsis and all with PJI (specificity 100%). Values below detection threshold of <0.01 did not rule out an infection with safety, for 25% of these patients had a prosthesis infection. Correlation between WBC and PCT was high.
IL-6 at a cut-off of 8 pg/ml had a sensitivity of 40% and a specificity of 80%. CRP was the only parameter to show a significant difference of the mean between the aseptic groups and patients with infection ($p < 0.05$). With a cut-off at 10 mg/l, CRP showed a sensitivity of 50% to predict a PJI, with a specificity of 77%.

DISCUSSION & CONCLUSIONS: CRP levels are easily elevated even from minor disorders, therefore we put the threshold at 10 mg/l, loosing sensitivity but increasing specificity. WBC and PCT are markers used for acute systemic inflammation. Our results indicate that periprosthetic infections remain a "local" problem, and these parameters were not suitable to detect early stages on infection or chronic low-grade inflammation. CRP is already widely acknowledged to be included in diagnostics of periprosthetic infections, and the results here confirm its ambiguous role. IL-6 showed similar results, and might in combination with CRP add some diagnostic reliability. However, no other means can replace the established diagnostic algorithm for PJI, including microbiology, histopathology and clinical evaluation.

ACKNOWLEDGEMENTS: This study was supported by funding from the Detusche Arthrose-Hilfe e.V. and the BonFor Research funding of the University of Bonn.

The use of Antimicrobial Peptides to prevent Implant Infections: Experimental Options and Review of Literature

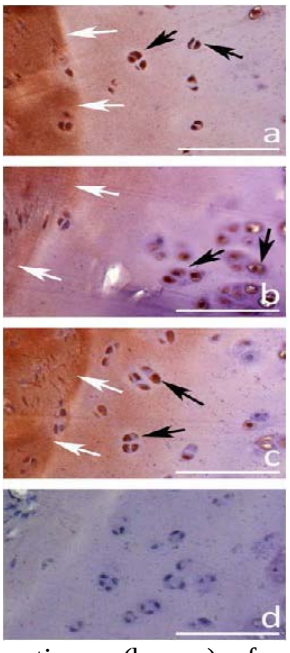
S.Sivananthan[1,2], E. Sherry[3]
[1]Worthing Hospital NHS Trust, UK, [2] Keele University, UK, [3] Hervey Bay Hospital, Australia.

INTRODUCTION:
Since their discovery it has been postulated that these naturally occurring peptides may have a role in preventing implant associated infections. Antimicrobial peptides have been isolated in bone and cartilage and appear to be part of natures immune system which has evolved to defend the body.

METHODS:
Literature search using online databases regarding the use of antimicrobial peptides for in-vitro and in-vivo studies was performed. In addition work performed in the author's institution under the MyJoint Project on isolation of antimicrobial peptides and coating options on implants is presented.

RESULTS:
Antimicrobial peptides are present in bone and cartilage. These molecules which include human beta defensin 2 may be a promising method with which to prevent peri-operative implant infection.

Fig. 1: Immunohistochemical stains for human beta defensins in nasal cartilage. Distinct



positive reactions (brown) for all three defensins involve the cartilage matrix and also the chondrocytes. (d) is the negative control with no staining. Bars = 100µm.

Various approaches have been described in the literature with regard to coating implant surfaces. The risks and benefits of these approaches were examined and found to be difficult to apply in a uniform, scalable and reproducible fashion.

DISCUSSION & CONCLUSIONS:
The field of study in antimicrobial peptides is in its infancy, yet it is already clear that these molecules are of extraordinary importance as a first line of defense against infection. As a system, antimicrobial peptides are not only able to recognize and kill pathogens, but interact with other elements of the innate and adaptive immune system to both induce and limit inflammatory reactions. The expression of human defensins in bone, nasal and auricular cartilage suggest that these molecules may be essential in the antimicrobial defenses of these tissues. However studies regarding the use of these molecules for clinical applications are still at the very early stage and more research is needed before clinical use can be envisaged.

REFERENCES:
[1] Stallmann HP et al., Injury, 2006, 37 S34-S40.[2] Kleine M et al., JBJS(A). 2011 May;93:840-6.[3] Zasloff M., Nature, 2002, 415:389-395. [4] Sivananthan S et al., J Craniofac Surg, 2010, 21(1):198-201.

ACKNOWLEDGEMENTS:
We thank the European Union for its financial support inside the 6th Framework Program for the scientific project MyJoint (FP-6-NEST-028861).

Antimicrobial Treatment of Implant-Associated Infections

W. Zimmerli[1] (werner.zimmerli@ksli.ch)

[1]*Basel University Medical Clinic, Rheinstrasse 26, Kantonsspital, CH-4410 Liestal, Switzerland*

Implants are highly susceptible to infection [1]. The infection rate is 0.5-2% after hip or knee arthroplasty and ~5% after total ankle arthroplasty [2, 3] . Implants are not only at risk for infection during the perioperative period, but remain susceptible to hematogenous seeding during their entire life-time. During *Staphylococcus aureus* bacteremia, patients with prosthetic joints have a risk of 34-39% to suffer from hematogenous seeding on their implant [4-5] . Therefore, even with an optimal peri-operative strategy of prevention, the total number of periprosthetic joint infections is steadily increasing. The treatment goals are eradication of infection and restoration of joint function [2]. Traditional treatment rules are staged replacement of the implant with meticulous surgical débri-dement of all foreign material, or life-long antibiotic suppression therapy for everybody. This simplistic approach has not only medical, but also non-medical reasons, such as liability and insurance (DRG-billing). According to modern con-cepts, 2-stage exchange for everybody or palliative life-long antibiotic suppression should be replaced by a more individual management according to well defined rules. The functional result is better after after less invasive surgery [6]. Therefore, not every patient should get the traditional 2-stage exchange. We propose a rational treatment algorithm which allows choosing the optimal surgical strategy for each patient [2]. Each surgical treatment option should be combined with a prolonged antibiotic treatment, preferably with an agent acting on slow-growing and adhering microorganisms. This requirement is fulfilled by rifampicin in staphylococcal infection. The excellent activity of rifampicin on implant-adhering microorganisms has been shown in vitro, in animal models, and in several clinical studies [7-11]. In order to avoid emergence of resistance, rifampin must always be combined with another agent. Traditionally, fluoroquinolones are excellent combination partners. However, due to increasing resistance of staphylococci, other drugs such as cotrimo-xazole, fusidic acid, linezolid [12] or dapto-mycin [13] have to be chosen.

CONCLUSIONS: Following the novel treatment concepts, using rifampicin-combinations against staphylococci and choosing the optimal surgical procedure, the chance for eradication of orthopedic implant-associated infection is 80-90%.

REFERENCES: 1. Zimmerli W et al. 1984) Pathogenesis of foreign body infection. Evidence for a local granulocyte defect. J Clin Invest 73: 1191-1200. 2. Zimmerli W et al. (2004) Prosthetic-joint infections. N Engl J Med 351: 1645-1654. 3. Kessler B et al. (2011) Risk factors for periprosthetic ankle joint infection: A hospital-based case-control study, submitted. 4. Murdoch DR et al. (2001) Infection of orthopedic prostheses after Staphylococcus aureus bacteremia. Clin Infect Dis 32: 647-649. 5. Lalani T et al. (2008) Clinical outcomes and costs among patients with Staphylococcus aureus bacteremia and orthopedic device infections. Scand J Infect Dis 40: 973-977. 6. De Man F et al. (2010) Infectiological, functional, and radiographic outcome after revision for prosthetic hip infection according to a strict algorithm. Acta Orthop 2010 (Epub ahead of print). 7. Widmer AF et al. (1990) Correlation between in vivo and in vitro efficacy of antimicrobial agents against foreign body infections. J Infect Dis 162: 96-102. 8. Zimmerli W et al. (1994) Microbiological tests to predict treatment outcome in experimental device-related infections due to Staphylococcus aureus. J Antimicrob Chemother 33: 959-967. 9. Zimmerli W et al. (1998) Role of rifampin for treatment of orthopedic implant-related staphylo-coccal infections: a randomized controlled trial. FBI Study Group. JAMA 279: 1537-1541. 10. Giulieri SG et al. (2004) Management of infection associated with total hip arthroplasty according to a treatment algorithm. Infection 32: 222-228. 11. Laffer RR et al. (2006) Outcome of prosthetic knee-associated infection: evaluation of 40 consecutive episodes at a single centre. Clin Microbiol Infect 12: 433-439. 12. Baldoni et al.(2009) Linezolid alone or combined with rifampin against methicillin-resistant Staphylococcus aureus in experimental foreign-body infection. Antimicrob Agents Chemother 53: 1142-1148. 13. John AK et al. (2009) Efficacy of daptomycin in implant-associated infection due to methicillin-resistant S aureus: importance of combination with rifampin. Antimicrob Agents Chemother 53: 2719-2724

Implant Infection (Antifouling and Antimicrobial Surface Coatings through Poly(2-methyl-2-oxazoline),)

C. Acikgoz[1], J. U. Lind[2], J. Nowakowska[3], M. Charnley[1], N. Khanna[3], R. Landmann[3], M. Textor[1]

[1]Laboratory for Surface Science and Technology, Department of Materials, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland, [2]Department of Micro and Nanotechnology, Technical University of Denmark, 2800 Lyngby, Denmark, Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, Basel

INTRODUCTION: Bacterial infection of implanted materials and devices is a major health care problem causing an adverse impact on the quality of life of patients and high costs. Numerous studies have been conducted to generate thin coatings that reduce bacterial adhesion on solid substrates. Particularly, coatings based on polymers play a significant role in the design and creation of antifouling and antimicrobial surfaces. Some coatings attempt to overcome the issue of biofouling by incorporating the active moiety into a biopassive background, providing the surface with both biopassive and bioactive activities. Herein we report the design of dual functional surfaces by combining nonadhesive properties of poly(2-methyl-2-oxazoline) (PMOXA) with an antibiotic moiety to kill bacteria adhering onto the surface. The nonadhesive properties of PMOXA modified surfaces have been investigated and shown to be as efficient as poly(ethylene glycol) based surfaces in suppressing the adhesion of proteins and bacteria.

METHODS: In our approach bioinert surfaces of PMOXA were prepared via Cu-catalyzed azide-alkyne cycloaddition reaction, known as click chemistry (Fig 1). Initially, PMOXAs incorporating alkyne functionality were synthesized via cationic ring opening polymerization technique. The living polymerization was terminated with ethyl piperidine-4-carboxylate and further hydrolysis with NaOH to obtain carboxyl terminated PMOXA. A monolayer of silane with azide functionality was prepared for covalent attachment of PMOXA to the surface. The course of the reactions was investigated by contact angle, XPS and ellipsometry.

RESULTS: Successful completion of click reaction between the azide terminated surfaces and PMOXA was confirmed using XPS analysis. Specifically, the XPS analysis of PMOXA modified surface showed an increase in the nitrogen signal at around 400 eV.

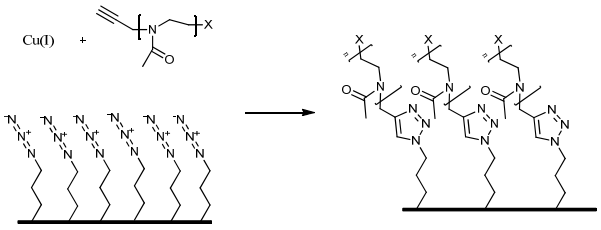


Fig. 1. Schematic illustrations of a silicon oxide surface functionalized with azide-terminated silane monolayer showing formation of triazole ring after click reaction with PMOXA.

The presence of covalently attached vancomycin on PMOXA-COOH coated surfaces was confirmed by antibody labelling. Both PMOXA and vancomycin functionalized PMOXA surfaces were tested using S. epidermis. The PMOXA itself was shown to be biopassive using plate counting technique, but the incorporation of vancomycin lead to increased biofouling, as seen by the presence of dead bacteria on the surface after staining the cells with fluorescent Live/Dead viability kid.

DISCUSSION&CONCLUSIONS: In this initial work we demonstrated that PMOXA can be used as platforms to covalently immobilize vancomycin preserving its functionality. However, there are a number of additional issues that we currently investigate: the site of vancomycin modification and the surface density of polymer chains on the surface.

REFERENCES: [1] Del Pozo, et al., N. Engl. J. Med. 361 (8) (2009) 787.[2] Zimmerli, et al., N. Engl. J. Med. 351 (2004) 1645.[3] Konradi, et al., Langmuir 24 (3) (2008) 613. [4] Rostovtsev,et al., *41* (14), 2596, (2002).

ACKNOWLEDGEMENTS: CCMX MatLife, BASF and RMS Foundation are acknowledged for their financial support.

Staphylococcus epidermidis infections of tumor megaprostheses

HT. Aro, N. Strandberg, S. Timlin

Orthopaedic Research Unit, Department of Orthopaedic Surgery and Traumatology, Turku University Hospital, Turku, Finland

INTRODUCTION: Metallic megaprostheses, allowing modular replacement of the resected skeletal segments, have largely replaced massive allografts in limb reconstructions of patients with malignant bone tumors. The megaprostheses have also revolutionized the treatment of long-bone metastases.

Megaprostheses provide fast functional recovery. This is psychologically important especially for patients suffering severe side-effects caused by adjuvant chemotherapy. Expeditious functional recovery is also the main surgical indication to improve the quality of life among patients with skeletal metastases.

The main perceived threat of megaprosthetic reconstruction is postoperative implant related infection. Infection can destroy all the main goals of the treatment and can ultimately result in amputation. Infection can disturb the execution of postoperative chemotherapy and thereby impair the prognosis of the patient.

We report the disastrous outcome of *staphylococcus epidermidis* infections in patients with tumor megaprostheses. This is a new clinical entity when compared with our past experience with massive allograft reconstructions.

METHODS: We have reviewed the clinical records of all the tumor patients (n=50) who underwent resection of long-bone tumor and skeletal reconstruction in our institution since 1995. All the cases of deep infections were analyzed for the pathogen. The ultimate outcome of the infection treatment was recorded.

RESULTS: There were five cases of deep infections (10%). All of them were caused by *Staphylococcus epidermidis*. Three of them ultimately resulted in amputation. All the amputations were related to the failed treatment of infected megaprostheses. None of the 30 patients with allograft or allograft-joint prosthesis composite reconstructions had amputation due to deep infection. The amputated cases were as follows:

- A 8-year-old girl with osteosarcoma of the distal femur had radical resection and reconstruction with a custom-designed megaprosthesis
- A 48-year-old woman underwent extra-articular radical knee resection for synovial sarcoma followed by reconstruction with a megaprosthesis covered with a microvascular muscle flap
- A 72 year-old man with a solitary kidney cancer metastasis of the elbow underwent resection of the elbow and reconstruction with modular megaprosthesis.

DISCUSSION & CONCLUSIONS: Our experience well agrees with the literature. Implant infections are not uncommon (11%-23%) in patients undergoing limb sparing surgery. [1-3] The causative pathogen is frequently *S epidermidis*. The reported rate of amputation has varied between 16% and 37%. [1-3] We have had fewer infections of massive allografts than reported previously (12.5%).[4] The three our patients who needed amputation for persistent infections shared the following features:

- reconstruction with megaprosthesis
- extended operation time or repeated surgeries due to the difficulties in soft tissue reconstructions requiring re-doing of microvascular anastomoses
- infection due to *S epidermidis* sensitive to the applied antimicrobial therapy
- antimicrobial therapy and repeated surgical debridement ineffective.

REFERENCES: [1] Jeys et al., J. Bone Joint Surg., 2005 87-A:842-849. [2] Biau et al., J Bone Joint Surg., 2006 88-A:1285-93 [3] Hardes et al., Arch Orthop Trauma Surg., 2006 126:289-96. [4] Delloye et al., J. Bone Joint Surg., 2007 89-A:579-87.

ACKNOWLEDGEMENTS: This study was supported by Turku University Hospital (EVO).

Experimental remarks for determine correct dosage silver Nano particle against infective microbes

S.J.Ashrafi[1*], M.F.Rastegar[2], G. Amoabediny[1], and S.A. Kumar[3]
[1]Dept of NanoBiotechnology, Faculty of new science and technology, Tehran Univesity, Teharn, Iran.
[2] Dep of Plant protection, Ferdowsi university of Mashhad, Mashhad, Iran.[3] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee,USA

INTRODUCTION: It is known that, silver species show different effects in anti microbial and toxicity tests. Therefore it is necessary to use standard protocol on the basis of which both biology and nanotechnology parameters are considered. Some of these parameters are: diversity in nano-silver synthesis methods, type of stabilizer, size, shape, size distribution and concentration of nano particles, type of culture media and component of them, condition of tests, effect of light or uv-light to nano-silver and its antimicrobial potentials, role of time in tests, etc. Each of these factors could cause the result of one test to become specific to the parameters of that test i.e, experimental conditions, type of nano-silver and type of microorganism , and thus will not reliable extended to the experiments with different parameters(1). In this work we present a one of these variable parameter about effect of against fungal plant pathogen *Pythium ultimum*.

METHODS: Commercial brand (nanocid, nanonasb pars COM, Iran) of silver nano particle (AgNps) use for all tests. Plant pathogen fungus, *Pythium ultimum* prepared form Myco collection of Ferdowsi university of Mashahd. Antifungal test carry out against mycelium and zoospore of this pathogen at lab condition (in vitro). Two conditions, solid and liquid investigated. Potato dexterous agar (PDA), corn meal agar (CMA) and water agar (WA) used as solid media. Potato glucose broth (PGB) as a liquid media for mycelium. For all test AgNps added to outoclaved and coled media appromixly at 50C. for zoospore test ,fresh fungus added to cannabis water media after 2dayes suspension of 10^6 prepared and dipped in different dosage of AgNps (5,15,30,60,90ppm). Zoospore germination investigated under light microscope fro 6 days. Several factor such as colony grow diameter, percentage of spore that could germinate, Wight of mycelium deliberated for the estimating the inhibitor dosage of AgNps correctly.

RESULTS: Variable result observed at all tests and methods, for solid condition fungus response to AgNps that combined with Medium (Fig 1). As seen in CMA media effective dosage is between 200-500ppm, for PDA fungus could even at 500ppm while for WA media inhibitor dosage is less than 50ppm because it could not growth in none of dosage in this media.

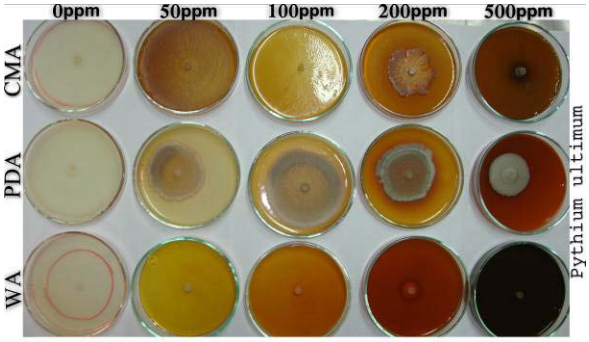


Fig. 1: different response of fungus to silver nano particle at 3 media at solid condition.

Zoospore germination inhibited in 5 ppm while in PGB media that there were both zoospore and mycelium fungus stopped in15ppm of AgNPs. Despite this differ liquid phase is more effective for pathogen controlling.

DISCUSSION & CONCLUSIONS about variation result in solid media our guess that is not related to nutrition effect of medium for fungus but related to effect of components on the nano particles. Like other report , The reason for this might be the inactivation of metallic silver when it comes in contact with blood plasma and the lack of durability of the coatings. The metallic silver also failed to improve the antimicrobial activity (2,3). At there we shown affect of just one of mentioned parameter in antimicrobial nano particle.

REFERENCES:[1]Ashrafi et al. 2010. American-Eurasian J. Agric & Environ Sci 7(1) 70-74. [2]Riley et al. 1995. Am J Med; 98:349–56.[3] Everaet et al. 1998. J Mat Sci-Mat in Med 9:147–57

Bacterial adhesion on cement : effects of bioactive polymers

T BAUER[1], V MIGONNEY[1]

thomas.bauer@apr.aphp.fr

[1] Laboratoire de Biomatériaux et Polymères de Spécialité rattaché à l'UMR 7052
Institut Galilée – Université Paris 13 – 93430 VILLETANEUSE

INTRODUCTION: The modification of implant or surgical cement surface with bioactive polymers is a very interesting way of preventing infection on arthroplasties by decreasing bacterial adhesion potential during surgery for arthroplasty. The aim of this work was to assess the results of bacterial adhesion on cement in which bioactive polymer was added.

METHODS: Different samples of surgical cement (methyle polymetacrylate) were used for the bacterial adhesion assessment using the same strain of Staphylococcus aureus. Polymers with different concentrations of methyle metacrylate (MMA) and sodium sulfonate (NaSS) were mixed with the cement and bacterial analysis was then performed. For each concentration of polymer different data were collected : bacterial adhesion, polymer elution, surface analysis, mechanical properies. All the results were compared with those obtained simultaneously with cement alone.

RESULTS: Polymers mixed in the cement significantly decreased adhesion of Staphylococcus aureus in all concentrations of polymer used. Polymers with high concentrations of NaSS showed a lower rate of bacterial adhesion. In the same time, elution was higher with high rates of NaSS and contributed to microscopic and macroscopic changing in the cement surface. With low concentrations of NaSS in the polymer, the elution of polymer on the surface of the cement was very low. In all the cases the adjunction of polymer significantly increased cement surface. Although this increased surface, bacterial adhesion was significantly inhibited showing a constant and efficient result with all the polymers mixed in the cement. The mechanical properties of the cement were modified in all the cases too.

DISCUSSION & CONCLUSIONS: Polymers mixed with surgical cement significantly modify the surface of the cement and decrease bacterial adhesion. Although this way of prevent infection of arthroplasties seems to be very attractive and reliable on implants, for the cement the benefits on bacterial adhesion still remain but the mechanical modifications of surface will lead to early failure with loosening. This way of prevention is very simple and cheap but seems to be difficult to apply on cement.

"Plasma-Click" based strategy for obtaining antibacterial surfaces on implants.

I.Braceras[1,2], P.A.Azpiroz[1,2], J.Oyarbide[2,3], N. Briz[1,2], N. Álvarez[1,2], G. Atorrasagasti[1,2], R.Muñoz[1,2], R.Fratila[3], E. Ipiñazar [1,2], and J.M.Aizpurua[3]

[1]*Tecnalia-Health, San Sebastian, E,* [2]*Ciber BBN, E,* [3]*Department of Organic Chemistry, University of Basque Country (UPV-EHU), San Sebastian, E.*

INTRODUCTION: Almost half of the nosocomial infections are related to the use of medical devices.[1] These infections, in addition, are particularly difficult to treat because of biofilm formation at the implant tissue interface, which constitutes a barrier that can diminish or disable the penetration of systemic antibiotics.[2] For this reason, implants with antibacterial surfaces are highly desirable.
In this study, a general strategy to functionalize implants with antibacterial surfaces is presented, with Vancomycin as the selected antibiotic, tested against *Staphylococcus Epidermidis*, associated with the most problematic prostheses related infections.[3]

METHODS:
The surface modification process comprised two independent operations:
1) Plasma polymerization of acrylic acid using an ion gun inverse magnetron source (IGIM) at low pressure. The chemical structure and $-CO_2H$ group density of the plasma deposited films were studied by FTIR spectroscopy and colorimetric techniques, respectively.
2) Click conjugation with azido-modified vancomycin. CO_2H groups from acrylic acid plasma polymer were extended by amidation with propargylamine using the conventional water-soluble carbodiimide procedure. This provided alkyne-terminated coatings suitable for "click" conjugation. Conversely, vancomycin was modified by HBTU- promoted amidation with 4-azidomethyl-benzylamine under protecting group-free conditions. Finally, (Fig. 1) the CuAAC "click" conjugation of alkyne-terminated plasma coatings and diluted aqueous solutions of azido-vancomycin were conducted at room temperature, using the Sharpless' CuSO4/sodium ascorbate catalyst.[4]

RESULTS: The plasma process was adapted to get different densities of CO_2H groups, as estimated by colorimetric methods (methyl orange test). Plasma polymerization was confirmed by FTIR analysis of the resulting poly(acrylic acid) coating film. The spectra exhibited no monomer signals and significantly broadened polymer C=O stretching bands (~1710 cm^{-1}). X-Ray photoelectron spectroscopy (XPS) revealed the presence of chlorine in the surface "clicked" with azido-vancomycin, confirming that the antibiotic was covalently bonded to the surface.
Finally, the antibiotic activity of the functionalized surfaces was confirmed against *Staphylococcus Epidermidis* bacteria cultures.

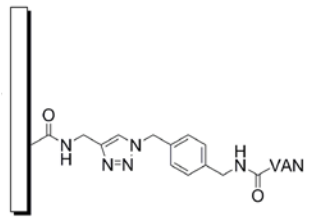


Fig. 1: Vancomycin "click" bound on the plasma polymerized surface.

DISCUSSION & CONCLUSIONS:
- A simple and versatile method for the functionalization of implant surfaces with antibiotic activity has been developed.
- The strategy described is feasible and could be extended to several other antibiotics.
- Furthermore, the amount of antibiotic at the surface can be adjusted depending on the implant specifications.

REFERENCES: [1] Richards M.J., et al., Crit. Care Med., 1999; 27: 887-892. [2] Cristina A.G., et al., Orthop. Clin. N. Amer., 1984; 15: 517. [3] Campoccia D, et al., Biomaterials, 2006; 27(11): 2331-2339. [4]Rostovtsev, V.V., et al. Chem. Int. Ed., 2002; 41: 2596-2599.

ACKNOWLEDGEMENTS: The authors are grateful for the financial assistance granted by the Spanish Ministry for Science and Innovation (MICINN). Project: BIOAVAN. (PSS-300100-2006-1 and PSS-300100-2007-4).

Design of polymeric coatings for drug release implants

E.Choinska, W.Swieszkowski, K.J.Kurzydlowski

Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland

INTRODUCTION: Bone infections are severe complications of open fracture [1]. The common method of treatment of this kind infection is prolonged (4–6 weeks) systematic administration high doses of antibiotics [2], but it often results in various toxic side effects. Therefore, implants which can locally release drug are advisable.
The aim of this study was to design biodegradable coatings of metallic implant for antibiotics delivery.

METHODS: The two different polymers: poly(D,L-lactide-co-glycolide) 50:50 (PDLGA) and poly(L-lactide) (PLLA) were used for coating preparation. Powder of gentamicin sulphate (GS) was used as a model antibiotic. The molecular weight of polymers was analyzed by GPC, the drug particles was measured by light scattered particles size analyzer. Samples for drug release were prepared as following: polished stainless steel pieces (20x30x2mm) were dip-coated with polymer/chloroform solution ultrasonically homogenized with 10% of gentamicin powder. The prepared samples was observed at SEM. Drug release test was carried out in PBS (pH=7.4) solution at 37^{o}C (5 samples per experiment). The amount of drug in PBS solution was detected using UV-VIS spectrophotometer [3].

RESULTS & DISCUSSION: Used in this study polymers have shown big differences in the molecular weight. It was 38 kDa for PDLGA and 156 kDa for PLLA. In order to obtain similar coatings thickness (about 33µm), different concentrations of polymers solution were used: 8% for PLLA and 50% for PDLGA. In both cases about 2mg of gentamicin per square centimetre of coating was obtained.
The size of gentamicin particles was in the range from 5 to 100µm, with average value 48µm. Applying such particles results in rough surface of the coatings (fig.1), what is advisable for better cells attachment. There weren't observed any differences between morphology of the two types of coatings. However, kinetics of in vitro GS release was significantly different for both materials (fig2).

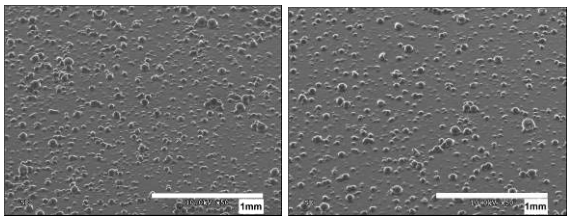


Fig. 1: SEM images of PDLGA/GS (left) and PLLA/GS (right) coatings

The high initial burst effect for PLLA coating can be explained by bad adhesion of the polymer to the GS particles, and also to the metallic substrate[4], which induced faster PBS penetration in the layer. The following period is effect of simple diffusion. In the case of PDLGA coating, thanks to the better adhesion of the polymer to the drug and to the substrate, the initial burst effect was decreased. Next, the diffusion and degradation mechanisms of release were observed. After 2 weeks the cumultative release started to be almost the same.

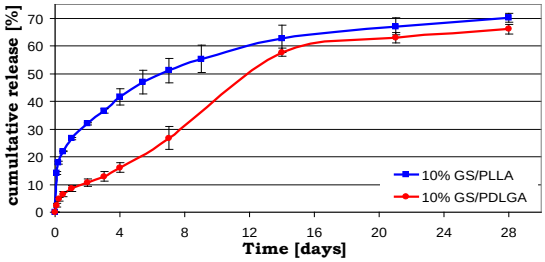


Fig. 2: Kinetics of gentamicin sulphate release

CONCLUSIONS:
Applying different polymeric coatings on metallic implants resulted in obtaining different rate of GS delivery. The control of the molecular weight of polymers and their adhesion to drug and substrate allow to design desirable kinetics of drug release.

REFERENCES: [1] K. Yokoyama et al., Injury, Int. J. Care Injured, 37: 554-560 (2006). [2] JK. Koort et al., Antimicrob Agents Chemother 49:1502–1508 (2005). [3]MR. Mirto, et al., Biomaterials 24: 79-87 (2003). [4]E. Choinska et al., Arch Metall Mater 55: 163-170 (2010)

ACKNOWLEDGEMENTS: This work was supported by grant no 5013/1090/1110.

In vitro studies of antibacterial activity of sol-gel bioglass containing silver

Lidia Ciołek[1], Joanna Karaś[1], Andrzej Olszyna[1], Ewa Zaczyńska[2] , Anna Czarny[2] , Bogusława Żywicka[3], e-mail: bioceramika@icimb.pl

[1] Institute of Ceramics and Building Materials, 9 Postępu Street, 02-676 Warszawa, www.icimb.pl , [2] Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, 12 Rudolfa Weigla Street, 53-114 Wrocław, [3] Medical University, 1 Pasteura Street, 50-361 Wrocław

INTRODUCTION: The aim of the study was to determine *in vitro* antimicrobial activity of bioglasses in the form of silver-containing powders. Physical and chemical properties of bioglasses produced by sol-gel method including grain morphology and surface semi-quantitative microanalysis were described in [1, 2], while the results of *in vitro* cytotoxicity were presented in publication [3]. This paper presents the results of *in vitro* antimicrobial activity of these bioglasses.

METHODS: There were four bioglasses produced with different content of silver and bioglass without this component as a reference material for research. Given the essential ingredients, there can be distinguished among them four aluminosilicates and one calciumsilicate bioglasses.

Studies of antimicrobial activity were performed by a dilution method, using precultures of test bacterial strains of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and the yeasts *Candida albicans*. Bioglasses were deposited on substrates at a concentration of 0,25 mg/ml, 0,5 mg/ml, 1,0 mg/ml, 2,5 mg/ml, 5,0 mg/ml, 10mg/ml, 50mg/ml and 100mg/ml. The 24-hole plates with the bacterial strains were incubated at the temperature (37 ± 1)°C, while the strains of yeasts at a temperature of (28 ± 1)°C. Action of nanopowders produced as compared to the above strains was identified after 18 h of incubation by counting live and dead bacteria. The broth cultures of various microorganisms without bioglasses constituted the reference.

RESULTS: *Fig. 1* shows the results of antibacterial activity of bioglasses in the form of nanoparticles on selected strains at a concentration of 2,5 mg/ml.

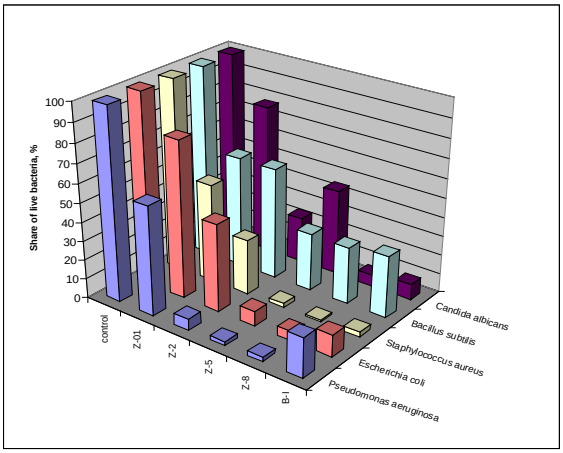


Fig. 1: The survival of strains determined after 18 h at a concentration of 2,5 mg/ml of bioglasses Z-01, Z-2, Z-5, Z-8 and B-I.

DISCUSSION & CONCLUSIONS: The studies of *in vitro* antibacterial activity in selected strains of bacteria and yeasts of produced bioglasses showed that bioglasses Z-5, Z-8 and B-I inhibit the growth of most micro-organisms during the tested period. Bioglasses Z-5, Z-8 and B-I showed the greatest activity against strains of *Staphylococcus aureus.*

REFERENCES: [1] Ciołek L., Karaś J., Olszyna A., Traczyk S., *Engineering of Biomaterials*, 2008, vol. XI, 77-80, 25-27, [2] Ciołek L., Karaś J., Olszyna A., *Prace Instytutu Szkła, Ceramiki, Materiałów Ogniotrwałych i Budowlanych* 2009, 3, 15-25, [3] Ciołek L., Karaś J., Olszyna A., Zaczyńska E., Czarny A., Żywicka B., *Engineering of Biomaterials*, 2009, vol. XII, 89-91, 91-93.

ACKNOWLEDGEMENTS: Scientific study financed from the science funds as a research and development project no. R08 010 02 by Polish Ministry of Science and Higher Education.

Plasma chemical oxidation enhances implant fixation and bone-implant contact in a rat model

Diefenbeck M[1], Mückley T[1], Schrader C[2], Schmidt J[2], Zankovych S[3], Bossert J[3], Jandt KD[3], Faucon M[4], Finger U[4]

[1]*Klinik für Unfall-, Hand- und Wiederherstellungschirurgie*, Universitätsklinikum Jena, Germany [2]*Innovent Technology Development*, Jena, Germany; [3]*Institute of Materials Science and Technology* (IMT), Friedrich-Schiller-University Jena, Germany, [4]*Königsee Implantate GmbH*, Aschau, Germany

INTRODUCTION: In the past, different approches have been used to enhance anchorage of titanium implants in orthopaedic surgery. Hydroxyapatite coatings, growth factors or bisphosphonates improved osseointegration and implant fixation so far. We decided on a different approach to enhance osseointegration: plasma chemical oxidation (PCO) was used to modify the surface of titanium implants. PCO converts the nm-thin natural occurring titanium-oxide layer on an implant to a 5 µm thick ceramic coating (TiOB-surface)[1]. The porous TiOB surface can be loaded with antibiotics, nanosilver or antiseptics.

METHODS: Implants were coated with a titanium-oxide (TiOB) surface by PCO. Bioinert TiOB-surfaces were produced at 220 V/1000 Hz and bioactive TiOB- surfaces at 280V / 1000 Hz in an electrolyses cell, respectively. A rat tibial implantation model with bilateral implantation of titanium rods[2] was employed to analyze the bone response to TiOB surfaces in vivo. 64 rats were randomly assigned to four groups of implants: (1) pure titanium (control), (2) titanium, type III anodization (controll II), (3) bioinert TiOB-coating and (4) bioactive TiOB-coating. After implantation periods of 3 weeks (n=8 per group) and 8 weeks (n=8 per group) mechanical fixation, peri-implant bone area and bone contact were evaluated in biomechanics and histology.

RESULTS: The bioactive TiOB-coating showed significant increased shear strength in pull-out tests and bone contact in histomorphometry after 8 weeks compared to all other groups.

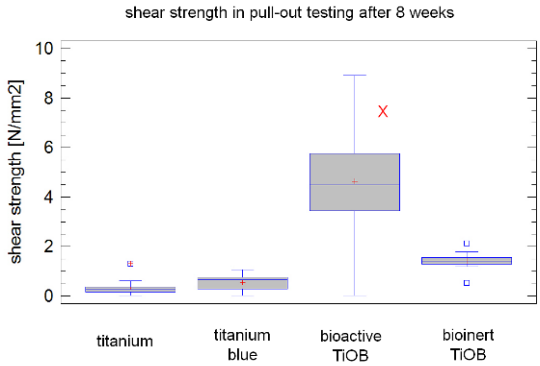


Fig: Shear strength in N / mm^2 after 8 weeks: Significant difference between the bioactive TiOB layer and all other groups. [95.0% confidence level (Fisher's least significant difference (LSD) procedure)].

DISCUSSION & CONCLUSIONS These first results of PCO in an animal model show that the bioactive TiOB modification has a positive effect on implant anchorage by enhancing the bone-implant-contact in normal bone. In our opinion, increased implant fixation is a first step for the prophylaxes of implants related infections. Next, we plan to evaluate bioactive TiOB surfaces loaded with antibiotics, antiseptics or nanosilver in an osteomyelitis animal model.

REFERENCES: [1] Schrader C, Bossert J, Finger U, et al. Poster at the 29th ICPIG, 2009, Cancun, Mexico

[2] Gao Y, Zou S, Liu X, et al. Biomaterials 2009; 30: 1790 – 1796

ACKNOWLEDGEMENTS: We thank the Thüringer Aufbaubank for the financial support of this research project (Verbund-Nr. 2008 VF 0048).

Preformed articulated knee spacer for the infected TKA: more than 10 years experience

R.Ferrari [1], C.C.Castelli [1] G.Gregis [2]

Ospedali Riuniti di Bergamo [1]*Departments of Orthopaedics and Traumatology*
[2] *Departments of Infectious Diseases*
Bergamo, Italy

INTRODUCTION: The treatment of TKA infection often results in a prolonged hospital stay a period of marked limitations of mobility for the patient and the prospect of a major reconstructive procedure with a compromised outcome. The aim in these cases is the eradication of infection, the restoration of full function and the prevention of recurrence.

METHODS: From march 2000 we routinely use the preformed articulated spacer in two stage procedure. The device is a PMMA spacer, gentamicine or gentamicine & vancomicine loaded, preformed by a factory, ready to use. It is produced in different sizes, designed as an ultracongruent condylar knee prosthesis with tested and standardized mechanical properties and antibiotic content and release's mechanism. Fifty consecutive patients mainly type A or B (Mc Phearson cl.) affected by late infection were enrolled in our prospective study without control group. A working extensor mechanism & ligamentous apparatus, with bone loss type I or II (Engh cl.) was mandatory. Post-op following std. rehabilitation programme as with primary TKA The second stage procedure was undertaken after a mean of 12 weeks. All the patients where followed up for a minimum of 24 months maximum 126. We evaluated between the stages of procedure: the quality of life, outcome, complication, bone loss, surgical approach at 2^ stage & type of revision implant, infection recurrence. The quality of life between the stages was evaluated with patient judgement on daily activies, post operative pain, ability to walk, use of crutches,Womac,IKSS.

RESULTS: Healing of infection process till today was observed in 92% of patients. Quality of life between the stages: no pain in 71% and fair in 29% of patients. Walking range: good in 52%, excellent in 24% fair in 16%. and poor in 8%. Seventy-six percent of patients went on using only one cructh, but of these ones at least, one third could walk without any crutch or support. Patients judged the result excellent or good in 76% of cases, fair in 16% poor in 8%. The range of motion remained unchanged between first and second stage or improved after definitive reimplantation. Mean IKSS clinical was 35.38 (clinical) & 37.96 (function) on presentation; it improved to a mean of 72.92 (clinical) & 76.04 (function) after the first stage and to a mean of 75.38 (clinical) & 80.58 (function) at the final review. Mean Womac (function and pain scores) was respectively 17.38 and 60.67 on presentation, it improved to a mean of 8.92 and 34.25 after the first stage and to a mean of 8.67 and 31.04 at final review. After the spacer removal no bone loss was observed. Any evidence of device wear & breakage, or renal malfunction & failure.

DISCUSSION & CONCLUSIONS: The use of preformed articulated knee spacer during two stage technique for infected tka improves the quality of life for patients between stages, increase patient compliance and cooperation reducing the social costs.

REFERENCES: [1].Haddad FS, et Al. J.B.J.S Br 2000, 82:807–812. [2].Ferhring T.K. et Al CORR 2000 Nov; (380):9-16. [3].Emerson R. H. et Al CORR 2002 Nov;(404):132-8.[4.]Hofmann AA, CORR 2005 Jan;(430):125-31.

Sequence changes in the isoforms of Staphylococcal MSCRAMMs Bbp/SdrE affect ligand specificity

MP Francis[1], V Vazquez[1], G Vannakambadi[1], M Höök[1]
[1]*Center for Inflammatory and Infectious Disease, Texas A&M Health Science Center - IBT, Houston, Texas, USA*

INTRODUCTION: *Staphylococcus aureus* are opportunistic bacteria responsible for a wide variety of infections including sepsis, osteomyelitis and orthopedic implant infections. A large panel of virulence factors is involved in Staphylococcal disease. Recent studies suggest that different types of infections involve varying sets of virulence factors. One class of virulence factors found in *S. aureus* are the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) that mediate Staphylococcal attachment to molecules in the extracellular matrix such as fibronectin, collagen and fibrinogen. In *S. aureus,* a subset of MSCRAMMs known as the Sdr family contains a characteristic Serine/Aspartate Repeat region and exhibit a common domain organization.

One member of the Sdr family is BSP-Binding Protein (Bbp), a protein originally isolated by searching for bone tissue binding proteins from a Staphylococcal osteomyelitis strain. Simultaneous gene sequencing identified a protein named SdrE that is very similar to Bbp. Subsequently, SdrE has been linked with invasiveness in Staphylococcal infection.

Bbp exhibits 87% identity to SdrE. Sequence alignment reveals high homology within all domains except for the N2N3 ligand binding domain, where identity is only 66%. The Bbp protein appears to represent one of several isoforms of SdrE, with further evidence being their lack of presence in the same strain.

Recent studies in our lab have shown that Bbp binds specifically to human fibrinogen (Fg) with high affinity. Bbp binding was tracked to a 15 amino acid sequence in the α-chain of Fg in residues 561-575.

METHODS: The ligand binding N2N3 domains of Bbp and SdrE were expressed in *E. coli* using the pQE30 vector. One liter cultures were pelleted, lysed, and centrifuged; the supernatant was then run on a Ni^{2+}-affinity column. SDS-PAGE and Coomassie staining showed purification of ~95%.

Recombinant proteins were tested for Fg binding first through an ELISA-type assay in which Fg was coated onto microtiter wells. Detection of bound protein was accomplished through protein-specific primary antibodies, HRP-conjugated secondary antibody and SigmaFAST OPD kit. Samples were read at 450 nm on a UV/Vis Spectrophotometer (SpectraMax 5 - Molecular Devices).

To confirm the specific Fg sequence and difference in affinity, a peptide was made corresponding to Fg α-chain residues 561-575. This peptide was able to abrogate binding of recombinant Bbp or SdrE to Fg in an Inhibition ELISA-type assay. This is the same assay as the ELISA-type assay except that the recombinant protein is incubated with the peptide, before addition to the Fg-coated microtiter wells.

Finally, Isothermal Titration Calorimetry (ITC) was performed. Proteins and peptide were diluted and dialyzed into 1x TBS. Experiments were carried out using a VP-ITC (MicroCal) at 30ºC.

RESULTS: While Bbp and SdrE show binding to Fg in each assay, their binding characteristics vary greatly. In the ELISA-type assay, Bbp and SdrE show apparent Kd's of 100 nM and 500 nM, respectively. In the Inhibition ELISA-type assay, Bbp shows a greater affinity for the Fg peptide with 80% inhibition with 3 µM Peptide compared to 15 µM peptide for SdrE. Finally, studying the binding characteristics with the gold standard of ITC, Bbp was found to have a Kd of 261 nM and SdrE a Kd of 6.6 µM.

DISCUSSION & CONCLUSIONS: The gold standard for binding studies shows a 25-fold reduction in binding affinity for Fg peptides from Bbp to SdrE. The sequence identity in the other regions shows that the differences in binding can be traced to sequence changes in the N2N3 domain. Experiments on other SdrE variants will be done to look for variance in ligand binding capability as well as the possibility of BSP binding.

Nanoencapsulation of Anti-Microbial Drugs

J. Gagnon (jacinthe.gagnon@unifr.ch)[1], P. Weber, K.M. Fromm (katharina.fromm@unifr.ch)[1]

[1]*Department of Chemistry, University of Fribourg, Fribourg, CH*

INTRODUCTION: Silver compounds and nanoparticles are gaining more interest from the scientific society as a replacement to antibiotics. For example, silver compounds may be used to coat implantable materials in order to prevent biofilm formation and other complications due to the presence of bacteria. However, these compounds may be too soluble and even toxic for the host. Encapsulation might be very advantageous in order to increase the stability and biocompatibility of silver drugs. In this research, cerium oxide, also called ceria, nanocapsules were synthesized due to the high stability and low toxicity of this material[1].

METHODS[2]: CeO_2 nanocapsules were synthesized first by making anionic polystyrene spheres, followed by the coating of these spheres with ceria, and removing the core via calcination. Silver nitrate was encapsulated inside these capsules by submitting the capsules to vacuum, followed by immersion in a saturated solution of $AgNO_3$ in ethanol[3].

RESULTS: Synthesized nanocapsules were composed of cerium oxide, as determined by powder XRD (not shown). From the TEM images (Figure 1) and from the disappearance of the polystyrene bands on the IR spectra (Figure 2), it is demonstrated that the core was completely removed after calcination resulting in empty capsules. These capsules are highly stable in acidic and basic solutions, as well as under high vacuum. Figure 3 shows the TGA of the capsules loaded with silver nitrate. The degradation of $AgNO_3$ is retarded to 800-900 ^oC when it is encapsulated compared to the free $AgNO_3$ which degrades at 400-450 ^oC.

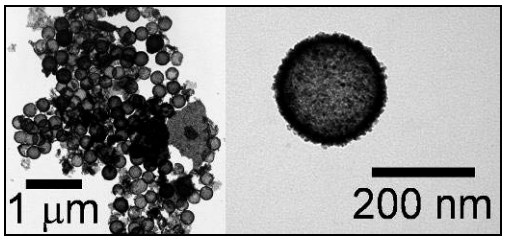


Fig. 1: TEM images of empty ceria nanocapsules.

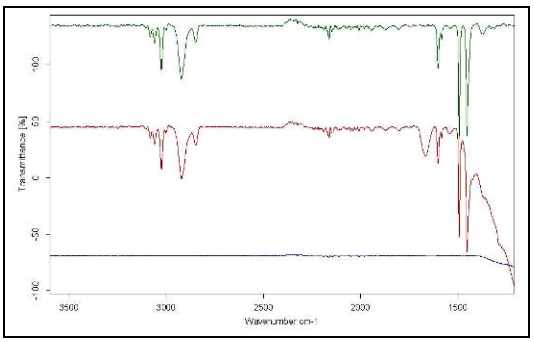


Fig. 2: IR spectra of polystyrene nanospheres before (green) and after (red) coating and CeO_2 nanocapsules (blue).

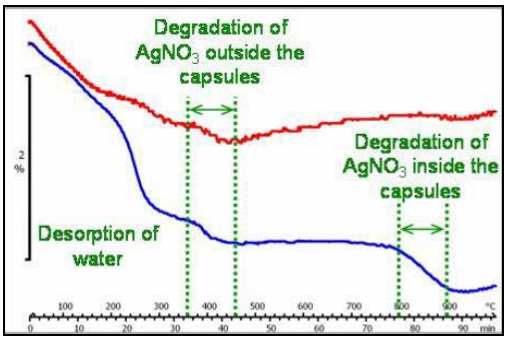


Fig. 3: TGA of empty ceria nanocapsules (red) and $AgNO_3$ encapsulated in nanocapsules (blue).

DISCUSSION & CONCLUSIONS: Ceria nanocapsules were successfully synthesized. TGA also gives promising results for the encapsulation of anti-microbial silver compounds. More experiments and analysis will be performed to study the encapsulation of silver nanoparticles and complexes and their release. Anti-microbial efficiency of these systems will also be analyzed. Finally, the biocompatibility in terms of cellular adhesion and toxicity will be studied in order to allow the implantation into patients.

REFERENCES: [1] Hardas et al., Toxicol. Sci., 2010, 116(2): 562-76. [2] Kartsonakis et al., J. Am. Ceram. Soc., 2008, 91(2): 372-8. [3] Kartsonakis et al., J. Sol-Gel Sci. Technol., 2007, 48: 24-31.

ACKNOWLEDGEMENTS: We are grateful to the Swiss National Science Foundation and the University of Fribourg for generously supporting this project.

Bactericidal Behaviour of Ti6Al4V after UV Irradiation in the Presence of Adsorbed Proteins

ML. González-Martín[1,3], MA. Pacha-Olivenza[3], AM. Gallardo-Moreno[1,3], C. Pérez-Giraldo[2,3], JM. Bruque[1,3]

[1]Dept. of Applied Physics, Faculty of Sciences, & [3]Dept. of Biomedical Sciences, Medicine School, University of Extremadura, [2]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Badajoz, Spain

INTRODUCTION: The ability of bacteria to colonize implants is facilitated by several bacterial surface proteins, termed MSCRAMMs which have a high affinity for extracellular-matrix components, as they recognize fibronectin, fibrinogen and albumin [1, 2].
Passive layer of Ti6Al4V is mainly composed of TiO_2. The photocatalytic activity upon ultraviolet light of this oxide has been exploited as biocide in different environmental areas. Our group has recently shown that UV-irradiation generates a residual bactericidal effect on the Ti6Al4V surface after illumination ceased, without compromising the biocompatibility of the alloy [3]. In this work we show that this antimicrobial effect is not suppressed when a layer of proteins coats the material which, in turn, is a closer situation to the *in vivo* context.

METHODS: 25 mm diameter disks of Ti6Al4V were kindly supplied by Surgival S.A. G15-T8 UV lamps, emitting predominantly at a wavelength of 254 nm, were kindly provided by Philips Iberica, Spain. Disks were studied before and after 15 h of UV-irradiation. The tested bacterial strains were: *Staphylococcus aureus* ATCC29213 (*S. aureus*) *Staphylococcus epidermidis* ATCC35984 (*S. epidermidis*4) and *Staphylococcus epidermidis* HAM892 (*S. epidermidis*2). Proteins employed were human albumin, 96-99% (Sigma-Aldrich), fibronectin, 0,1% solution (Sigma-Aldrich) and a protein pool. Adhesion experiments were carried out with the help of sterile silicone chambers, fixed to the Ti6Al4V surface. The protein solutions were added to the chamber and allowed to adsorb on Ti6Al4V for 15 min. Then, the protein solution was removed and a bacterial suspension was added and allowed to contact the alloy for 60 min. Experiments were carried out at 37ºC, under orbital shaking of 20 rpm. After the adhesion process, the viability of adhered bacteria was evaluated with a staining-based method (Live/Dead Baclight L-7012).

RESULTS: *Figure 1* shows the viability results of *S. aureus* after adhesion on Ti6Al4V covered or not with proteins, and before or after being UV-irradiated. Viability of adhered bacteria on no UV-irradiated samples is 100%, despite the extension of this adhesion is lower on protein coated Ti6Al4V than on the bare surface, being lower for albumin and the protein pool than for fibronectin, possibly due to the two fibronectin binding protein FnBPA and FnBPB localized on the bacterial cell surface [1, 2] (*Fig. 1*, green). However, bacteria adhered to the irradiated surfaces showed an almost complete loss in viability (*Fig. 1*, red).

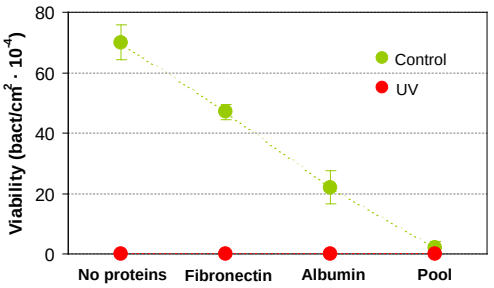


Fig. 1: S. aureus viability on Ti6Al4V before (green) or after (red) UV irradiation, with or without proteins adsorbed.

DISCUSSION & CONCLUSIONS: Despite the extension of bacterial adhesion on Ti6Al4V is strongly dependent on the presence of adsorbed proteins on its surface, the bactericidal effect displayed by the alloy after being UV-irradiated is not suppressed by the presence of this adsorbed protein layer.

REFERENCES: [1]Williams et al., Calcif Tissue Int, 2002;70:416-21. [2]Dziewanowska et al., Infect Immun, 1999;67:4673-8. [3]Gallardo-Moreno et al., Biomaterials, 2010;31:5159-68.

ACKNOWLEDGEMENTS: Authors thank to "Consejería Economía, Comercio e Innovación, Junta Extremadura" (GRU10149, PRI08A124), "Ministerio de Ciencia e Innovación" of Spain (MAT2009-14695-C04-(01,04) and FEDER.

The interdisciplinary approach to prosthetic joint infections: Results of 147 consecutive cases

S. Gravius[1], M. Wimmer[1], T. Randau[1], T. Hoppe[2], S. Petersdorf[2], N. Kraska[1], D.-C. Wirtz[1], G. Pagenstert[1,3]

[1] *Clinic for Orthopedics and Trauma Surgery, University of Bonn, Germany;* [2] *Institute of Medical Microbiology, Immunology and Parasitology, Universiy of Bonn, Germany;* [3] *Behandlungszentrum Bewegungsapparat, Orthopedic University Clinic Basel, Switzerland*

INTRODUCTION: Periprosthetic joint infections (PJI) after total joint replacements represent a severe complication. A valid and standardized diagnostic procedure including laboratory, microbiological and histopathological findings is essential.
This study evaluates our therapy algorithm for patients with prosthetic joint infections. In addition to a standardized diagnostic procedure and therapy algorithm, special emphasize was put on establishing an interdisciplinary case discussion including orthopaedic surgeons and clinical microbiologists to guarantee an optimized therapy for our patients.

METHODS: Between 2006 and 2010, 147 consecutive patients with proven PJI of the hip or knee were treated according to our PJI algorithm, adopted and modified from [1]. No patients were excluded due to risk factors, previous infections, prior operations, unsuccessful treatment attempts in the past, or presenting in a septic condition to represent the full spectrum of PJI. All patients underwent standardized diagnostics, including aseptic joint aspiration and biochemical and microbiological analysis of intraarticular fluid [2], intra-operative tissue specimens for histology and microbiology and serum infection parameters. Aligned to the "Liestaler therapy algorithm" [3] we decided upon the therapeutic procedure depending on Duration of infection, local and systemic risk factors, mechanical stability and pathogen. Patients were treated surgically with either debridement and retention or two stage exchange (with or without spacer) and interdisciplinary case discussions were held to adjust antibiotic and supportive therapies. For the outcome evaluation we evaluated the infection free survival of all patients treated, and recorded changes in therapy regime and associated complications with an average follow-up of 29.2±11.3 month.
RESULTS: We included acute (19.7%) as well as chronic (80.3%) infections and patiens with signs of SIRS (13.6%) and sepsis (4.1%). In 108 cases (73.5%) a causative germ could be identified, and 35.4% showed polymicrobial infections. In 47.3% the causative pathogen was rated as "difficult to treat". The histopathological findings revealed the qualitative prove of a periprosthetic joint infection in 114 cases (77%). We performed 27 times operative debridement with retention and a two stage revision in 120 cases (81.6%). In 50 of those a spacer was implanted. An average of 3.3±3.6 additional revisions had to be performed to consolidate the infection. Intravenous antibiotics were administered for 28.3±18 days for debridement with retention followed by an oral therapy of 97.4±49.4d. The antibiotic therapy had to be adjusted in 62 cases (41.9%) based on microbiological case discussions.
105 (71.4%) patients included in our study were graded as "definitely free of infection" and 8 (5.4%) as „probably free of infection". 5 patients (3.4%) died as result of a PJI-associated sepsis.

DISCUSSION & CONCLUSIONS: With this comprehensive data set we are able to identify "patients at risk" for treatment failure to be those with a septic or preseptic status prior to the start of treatment and patients with germs rated as "difficult to treat" or polymicrobial infections. The choice of treatment needs to be depending on all availale factors, and only in an interdisciplinary approach, PJIs can be treated successfully.

REFERENCES: [1]Zimmerli W et al. N Engl J Med. 2004 Oct 14;351(16):1645-54. [2]Trampuz A et al. Am J Med. 2004 Oct 15;117(8):556-62 [3]Giulieri SG et al. Infection. 2004 Aug;32(4):222-8.

Inkjet-Printed Antibiotic- and Calcium-Eluting Bioresorbable Nanocomposite Micropatterns for Orthopaedic Implants

Y. Gu[1], X. Chen[2], J.H. Lee[1], D. Monteiro[1], H. Wang[2], W.Y. Lee[1]

[1]*Chemical Engineering and Materials Science and* [2]*Chemistry, Chemical Biology and Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030, USA*

INTRODUCTION. We have explored the possibility of using antibiotic- and calcium-eluting bioresorbable micropatterns as a new pathway to engineer orthopaedic implant surfaces with both wound-healing and infection-preventing functions (Fig. 1).

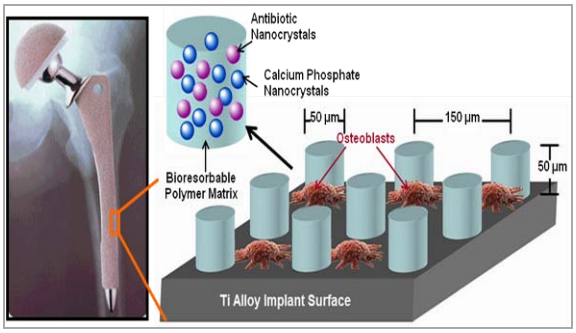


Fig. 1: Micropattern concept designed to both combat biofilm formation and promote osteoblast development

METHODS. The micropatterns consisted of a periodic array of ~50 µm circular dots separated by ~150 µm. The composition of the micropatterns was controlled by formulating inks with rifampicin (RFP) and poly(d,l-lactic-co-glycolic) acid (PLGA) dissolved in an organic solvent and ~100 nm biphasic calcium phosphate (BCP) nanoparticles suspended in the solution. During printing, RFP and PLGA co-precipitated to form a nanocomposite structure with ~10-100 nm RFP and the BCP particles dispersed in the PLGA matrix.

RESULTS & DISCUSSION. The rate of RFP release was strongly influenced by the RFP loading in the micropattern particularly during the first day, and could be controlled to be steady over 1 week (Fig. 2). The addition of BCP nanoparticles accelerated RFP release. The RFP-containing micropatterns effectively prevented the formation of *S. epidermidis* biofilm colonies due to their ability to kill bacteria before they have a chance to form colonies on the patterned surface. (Fig. 3) The BCP-containing micropatterns printed on the TiAl6V4 alloy surface significantly accelerated osteoblast cell differentiation, as measured by alkaline phosphatase expression (ALP) and calcium deposition.

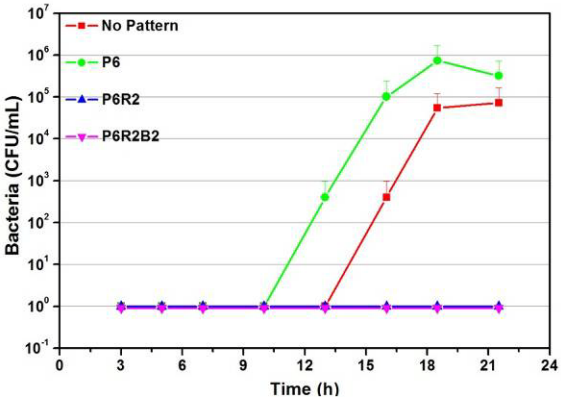


Fig. 2: Bacteria growth in microfluidic culture as measured by effluent agar plating. Note P6R2B2 is printed from 6 wt. % PLGA, 2% RFP, 2% BCP, remainder organic solvent.

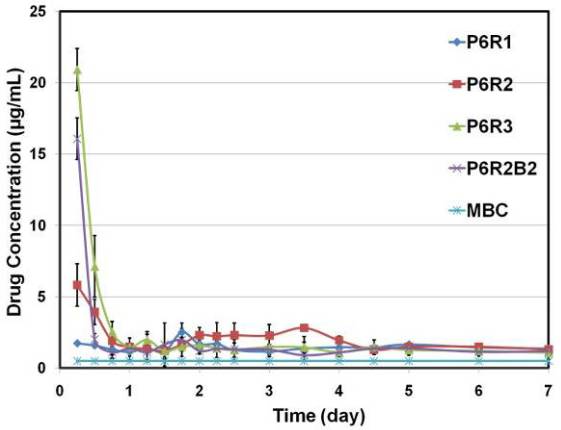


Fig. 3: Long-term micropattern RFP release profile (MBC ~ 0.5µg/mL for S. epidermidis)

CONCLUSIONS. The microfluidic dynamic culture showed the ability of the RFP-containing micropatterns to completely kill *S. epidermidis* as an effective means of preventing biofilm colony formation. The static osteoblast culture suggested that the presence of BCP nanoparticles significantly enhanced ALP activity and calcium deposition without compromising cell proliferation.

ACKNOWLEDGEMENT. NSF-Biomaterials (DMR 1005902).

A new animal model for implant-associated infected non-unions of the tibia and bacteria detection with fluorescence *in situ* hybridization

C.Henkenbehrens[1,2], V.Alt[1,2], K. Lips[2], D. Muhrer [1,2], U. Sommer[2], E. Domann[3] , R. Schnettler[1,2]

[1]*Department of Trauma Surgery, University Hospital Giessen-Marburg, Giessen, Germany,*
[2] *Laboratory of Experimental Surgery, Justus-Liebig-University Giessen, Germany,*
[3] *Institute of Medical Microbiology, University Hospital Giessen-Marburg, Giessen, Germany*

INTRODUCTION: Today, there is no adequate animal model to mimic the difficult clinical situation of infected non-union of the tibia after intramedullary stabilization. The purpose was to establish an rat model of implant-related infected non-unions of the tibia in rats. Additionally, it was evaluated if detection of bacteria by fluorescence in situ hybridisaion (FISH) technique is possible in bone infection.

METHODS: In 17 rats osteotomy of the midshaft of the tibia was performed and stabilized with an intramedullary device. There were 2 study groups were: group 1: contamination of the osteotomy site with 104 colony forming units (CFUs) of *Staphylococcus aureus* (11 animals), group 2: no bacterial contamination (6 animals). The animals were sacrificed after 42 days and bone healing and infection were assessed clinically, by X-ray, micro-CT, and microbiological methods including FISH technique using EUB and STAPHY probes. Histology and scanning electron microscopy (SEM) for biofilm formation were performed.

RESULTS: In all animals of the control group uneventful bone healing after 6 weeks without any signs of local infections was detected. 10 of 11 (90.9%) animals of group 1 with bacterial contamination showed infected non-union formation with positive clinical, radiological and microbiological infection signs of the tibia but without any systemic infection signs (Fig. 1). FISH technique could identify bacteria in the infected bone (Fig. 2). All explanted intramedullary implants from the infected animals showed positive biofilm formation in SEM.

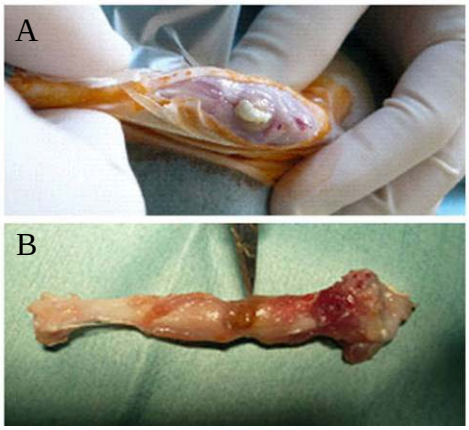


Fig. 1: Infected non-union of the tibia with pus formation (A) and non-healed osteotomy in the mid-diaphysis of the tibia (B).

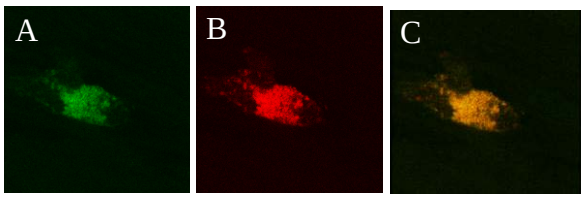


Fig. 2: Confocal laser scans with detection of bacteria by EUB0338 (A) and STAPHY (B) probe in trabecular bone. Merge of both images shows exact correlation of the probes (C).

DISCUSSION & CONCLUSIONS: The current work shows that it is possible to reliably induce implant-associated infected non-unions after intramedullary stabilization of a tibia osteotomy and bacterial contamination with Staphylococcus aureus. Furthermore, it was found that detection of bacteria by FISH technique is possible in bony tissue in implant-related bone infections. Both the model and the FISH technique are of interest for improvement of prevention, treatment and diagnosis of implant-related bone infection in the future.

ACKNOWLEDGEMENTS: This work was funded by OTC Foundation, Bern, Switzerland.

Antibacterial Activity of Antibiotic loaded Thermo-responsive Hyaluronan Hydrogel

N. Horn[1], C. Lutton[1,2], D. Eglin[1], R.G. Richards[1], T.F. Moriarty[1]
[1]AO Research Institute, AO Foundation, Davos, CH, [2] Institute of Health and Biomedical Innovation (IHBI), Queensland, Australia.

INTRODUCTION: A biodegradable antibacterial carrier, easily applied as a temporary coating on orthopaedic implants or for controlled delivery of antibiotic to a traumatic wound, would be advantageous to prevent infection via the spatio-temporal release of antibiotics. This study reports the preparation and characterisation of poly(N-isopropylacrylamide) grafted hyaluronan hydrogel compositions loaded with antibiotics.

METHODS: The synthesis of poly(N-isopropylacrylamide) grafted hyaluronan has been described in details elsewhere [1]. Briefly, azido terminated poly(N-isopropylacrylamide) (N_3-PNIPAM) with number average molecular weight (Mn) equal to 25 $x10^3$ $g•mol^{-1}$ was prepared by RAFT polymerization. Azido functionalized ciprofloxacin (N_3-Cip) was prepared by reacting ciprofloxacin with 11-azido-3,6,9-trioxandecane-1-amine in the presence of EDC/NHS in N-Dimethylsulfoxide. Then, several thermo-responsive hyaluronan gel compositions were prepared by reacting N_3-PNIPAM and hyaluronan propargylamide via copper(I)-catalyzed azide-alkyne cycloaddition [1]. A 1st thermo-responsive gel composition was prepared without grafting of N_3-Cip, a 2nd composition contained 1.4 mM/g grafted ciprofloxacin as characterized by ^{1}H NMR, a 3rd and 4th compositions were loaded respectively with 1.4 mM/g N_3-Cip and ciprofloxacin. *Antimicrobial activity.* Activity was tested by adding 120 ul of gel in the a hole punched in the center of a cation adjusted Mueller Hinton agar containing E. coli NCTC12241 throughout the bulk of the agar. Zones of inhibition (ZOI) were measured for each plate. For the PEEK experiment discs were seeded with E. Coli which were allowed to attached for 1 hour. Gels were then added on top of the discs and allowed to incubate for a further hour. Bacterial counts were then taken.

RESULTS: The blank gel showed no ability to kill bacteria, Figure 1. Addition of either ciprofloxacin or the N_3-Cip to the blank gel resulted in zones of inhibition, although the modified antibiotic had a significantly reduced zone ($p<0.001$). The gel with bound antibiotic also had a zone of inhibition demonstrating its antimicrobial ability. This zone was significantly less than either of the unbound antibiotic zones ($p<0.001$). The same trend was observed for the PEEK disk experiment, Figure 2. Blank gel had no effect where-as the presence of antibiotic in the gels was able to reduce the number of surviving bacteria. The gel with bound antibiotic showed the ability to kill bacteria on contact.

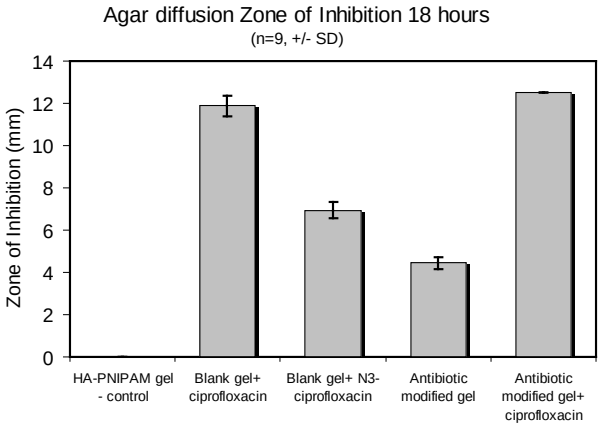


Figure 1. Plot of ZOI vs gel composition

DISCUSSION & CONCLUSIONS Antibiotic loaded hydrogel compositions were able to kill bacteria on a relevant implant surface and significant activity was observed over two days and therefore the local application into a vascular compromised wound could be beneficial preventing infection. Further tests will be performed to fully characterize and understand the antibacterial activity.

REFERENCES

1. Mortisen D. *et al.*, Biomacromolecules 11: 1261-72 2010.

Predictive Factors for Osteomyelitis in Open Tibia Fracture

Kamarul, T ; Mansor A; Hossain, MG; Syima, NN; Kanthan, SR
National Orthopaedic Centre of Excellence for Research and Learning (NOCERAL), University of Malaya, Malaysia
tkzrea@yahoo.com

INTRODUCTION:

Open fractures are associated with higher rates of osteomyelitis usually as the result of tissue contamination during an injury. Although certain patient risk factors contributing to the likelihood to developing osteomyelitis have been described, [1, 2,] the predictive factors that resulted in these conditions have not been previously established. Using data obtained for the past 6 years in University of Malaya Medical Centre (UMMC), we were able to determine the risk factors and relative risk to developing osteomyelitis as the result of open tibia fractures in patients managed in our centre.

METHODS:

A total of 312 open tibia fracture were treated between January 2001 and December 2006 in UMMC, of which 262 patients were available for review with a minimum of twelve months follow up post injury. Questionnaires and data regarding patient information were obtained from past medical records and where possible, interview with patients were conducted. Factors influencing osteomyelitis were examined using parametric statistical analysis. Data were analyzed using statistical software SPSS (version15.0).

RESULTS:

A total of 262 open tibia fractures were analyzed in this study. The prevalence of osteomyelitis among the study population was 11.1% for patients age between 16-80 years. Combinations of forty-five (v = 45) different factors were analyzed to determine their predictive probability associated with the development of osteomyelitis among the cases of tibia fracture. A summary for relative risk ratios to develop osteomyelitis for open tibia fractures is summarized in table 1.

Table 1 showing predictive factors for osteomyelitis of open tibia fracture.

Factors contributing to osteomyelitis	Increase in risk	p-value
Age (more than counterpart)	2.9 %	0.023
Stay in hospital (days more than counterparts)	5.1 %	0.000
Time to surgery (hours before first debridement)	6.0 %	0.042
Length of antibiotics (days more than counterparts)	5.7 %	0.001
Smoking	2.14 fold	0.045
Alcohol consumption	2.78 fold	0.047
Non- union	2.88 fold	0.014
Type III B vs Type II	3.77 fold	0.048
Type III B vs Type III A	4.35 fold	0.027
Type III B vs Type I	4.17 fold	0.042

DISCUSSION:

The present study has identified various risk factors and their predictive probability for the development osteomyelitis following open tibia fracture. The most significant of these is the degree of open fracture, which correlates to the amount and, extend of tissue damage. The results of this study would allow high-risk patients to be recognized early in order for appropriate timely measures to be taken, ensuring better patient care to be provided.

REFERENCES:

[1]Patzakis MJ et. al. Clin Orthop 1989; 243:36–40.[2]Adams CI et. al. Injury 2001; 32: 61-65.

Design of silver containing plasma polymer films for a controlled response to bacteria and mammalian cells

E. Körner, D. Hegemann

Empa, Swiss Federal Laboratories for Materials Testing and Research, Lerchenfeldstrasse 5, 9014 St. Gallen / Switzerland.

INTRODUCTION: The effectiveness and efficacy of release-based antibacterial products is determined by the supply of the active compound such as e.g. silver ions. However, cytotoxicity and hindered healing effects are also associated with antibacterial products. It is thus important to adjust the design of the antibacterial product depending on the field of application and the duration of the application. Therefore, silver (Ag) nanocomposite coatings were designed having different Ag^+ ion release profiles (small amounts of nano-scale Ag already show antibacterial properties) operational for short-term and extended to long-term applications.

METHODS: Functional hydrocarbon plasma polymer coatings with embedded Ag nanoparticles were deposited using an asymmetric RF plasma reactor at low pressure (10 Pa)[1]. The plasma polymer is produced with a reactive gas/monomer mixture of CO_2/C_2H_4. Ar was added in order to sputter Ag atoms from the Ag cathode and form nanoparticles in the growing polymer matrix. The influence of the gas ratio and different power inputs were investigated at constant pressure. The Ag content and the Ag^+ ion release are quantified with ICP-OES. Bacterial assays were performed with gram- and gram+ bacteria. The cytocompatibility of the coatings was tested with 3T3 mouse fibroblasts and compared to the antibacterial effectiveness.

RESULTS: The matrix functionality is an important element in the design of the coating showing a clear dependence on the CO_2 ratio in the process gas. An increase of the CO_2 ratio leads to a higher incorporation of oxygen functionalities. The Ag content as well as the Ag particle size was adjusted by means of the power input and the gas mixture. A strong dependence of the Ag^+ ion release on the incorporated Ag was measured.
A homogeneous Ag nanocomposite coating (Fig: 1) reveals a Ag ion release boost within the first 24 hours, whereas a Ag nanocomposite with Ag gradient (Fig. 1) can be tuned for uniform Ag ion releasing properties over a certain period (days to weeks)[2].

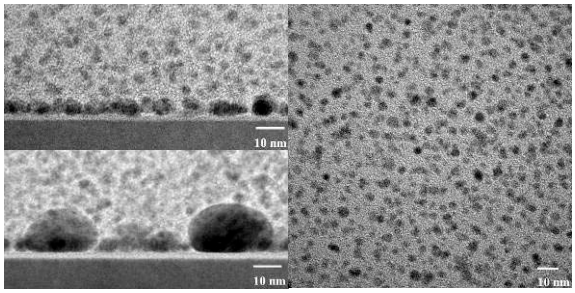


Fig. 1: Ag nanoparticle distribution for coatings prepared with a gas ratio CO_2/C_2H_4 6:1 and a power input of 50 W showing a) a homogenous particle growth, b) a Ag reservoir within the first stage close to the substrate interface and c) a homogenous bulk dispersal

DISCUSSION & CONCLUSIONS: The coatings exhibit an excellent effectiveness against the gram- bacteria. Although these coatings show the lowest Ag concentration and thus smallest Ag release within the examined range, no bacterial surface contamination could be observed. A higher amount of Ag, on the other hand, was found to be required for the gram+ bacteria. The coatings with a Ag content lower than 0.1 g/cm^3 or with a adjusted Ag gradient design were found to be cytocompatible.
Plasma technology was found to be a versatile tool to produce and design effective antibacterial and cytocompatible coatings which can be adjusted for various applications.

REFERENCES: [1] E. Körner, G. Fortunato, D. Hegemann, *Plasma Process. Polym. 6,* **2009**, 119-125. [2] E. Körner, M.H. Aguirre, G. Fortunato, A. Ritter, J. Rühe, D. Hegemann, *Plasma Process. Polym. 7,* **2010**, 619 -625.

ACKNOWLEDGEMENTS: This work is part of the EU-funded project EmbekI "Development and analysis of polymer based multifunctional bactericidal materials", grant #211436 of the seventh framework program.

A simple acoustic technique to assess the setting time of antibiotic loaded calcium sulphate

P.A.Laycock[1], M. Brayford[1], J.J.Cooper[1]

[1]*Product Development, Biocomposites Ltd, Keele Science Park, Keele, Staffordshire, UK*

INTRODUCTION: Current methods for determining the set times of calcium based bone cements include visual examination, ultrasound[1] or Vicat and Gillmore needle apparatus. This work developed a new simple acoustic technique to measure the setting times of a calcium sulphate bone cement loaded with different antibiotic mixes.

METHODS: A 100% pure, synthetic, calcium sulphate (Stimulan®, Biocomposites Ltd) in the form of a 'rapid cure' 10cc kit was mixed with 6ml of 4 different antibiotic mixing solutions. The solutions used were Tobramycin 80mg/2ml (Hospira, Inc.), Gentamicin 80mg/2ml (Hospira, Inc.), Tobramycin 80mg/2ml with 0.5g Vancomycin Hydrochloride (Hospira, Inc.) and Gentamicin 80mg/2ml with 0.5g Vancomycin Hydrochloride. A digital stopwatch was started as soon as the mixing solution was introduced into the Stimulan powder, which was then mixed for 30 seconds, rolled into a ball and allowed to cure undisturbed. Each mix was repeated for 3 experiments.

A new method was developed to quantify the setting times specifically of small volumes of synthetic bone cements. The method adopts an acoustic technique. A microphone connected to the sound card of a PC is used to pick up the sound the paste makes when dropped from a fixed height (30mm) onto a thin metal plate (Figure 2). As the paste hardens the frequency rises and the sound changes from a dull 'thud' to a higher pitch note until it reaches a similar frequency obtained with a fully set paste (24 hrs). The software used to analyse the frequencies was TrueRTA™ (True Audio) and the paste was said to be set when the 2 and 5 KHz frequency bars achieved a level of >5 dBu amplitude. See (Figure 1).

RESULTS:

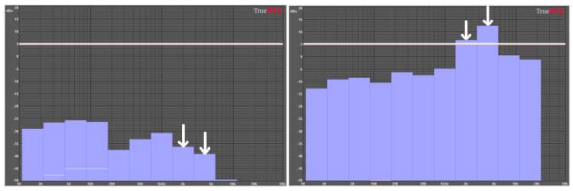


Fig. 1: Frequency profile of a paste prior to setting (left) vs. immediately on setting (right).

Fig.2:Apparatus used containing microphone, plate and sample drop height guide.

Table 1. Setting times (Minutes:Seconds) of Stimulan mixes (n=3)

Stimulan +	S1	S2	S3
Tobramycin	10:25	9:58	10:09
Gentamicin	4:04	3:47	3:50
Tob/Vanc	9:09	8:45	9:01
Gent/Vanc	3:39	3:45	3:38

DISCUSSION & CONCLUSIONS: A new simple acoustic test has been developed and used to assess the effect of different antibiotic additions on the setting time of a 'rapid cure' calcium sulphate bone cement (Stimulan®). The test gives reproducible and consistent results and can be used with very small quantities of cement.

REFERENCES: [1] Carlson J., et al. Biomaterials, Volume 24, Issue 1, January 2003, Pages 71-77, An ultrasonic pulse-echo technique for monitoring the setting of $CaSO_4$-based bone cement.

EFFECTS OF ANTIBIOTIC ADDITION ON THE SETTING TIME OF CALCIUM SULPHATE BONE CEMENT

P.A.Laycock[1], M. Brayford[1], J.J.Cooper[1]

[1]*Product Development, Biocomposites Ltd, Keele Science Park, Keele, Staffordshire, UK*

INTRODUCTION: The well-known advantages[1] of using high purity calcium sulphate as a bone graft substitute make it an ideal vehicle in the local delivery of antibiotics. It is totally resorbable therefore does not need a second surgery for removal and there are no limitations concerned with heat sensitive antibiotics.[2] The advantages of local delivery are a more concentrated measure of antibiotic actually reaching the infected site over a much shorter time period (adequate intravenous administration lasts six weeks) and therefore having a greater effect on infection, as well as reducing toxicity associated with oral and systemic delivery.[3]

To date, many studies focusing on calcium sulphate have been concerned with elution profiles of antibiotics. Most of the common effective antibiotics used to treat osteomyelitis, have different effects on the setting time of calcium sulphate.

METHODS: A 100% pure, synthetic, calcium sulphate (Stimulan®, Biocomposites Ltd) in the form of 'Standard cure' and 'rapid cure' 10cc kits were mixed with 6ml of 4 different antibiotic mixing solutions. The solutions used were Tobramycin (Hospira, Inc.) 80mg/2ml with 0.5g Vancomycin Hydrochloride (Hospira, Inc.), Gentamicin 80mg/2ml (Hospira, Inc.) with 0.5g Vancomycin Hydrochloride, Vancomycin Hydrochloride with 6ml sterile water and Ciprofloxacin Hydrochloride (Medisca) with 6ml sterile water. A digital stopwatch was started as soon as the mixing solution was introduced into the Stimulan powder, which was then mixed for 30 seconds, rolled into a ball and allowed to cure undisturbed. A separate test was carried out using the Tobramycin and Vancomycin Hydrochloride mix with varying liquid volumes to monitor the effect of liquid content on the setting times. Setting times were determined using a recently developed acoustic frequency monitoring technique which assesses the change in pitch given by the cement ball when dropped onto a metal plate.

RESULTS:

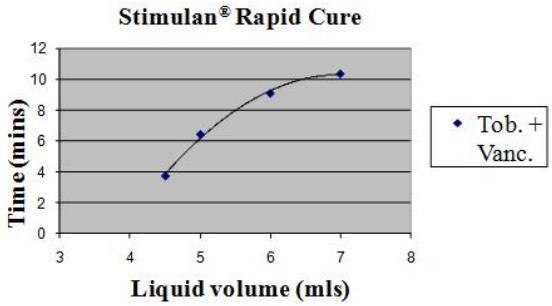


Fig. 1: Effect of varying the liquid volume on the setting time of antibiotic loaded calcium sulphate. N.B.7ml gave a rather wet mix while 4.5ml gave a dry mix. Volumes outside these values result in inconsistent setting outcomes.

Table 1. Setting times (Minutes:Seconds) of Stimulan mixes

ABX	Rapid cure set time	Std cure set time
Tob + Vanc	9:09	55:53
Gent + Vanc	3:39	30:50
Vancomycin	2:00	7:46
Ciprofloxacin	>1hr 45 min	>2 hrs

DISCUSSION & CONCLUSIONS: The addition of various antibiotics to 'standard' calcium sulphate bone cement can significantly retard the setting reaction thus increasing the set time. The setting time is also dependent upon the volume of liquid added with the time reducing as the liquid volume decreases. A new, synthetic, 'rapid cure' calcium sulphate bone cement has been developed which represents a promising option for addressing the inconvenience of delayed setting times. The effect of Ciprofloxacin Hydrochloride on the setting times requires further investigation.

REFERENCES: [1] Helgeson M. D., et al. Orthopedics. 2009, 32(5): 323.[2] Karr J. C., et al. Journal of the American Podiatric Medical Association. 2011, 101(2): 147. [3] Gitelis S., Brebach G. T. Journal of Orthopaedic Surgery. 2002, 10(1): 53.

Effect of 405nm High-Intensity Narrow-Spectrum Light on Osteoblast Function

R. McDonald[1], M. Maclean[2], J.G. Anderson[2], S.J. MacGregor[2], M.H. Grant[1]

[1]*Bioengineering, University of Strathclyde, Glasgow, Scotland,* [2] *The Robertson Trust Laboratories for Electronic Sterilisation Technologies, University of Strathclyde, Scotland*

INTRODUCTION: A significant portion of medical devices fail due to acquired infection, with infection rates after arthroplasty surgery between 1-4%, and considerably higher after revision surgery. To reduce the associated costs of infection, a new preventative method is required. High intensity narrow spectrum (HINS) 405 nm light is a new technology shown to have bactericidal effects on a range of medically important bacteria[1]. The effect of HINS-light on osteoblasts and bacteria were investigated to determine the potential of this technology to contribute to infection prevention in operating theatres, during surgery and post-operative dressing changes.

METHODS: Osteoblasts were seeded at $2x10^4$ cells/cm^2 and allowed to adhere for 24 hr before being exposed to HINS-light at intensities from 0.8 to 15 mWcm^{-2} for 1 hr (2.9 – 54 Jcm^{-2}). Expression of alkaline phosphatase (ALP) was measured at 24 and 72 hr post exposure and collagen synthesis was measured spectrophotometrically after staining with picric Sirius red. Expression of osteocalcin was assessed by ELISA at 24 and 72 hr post exposure. Cell morphology was assessed by SEM. The bactericidal effects of the maximum intensity which did not inhibit osteoblast function were investigated using a range of bacterial species. Bacteria were exposed in phosphate buffered saline (PBS) and on bacteriological agar (BA) plates, and surviving populations enumerated with results reported as % kill relative to control populations.

RESULTS: Low intensities of HINS-light (up to 5 mWcm^{-2}) were shown to have no significant effect on osteoblast function. Expression of ALP, synthesis of collagen, and osteocalcin expression showed no significant decrease relative to control at any time point after 1hr exposure to HINS-light intensities at or below 5 mWcm^{-2}. Exposure to 15 mWcm^{-2} HINS-light for 1-hr caused a significant 46% decrease in ALP expression at 24hr post exposure, with no recovery occurring by 72hr. Osteocalcin expression was found to be decreased by 56% at 72hr post exposure, and collagen synthesis by 35%. SEM of exposed osteoblasts showed obvious signs of damage to the cell membrane when exposed to higher intensities of 15 mWcm^{-2} for 1-hr (Figure 1).

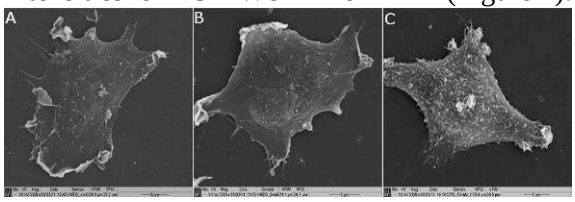


Fig 1: Effect of a 1-hr exposure to 5 and 15 mWcm^{-2} (B and C) HINS-light on osteoblast morphology. Control (A). Scale bars are 5µm.

Following these results, 5 mWcm^{-2} for 1-hr was determined as the maximum safe exposure for the osteoblasts and was applied to various bacterial species with varying results (Table 1).

Table 1. Inactivation (% kill) of bacteria exposed to 5 mWcm^{-2} HINS-light for 1 hour.

	In PBS suspension	On agar surface
S. epidermidis	**99.0 (±0.2)**	**40.0 (± 22.2)**
S. aureus	**54.1 (± 14.5)**	**21.6 (± 2.8)**
MRSA	**47.8 (± 14.6)**	**82.8 (± 14.9)**
P. aeruginosa	**18.6 (± 3.1)**	**76.2 (± 13.4)**
A. baumannii	**12.3 (± 2.3)**	**27.0 (± 8.0)**

DISCUSSION & CONCLUSIONS: One hour exposure to intensities of HINS-light of 5 mWcm^{-2} and below have been shown to be safe for mammalian tissue. This dose has also been shown to have considerable bactericidal effects, suggesting that HINS-light has potential to be developed for safe use in the hospital environment for maintaining the sterility of surfaces and mammalian tissues.

REFERENCES: [1] M. Maclean et al. Applied and Environmental Microbiology 75(7), 1932-1937 (2009)

ACKNOWLEDGEMENTS: RM is supported by the EPSRC.

The use of an antibiotic - impregnated biodegradable bone scaffold to prevent osteomyelitis

J. M[c]Laren[1], K. Shakesheff[2], R. Quirk[3], A. Goodship[4], R. Bayston[1] and BE. Scammell[1]

[1] *Division of Orthopaedic and Accident Surgery, University of Nottingham, C Floor, West Block, Queen's Medical Centre, Nottingham, NG7 2UH, UK* [2] *Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK* [3] *RegenTec Ltd, Biocity Nottingham, Pennyfoot Street, Nottingham, NG1 1GF, UK* [4] *The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK*

INTRODUCTION: Nottingham University has patented an injectable bone graft that forms a porous scaffold with mechanical properties similar to cancellous bone and is non-toxic to surrounding tissue. It consists of poly-lactic co-glycolic acid combined with a plasticizer and a ceramic component, which can be used to deliver antibiotics to contaminated bone, immediately after the initial debridement of the wound without the usual wait and second surgery at 6-9 weeks post injury.
Here we report on the release of the antibiotic from the scaffold material and the *in vivo* study.

METHODS: Both gentamicin (4% w/v) and clindamycin (2.5% w/v) were combined within the scaffold material and the rate of release tested against *Staphylococcus aureus* (F2789) using the established method of serial plate transfer tests. The mechanical strength and porosity of the material was measured to ensure that there were no negative effects on the mechanical properties introduced by adding the antibiotic combination.
The material was then tested in a large animal model, where a critical size defect (8mm x 15mm) was created in the cancellous bone of the femoral condyles of 4-5 year old sheep. *Staphylococcus aureus* (F2789) was introduced into the defect and then the scaffold material, either with or without antibiotics, was packed in. Sheep were sacrificed at either 2 or 13 week time-points and samples were taken for culture and for histology and imaging. Bone was scanned using micro-computed tomography and prepared for histological analysis.

RESULTS: The antibiotic combination provided in vitro inhibitory activity persisting for over 21 days (the target duration) without emergence of resistance. No significant effect of this combination / concentration was seen on the mechanical properties.

Sheep that were infected with bacteria and did not receive antibiotics within the scaffold were notably ill, showing weight loss and requiring analgesia for the duration of the study. Samples collected from the defect contained bacteria, and were shown to be the strain of *S aureus* (F2789) introduced during surgery. At both 2 and 13 weeks, samples harvested from sheep that received bacteria and antibiotic - impregnated scaffold contained no bacteria and these sheep remained healthy throughout the study.
Micro-computed tomography showed that bone growth was over 50% in animals infected with bacteria and then treated with the scaffold containing antibiotics. This was also seen in the control group (no bacteria, see Figure 1).

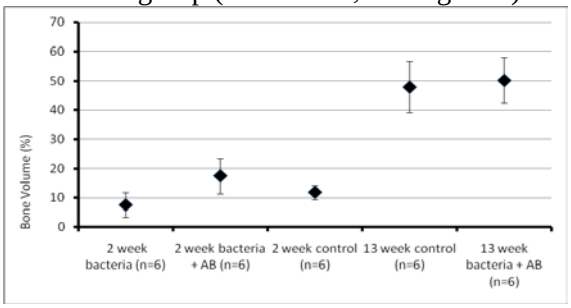


Fig.1: Growth of bone within the defect site. At 2 weeks, there was no significant difference but at 13 weeks 50% bone ingrowth was seen in both controls and the treatment group.

DISCUSSION & CONCLUSIONS: We successfully induced a localised bone infection in a large animal model of trauma. The scaffold containing the antibiotic combination showed in vitro activity against *S aureus* for a long enough period to eradicate the contamination, as well as having no significant effect on mechanical properties. The antibiotic - impregnated scaffolds were effective in treating *S aureus* infections in sheep, whilst supporting bone growth and repair within the defects.

Radiograph Morphometry of Experimental Osteitis in Rabbits treated with Antibiotics-combined Human Bank Bone or Bone Cement

Meliha Kujundzic[1], K. Kaudela[2], Ursula Burner[2], H. Winkler[3], H. Plenk Jr.[1]

[1]*Bone & Biomater. Res., Inst. Histology & Embryology, Medical Univ.of Vienna, A*
[2] *European Cell and Tissue Bank, 4600 Wels, A*
3 Osteitis Center Doebling, 1190 Vienna, A

INTRODUCTION: Systemic administration and/or local release of antibiotics from bone cement are common treatments of osteomyelitis and the resulting osteitis in fracture repair. To avoid subsequent removal of bone cement, antibiotics-impregnated biodegradable human bank bone could be an alternative. Using an established experimental model [1, 2], the performance of this alternative treatment has been tested [3]. In the present study the so far subjective estimation of radiographic bone changes was supplemented by computerized morphometry, combined with histomorphology.

METHODS: In 16 New Zealand rabbits each (f, 1.7-2.0 kg bw) the right tibia was infected by *staphylococcus aureus* (MRSA) and divided in groups A+B, or *pseudomonas aeruginosa* and divided in groups C+D. After 4 weeks, established chronic osteomyelitis was treated with Vancomycin- (groupA) or Tobramycin- (groupC) impregnated bone allografts (Osteomycin®V and T), or bone cement combined with Vancomycin (groupB) or Gentamycin (groupD) (Palacos®V and G). After 7 weeks with clinical, serological and radiological control the animals were sacrificed and the tibiae retrieved for microbiological and pathohistological evaluation. Morphometric measurements on standardized, calibrated radiographs, using Adobe-Photoshop CS5 and the analyze function, were statistically evaluated.

RESULTS: After 4 weeks of serologically proven osteomyelitis, distinct signs of osteitis (osteolysis, cortical remodelling, periosteal reactions) could be observed on the radiographs (Fig.1). After 7 weeks treatment, different amounts of diaphyseal thickening, but also of more homogenous densification were visible in a region of interest corresponding to the histological samples (Fig.1). Undecalcified cross ground sections showed more or less intense cortical remodelling, and islands of inflammatory cells in all 4 groups. Bone allografts were undergoing osteoclastic resorption, while a fibrous membrane with foreign body giant cells surrounded bone cement. Morphometric measurements yielded in groups A to C only general trends to increased bone areas at 11 weeks, but no significant evidence of healing.

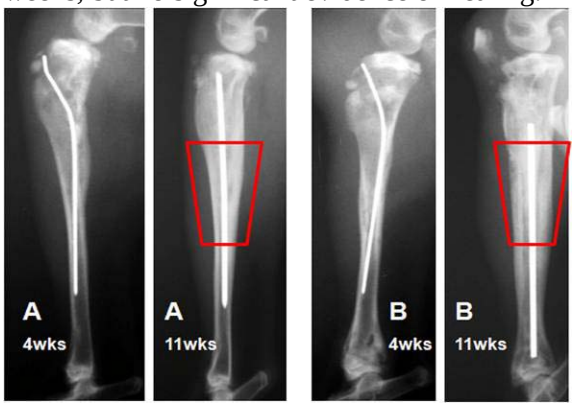


Fig. 1: Representative radiographs, groups A and B, at beginning (4wks) and end of treatment (11wks).

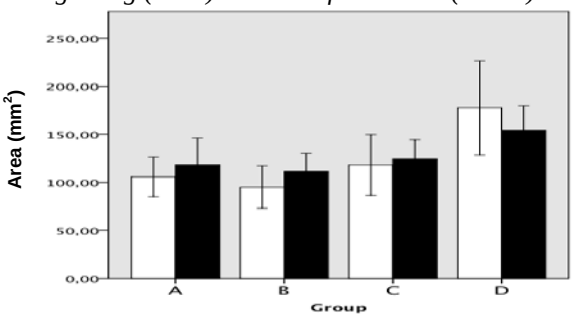


*Fig.2: Bone areas (means± 95% confid.intervals), as outlined in Fig.1, at 4 and **11 wks** in groups A to D.*

DISCUSSION & CONCLUSIONS: The previous estimation of improved radiological healing in bone allograft groups, as compared to bone cement groups [3], could not be confirmed by radiograph morphometry. Determination of grey-level histograms in cross sectional planes corresponding to levels of pathohistological sections seems a more promising approach and will be presented.

REFERENCES: [1] Faber et al.,Antimicrobial Agents Chemotherapy, 2005, 49, 2438-2444. [2]Adams et al.,J.Orthop.Res. 2009, 27, 701-709.[3]Kaudela et al.,Trans.19thEATB, 89 (2010).

Following the mechanism of biofilm formation tailoring surface modificationon impedance sensors

L. Montero[1], O. Estrada[1], J. Gilabert[1] , V. Ramos-Pérez[1], R. Villa[2] S. Borrós[1*]

[*] *Corresponding author*

[1]*Grup d'Enginyeria de Materials (GEMAT),Institut Químic de Sarrià-Universitat Ramon Llull, Barcelona, Spain)*

[2] *National Center of microelectronics, IMB-CSIC, Campus de Bellaterra, Barcelona, Spain*

INTRODUCTION: Bacterial adhesion to surfaces is a crucial step in biofilm formation and associated problems, such as in biomaterials implant surgery. One of the first steps in biofilm formation is transport of the bacterial cells toward the surface. Once brought within the range of the interaction forces, bacteria can come in close contact with a substratum surface and adhere. Initially, adhesion is reversible but over time adhesion becomes irreversible. We intend to prevent adhesion and to detach adhering microorganisms by changing the structure and topography.

METHODS: We present the ultrathin films deposition of the hydrogel poly(hydroxyethyl methacrylate) (pHEMA) by initiated chemical vapor deposition (iCVD) to improve the mechanical properties of the microelectrodes. It is able to deposit thin films of application-specific polymers in one step without using any solvents. It allows engineering polymers to be made with specific microscale properties and active biomolecules such antimicrobial peptides covalently grafted on the surfaces antibiotics as bacitracin grafted on the surfaces. The hydrogel here presented enhances the surface resistance of the post-processed microelectrodes due to the nanoscale mesh that is small enough to allow the diffusion of the electrolyte hence the microelectrode is not altered. This sensor was produced locally in the Clean Room facilities at the National Center of Microelectronics in Barcelona (Spain). To ensure that a robust biofilm is developed over no-treated surfaces has established the optimized conditions of *Staphylococcus epidermidis* cultures to guarantee the mid-log phase growth and the bacterial viability over the surfaces. The final visualization has been stained ith the BacLight Live/Dead stain and examined with apotome microscope technique.

RESULTS: The sensors with pHEMA keep their impedance and demonstrate that this coating is stable and robust for the biofilm detection. Also the effect of modified surfaces with the antibiotics is further confirmed by microscope visualization *(Figure 1)*. The consequence of the bacterial contact with antimicrobial compounds grafted with iCVD technique affect the cell viability of *Staphylococcus epidermidis.*

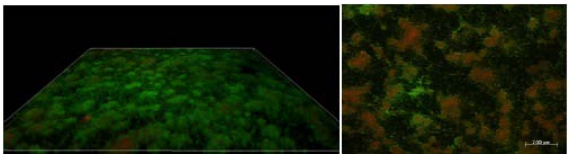


Fig. 1: Effect of surface modification on biofilm cultured and labelled Live/Dead stain wherein live bacteria fluoresce green and dead bacteria fluoresce red. No surface modification (left) vs. Surface modification (right

On the other hand, the sensor is able to maintain its response along the time following the microorganisms growth and the biofilm deposition. Further experiments are running in order to study the bioelectric effect on the biofilm and its synergism with surface modification.

DISCUSSION & CONCLUSIONS: The modification proposed in this work allows making long-term measurements on biofilm growth. The surface can be easily tuned to affect the biofilm viability on sensors. The information derived from the study could be use to design implant surfaces resistant to biofilm growth.

REFERENCES: Baxamusa, S.H. et al., 2008a. *Biomacromolecules*, 9(10), 2857-2862. Chan, K. & Gleason, K., 2005. *Chemical Vapor Deposition*, 11(10), 437-443.

ACKNOWLEDGEMENTS: This work was partially supported through grant TEC2009-14779-C02-02 of Spanish Science and Innovation Minsitry: OE, would like to thank the Spanish Ministry for a PhD fellowship. LM recognizes the support of Institut Químic de Sarrià.

Surface modification of titanium by Phosphonate monolayers Releasing Bactericidal Species

P.H. Mutin[1], J. Amalric[1], G. Guerrero[1], A. Ponche[2], J.-P. Lavigne[3]
[1]*Institut Charles Gerhardt, CNRS-UM2, Montpellier, FR;* [2]*Institut de Chimie des Surfaces et Interfaces, Mulhouse, FR,* [3]*INSERM ESPRI 26, UFR Médecine, Nîmes, FR*

INTRODUCTION: The adhesion of bacteria on surfaces and the subsequent development of bacterial biofilms is the cause of a wide variety of diseases and infections [1]. Bacteria in a biofilm are significantly more resistant to host defenses and antibiotics. A promising strategy for the prevention of biofilm formation is to modify the surface of an existing biomaterial with an antibacterial coating. Organic monolayers terminated by hydrophilic groups have already been used to decrease bacterial adhesion. Phosphonate monolayers are good candidates to modify the surface of most inorganic biomaterials (titanium, stainless steel, apatite, etc.) [3]. Here, we will present two phosphonate based nanocoatings able to release bactericidal species, silver ions (Ag^+) [4] and nitric oxide (NO).

METHODS: Monolayers terminated by silver thiolate (**Ti-SAg**) and diazeniumdiolate (**Ti-DADO**) groups were deposited in two steps on titanium (Fig.1). The coatings were characterized by XPS, grazing-incidence FTIR and water contact angle measurements.

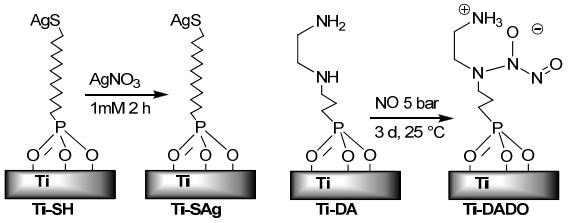


Fig. 1: Schematic representation of the monolayers deposited on titanium.

RESULTS: In the case of **Ti-SAg** sample, the presence of silver-thiolate groups was evidenced by XPS. The amount of silver was estimated to 3.5±1 Ag nm^{-2}, corresponding to about 0.6 nmol Ag cm^{-2} or 60 ng of silver per cm^2. The release of NO by the **Ti-DODA** sample under physiological conditions of pH and temperature (pH 7.4 and 37°C) was quantified by chemiluminescence. The total amount of NO released after was 0.16 nmol cm^{-2}, corresponding to 1.0 NO nm^{-2}, with a half-time of release of 20 hours.

The samples were incubated for 1 h at 37°C in a suspension of *E. coli* in Mueller-Hinton medium containing about 10^5 CFU mL^{-1}, rinsed with sterile saline solution to remove non-adherent bacteria, and incubated for 24 h in sterile AP medium. The amount of viable adherent bacteria recovered on the surface of the **Ti-SAg** and **Ti-DADO** samples was about 10^4 times lower than on the bare Ti samples or the **Ti-SH** and **Ti-DA** samples (Fig. 1). In the same way, the biofilm density estimated by the crystal violet method after incubation for 3 days at 37°C in a *E. coli* suspension in Mueller-Hinton medium (ca 10^5 CFU mL^{-1}) was decreased by about 80% on the **Ti-SAg** and **Ti-DADO** samples.

DISCUSSION & CONCLUSIONS: Despite the extremely low amount of bactericidal species involved, phosphonate monolayers releasing Ag^+ ions or NO can significantly decrease bacterial adhesion and biofilm formation at the surface of titanium. Moreover, in the case of monolayers releasing Ag^+ cations, the inhibition of bacterial adhesion was maintained after sterilization with ethylene oxide, and cytotoxicity tests on pre-osteoblast cells showed that the coating was not toxic.

.

REFERENCES: [1]J. W. Costerton, L. Montanaro and C. R. Arciola, Int. J. Artif. Organs, 2005, 28, 1062. [2]P. H. Mutin, G. Guerrero and A. Vioux, J. Mater. Chem., 2005, 15, 3761. [3]E. M. Hetrick, J. H. Shin, H. S. Paul and M. H. Schoenfisch, Biomaterials, 2009, 30, 2782. [4]J. Amalric, P. H. Mutin, G. Guerrero, A. Ponche, A. Sotto and J.-P. Lavigne, J. Mater. Chem., 2009, 19, 141.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge financial support by CNRS, INSERM and Montpellier 2 University.

A REPRODUCIBLE OSTEOMYELITIS MODEL IN RABBITS

JCE Odekerken, TJM Welting, JJC Arts, GHIM Walenkamp.
Department of Orthopaedic Surgery, Research School CAPHRI, Maastricht University Medical Center.

INTRODUCTION: In this study a reproducible rabbit osteomyelitis model, resembling clinical orthopaedic implant infections, is being developed. The model will be utilized for evaluating the efficacy of novel antimicrobial coatings on titanium substrates. We here report on a pilot study that evaluates surgical methods, imaging techniques and osteomyelitis outcome.

METHODS: In the rabbit osteomyelitis model a titanium rod was placed in the proximal metaphysis of the tibia [1,2]. The implants were contaminated with various inocula of *Staphylococcus aureus* (ATCC 49230) to induce osteomyelitis. Correct positioning of the implant and the degree of infection were evaluated by X-ray, µPET and µCT imaging (fig. 1). Health status was monitored by hematological analyses, weight and temperature of the animal. After 42 days of follow-up, animals were sacrificed. The tibia and titanium implants were evaluated by histological staining on bone integration, biofilm formation and clinical signs of infection in the surrounding tissue.

RESULTS: Rabbit cadaveric studies were performed to evaluate surgical approaches for the implantation procedure. To avoid damage to intra articular lesions and to avoid septic arthritis we found that surgery through the patella tendon is preferable to the medial parapatellar artrotomy. The tibia was reamed by hand drilling at the place where in human fracture treatment the intramedullary nail is inserted. The proximal tibiae of rabbits were contaminated with different exponentially growing *S. aureus* inocula.

During follow-up, several imaging techniques were evaluated for their potential to evaluate the infection in this model. Using µCT we were able to obtain additional information on implant position, bone remodeling and osteolysis due to the implant infection. ^{18}F-FDG µPET imaging on the other hand provided additional information on differentiation between inflammation (post operative effects) and bacterial infection of the implant area. These data, combined with hematological analyses, bacterial culturing of post mortem tibia samples, X-ray imaging and histology, are expected to strengthen this animal model for use in orthopaedic implant related infection studies, as well as antimicrobial coating evaluation.

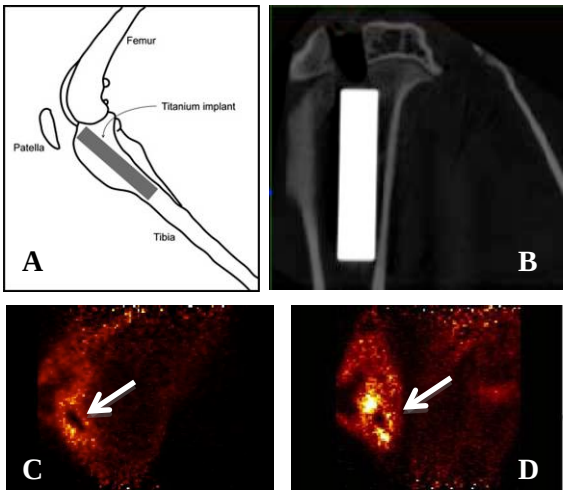


Figure 1: A: Schematic representation of the rabbit osteomyelitis model, depicting the titanium implant in the proximal end of the tibia. B: µCT-image of the tibial implant. C-D: µPET.-image of the implant area (arrow) C: Sagital plane D: Coronal plane.

DISCUSSION & CONCLUSIONS: Combined data derived from approaches described above, will give a comprehensive overview of relevant osteomyelitis parameters when testing efficacy of antimicrobial coatings.

REFERENCES: [1] Vogely HC *et al.* J Orthop Res 2000 may;18(3):485-93. [2] Moojen DJ *et al.* J Orthop Res 2009 Jun;27(6):710-6.

ACKNOWLEDGEMENTS: This study is a part of the NANTICO project, financed by the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

A new generation of antimicrobial polymers

M. Oster [1, 2], N.M. Neisius [1], S. Gaan [1]

[1] *EMPA, Swiss Federal Laboratories for Materials Science and Technology, Advanced Fibers Department,* [2] *European School of Chemistry, Polymers and Materials Science (ECPM), University of Strasbourg, Strasbourg, France*

INTRODUCTION: In the past years the ROMP (**R**ing **O**pening **M**etathesis **P**olymerization) [1] of norbornene derivatives has become a versatile tool to obtain tailored polymers with interesting functional groups.
Although ruthenium complexes have already been used for the preparation of "biological polymers" [2], they have not yet been used, to the best of our knowledge, to prepare polymers with antimicrobial properties
The aim of this research is to develop stable, well-defined antimicrobial polymers with different biological as well as physical properties by means of ROMP.

METHODS: Once the monomer precursors are prepared by simple and efficient chemical transformations, a ROMP [1] is performed on these monomers to obtain amino functionalized macromolecules. The polymers bearing tertiary amines are subsequently transformed into quaternary amines [2]. It is well known that quaternary ammonium compounds are not only active against many fungi and viruses, but also exhibit interesting disinfectant and antimicrobial properties. Therefore, they are widely used to inhibit microbial growth.
In this project, we would like to study their biological properties when grafted to a new type of polymeric backbone.

RESULTS: The thermal decomposition of the dicyclopentadiene was realized to form cyclopentadiene which was then immediately used for the Diels-Alder reaction [3]. The appropriate norbornene derivative product was obtained with a yield of 96%. After drying and characterisation, the product was modified with different amino compounds to obtain adequate imide monomers. One of such modification resulted in a functional monomer with sufficient yield which could be further modified. Dimethylamino-propylamine [4] was used as a reactant and solvent and added dropwise to modify 5-norbornene-2,3-dicarboxylic anhydride. The solution was refluxed with stirring for several hours. The reaction was monitored using GC-MS analysis until full conversion was completed. After usual work up and recrystallization of the crude product, we isolated the desired product as a pure colourless solid in nearly 70% yield. ROMP using 1st generation Grubb's catalyst was tried in order to see the influence of the amino groups on the kinetics and performance of the polymerization reaction [1]. Later on, different types of ru-alkylidene complexes will be tested.

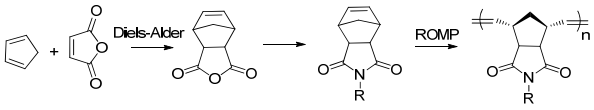


Fig1. General reaction scheme of the preparation of different functional polymers.

DISCUSSION & CONCLUSIONS: Until now at least one monomer could be synthesized successfully within two steps in an overall yield of 67%. Thus, first polymerization reaction could be carried out in recent future and would be reported in our presentation. Once we have developed such polymers, further research needs to be done regarding efficacy of various antimicrobial functional groups that can be grafted to the polymers in order to get good biological and physical properties of the macromolecules.

REFERENCES: (1) Sutthasupa, S.; Sanda, F.; Masuda, T. *Macromolecules* **2009**, *42*, 1519. (2) Thebault, P.; Guittard, F. *J. of Fluorine Chem.* **2010**, *131*, 592. (3) Goll, J.M.; Fillion, E. *Organometallics* **2008,** *27*, 3622. (4) Lu, H.; Cheng, J. *J. Am. Chem. Soc.* **2008**, *130*, 12562.

ACKNOWLEDGEMENTS: I would like to thank EMPA and the Advanced Fibers team for their help and financial support. I would also like to thank the ECPM for the opportunity to do such a project in EMPA.

Medical coating innovation: Antimicrobial PVD TiN

C. Piñero[1], A. Janssen[1], G. Sinicco[1], A. Ewald[2]

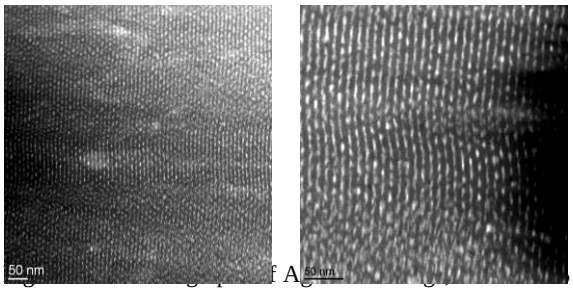
[1] *OC Oerlikon Balzers AG , Headquarters, Iramali 19, LI-9496 Balzers*

[2] *Dept. of Functional Materials in Medicine and Dentistry (FMZ), University Hospital of Wuerzburg, Pleicherwall 2, D-97070 Wuerzburg*

INTRODUCTION: The use of antibiotics to prevent infections when bound to or incorporated into orthopedic and medical devices has met with limited success. Furthermore the high incidence of nosocomial infections has promoted the development of new antibacterial products in order to diminish the occurrence of these diseases [1]. Therefore Oerlikon Balzers has developed a new antimicrobial wear resistant PVD Ag/TiN coating for orthopaedic and medical devices.

METHODS: Ag/TiN coatings were produced using PVD techniques. Hardness, colour, roughness, substrate temperature during coating deposition, antibacterial action and process flexibility for adjusting the antibacterial action were the highly significant parameters that were considered in order to develop the most suitable deposition process for the production of Ag/TiN coatings and their most efficient variants. Coatings having silver concentrations of about 0.1 up to 30 at% were synthetized and characterized on different steel substrates - including medical quality steel samples. Coating composition and structure were investigated by secondary ion mass spectrometry (SIMS), X-ray diffractometry (XRD) and transmission electron microscopy (TEM) techniques. Bacterial activity tests (performed with E. coli, S. epidermidis and S. aureus) on Ag/TiN-coated medical steel substrates were accomplished by FMZ.

RESULTS & DISCUSSION: We found that the most suited deposition process for the synthesis of the antibacterial Ag/TiN coatings was a combined arc/sputter ion plating process (AIP/MSIP). The presence of separated Ag and TiN phases in the coating structure could be confirmed by XRD-examinations. Characteristic Ag peaks were detected in XRD spectra of the synthesized antimicrobial Ag/TiN coatings. However the observed Ag peaks were wider than typical characteristic ones, particularly by coatings having lower silver concentrations. Wider Ag peaks can be explained by the nanometer grain size of the silver particles, which was confirmed by TEM examinations. TEM pictures (Fig. 1) show the formation of island shaped agglomerations having grain sizes of about 8-10 nm x 3-4 nm in Ag/TiN coatings containing 2,5 at% and 6,5 at%.



Ag, right 6.5 at% Ag

Experiments examining the antimicrobial activity of these coatings reveal very promising results using E. coli (Fig. 2)

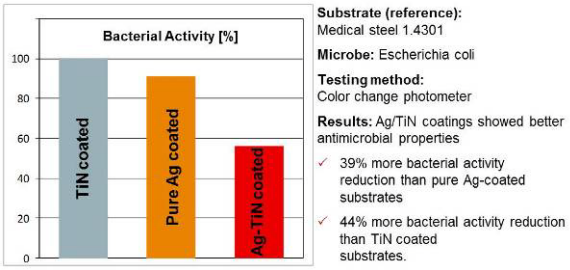


Fig. 2: antimicrobial activity of Ag/TiN coatings

Further coating variants designed as yet attained about Log 1 by Log-reductions using S. epidermidis as microbe and medical steel 1.4542 as reference.

CONCLUSIONS AND FURTHER WORKS: Developed Ag/TiN coatings showed better antibacterial action than pure PVD Ag sputtered coating and TiN coatings without silver. Further investigations for adjusting antibacterial action and long-time antibacterial according to particular customer requirements will be performed in cooperation with interested customers.

REFERENCES: [1] Ewald A. and et al.; BioMedical Engineering OnLine, 2006, 5:22

Mechanically responsive and antibacterial plasma polymer coatings for soft biomaterials

L. Ploux[1], E. Kulaga[1], T. Bourgade[1], V. Roucoules[1]

[1]*Material Science Institute of Mulhouse (IS2M), CNRS, Mulhouse, FR*

INTRODUCTION: For fighting persistent infections, there is a need to develop multifunctional systems with immobilized antibacterial agents, which exhibit long term durability and reduced development of drug-resistant mutation. Especially in the field of textile and other soft biomaterials used in gynaecological and abdominal wall reconstructions, this development is a challenge, due to the mechanical stresses that naturally occur at the implant location. Besides, the development of surfaces with stimuli-responsive properties, also known as smart surfaces, has been actively pursued in the past few years. In this project, a smart coating with antibacterial properties and dedicated to soft biomaterials was developed. External mechanical stimuli allowed to control the drug release properties.

METHODS: Maleic Anhydride Plasma Polymer (MAPP) film was deposited on the surface of polypropylene mesh. Silver (Ag^+) was loaded into the reservoir by simply dipping the coated substrate into a silver nitrate solution. Silver cations formed electrostatic pairs with carboxylic groups previously created by hydrolysis reaction of maleic anhydride groups. The carboxylic-bound Ag^+ ions were reduced to zero-valent AgNPs using $NaBH_4$ aqueous solution as a reductant. To immobilize bactericides, prevent from continuous leaching of antibacterial agent and control the release of Ag^+, a second thin plasma polymer film was deposited on the existing system. This plasma polymer layer had the property to induce cracks under stretching. The properties of the coating, including cracks properties under stretching and release properties of silver (Ag^+) through the cracks, were analysed by XPS, FTIR, SEM, AFM, confocal microscopy and UV-visible spectroscopy. Antibacterial efficiency was evaluated (i) on bacteria living in the surroundings on agar plates and in bacterial suspensions, (ii) on bacteria adherent on the ceramics by confocal microscopy.

RESULTS: Besides the physical-chemical characterisation of the polymer coating at different scale, we demonstrated that cracks were induced in the second plasma polymer coating by mechanical stimulation, and that density and size of cracks were controlled during this process. We also showed that, when the material was no longer elongated, cracks were closed until mechanical strength appeared again (Fig.1). This gave the opportunity to control the flux of the antibacterial agent by the mechanical stimulation. The microbiological assays on *Escherichia coli* species allowed to highlight the efficiency of the antibacterial agent delivered through the cracks.

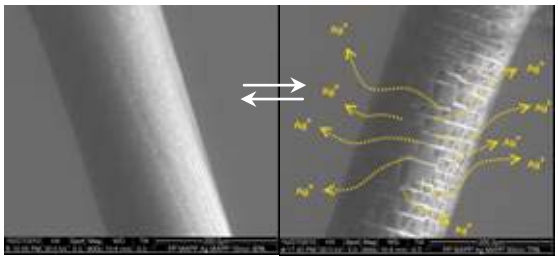


Fig. 1: Reversible releasing process.

DISCUSSION & CONCLUSIONS: Mechanically sensitive architectures made of macromolecules and bioactive agents are constructed on the modified polypropylene substrate, using solvent-free steps. Here, the behavior of the films is presented in terms of elongation-dependent releasing measurements. This work puts a light on original approach offering a great opportunity to readdress the persistent challenge of obtaining effective and long-lasting antibacterial coatings.

ACKNOWLEDGEMENTS: This work was realised with the financial support of the Region Alsace (AntiMicroBioMat project).

A New Antibiotics-Impregnated Microporous Bioceramic

L. Ploux[1], M. Mateescu[1,2], L. Guichaoua[2], K. Anselme[1], E. Champion[2], D. Chulia[2], M. Viana[2], R. Chotard[2]

[1]Material Science Institute of Mulhouse (IS2M), CNRS, Mulhouse, FR, [2]Laboratory of Science des Procédés Céramiques et de Traitement de Surface, CNRS/Univ Limoges, Limoges, FR

INTRODUCTION: For fighting persistent infections, bioactive biomaterials able to prevent bacterial adhesion and to kill just adhered bacteria must be developed. Antibiotic-loaded implants are of particular interest, since they allow using lower antibiotics doses than with systemic treatment whilst providing high enough concentrations of antibiotics at the site of infection. Calcium phosphate ceramics, commonly used as bone substitutes, are good candidates as such drug carriers. The kinetics of drug delivery is essentially modulated by the porous architecture of such implants. The purpose of this study was to develop bioceramics with a well-defined porous structure in order to control drug loading and release kinetics. Short- and middle-term antibacterial efficiencies were analyzed for bacteria growing in surrounding suspension as well as for bacteria adhering onto ceramics.

METHODS: Hydroxyapatite materials with an interconnected framework of micropores were obtained through a heterocoagulation colloidal process, using polymer microspheres as templates (core) and hydroxyapatite nanoparticles as inorganic building blocks (shell). The core-shell structures suspension was consolidated and polymer templates were removed by calcinations. The size, volume and distribution of pores were tailored by the size and volume ratio of the core and shell particles. Two different materials were selected for further use. Their drug loading ability was studied using tetracycline and loading process by vacuum immersion in antibiotic solutions with various concentrations and immersion times. In vitro drug release under controlled conditions was determined using a flow-through dissolution apparatus. Antibacterial efficiency was evaluated (i) on bacteria living in the surroundings on agar plates and in bacterial suspensions, (ii) on bacteria adherent on the ceramics by confocal microscopy.

RESULTS: The two materials tested were obtained after calcinations at 1100°C (HA-1100) and 1220°C (HA-1220) respectively, leading to variations in the porosity and interconnections sizes and rates (HA-1100>HA-1220). The amount of loaded antibiotic was higher for ceramics with higher porosity and more interconnections. The release was faster for HA-1100 than for HA-1220, which is in accordance with the higher porosity and interconnection rates of HA-1100 compared to HA-1220. Microbiological tests demonstrated antibacterial efficiency (fig.1) both for bacteria living in the surroundings and for adherent bacteria. While growth of adherent bacteria was affected by antibiotics, adherent bacteria were present in similar numbers on unloaded and loaded materials. Antibacterial properties were higher with HA-1100 vs. HA-1220. They were not affected by proteins previously adsorbed onto the drug-loaded materials, confirming the possibility to maintain antibacterial efficiency in real biological environment.

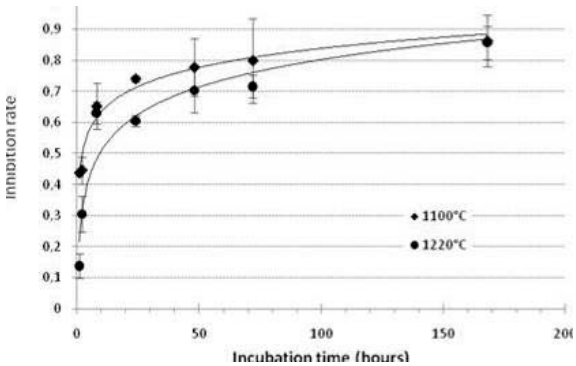


Fig. 1: Inhibition rate of planktonic bacteria in the surroundings of loaded bioceramic.

DISCUSSION & CONCLUSIONS: We demonstrated the possibility of varying the antibacterial efficiency of microporous hydroxyapatite by controlling the quantity of loaded antibiotics and its release through the control of the porous microstructure.

ACKNOWLEDGEMENTS: This work was realised with the financial support of ANR (BiocerPorDDS project).

Characterisation of *Staphylococcus aureus* from orthopaedic device related infections

V. Post[1], T.F. Moriarty[1], R.G. Richards[1]

[1]*AO Research Institute Davos, AO Foundation, Davos, CH,*

INTRODUCTION: Musculoskeletal infections are one of the most common complications associated with surgical fixation of bones fractured during trauma[1,2]. The most common pathogen causing these implant-associated infections is *Staphylococcus aureus*, a Gram positive microorganism that is frequently part of the normal human skin flora. Adherence of *S. aureus* to extracellular matrix protein coated devices occurs through specific factors known as adhesins or MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules - Table 1). The aim of this study was to characterise *S. aureus* isolates specifically cultured from infections associated with fracture fixation devices with respect to the repertoire of MSCRAMMs or other virulence factors and the clonality of these strains.

METHODS: *S. aureus* isolates from different patients with infections associated with implant devices were typed and characterised by *agr* (accessory gene regulator) group and Randomly amplified polymorphic DNA (RAPD) PCR[3]. The most relevant MSCRAMMs and virulence factors present in the isolates were screened for by PCR[4] (listed in Table 1).

Table 1. Most prevalent MSCRAMMs and their functions

Gene	Function
icaA	encodes proteins for synthesis of polysaccharide intercellular adhesion
clfA/B	encode clumping factors A and B
eno	encodes laminin binding protein
ebpS	encodes elastin binding protein
fib	encodes fibrinogen binding protein
cna	encodes collagen binding protein
fnbA	encodes fibronectin binding protein A
fnbB	encodes fibronectin binding protein B
bbp	encodes bone sialoprotein binding protein
sdrC/D/E	Fibrinogen-adhesin genes

RESULTS: Isolates could be typed by RAPD-PCR into three main groups (A-C in Fig. 1). Group B isolates fell into two subgroups (B1 and B2). All four *agr* groups were present (Table 2). Within each *agr* group most isolates possessed no consistent pattern of virulence factors and MSCRAMMs (Table 2).

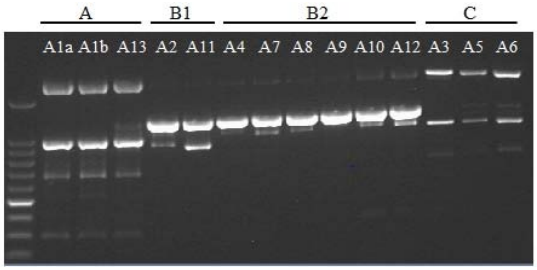


Fig. 1: RAPD profiles of S. aureus *isolates using primer EF007*[3].

Table 2: Prevalence of the patterns found within the agr groups

Agr type	No. of isolates	*MSCRAMMs*						
		cna	*ebpS*	*sdrE*	*bbp*	*fib*	*fnbA*	*fnbB*
I	1	+	+	+	-	-	-	-
	1	-	-	-	-	+	-	-
	2	-	-	+	-	+	+	+
II	1	-	+	+	-	+	+	+
	1	-	+	-	-	+	+	-
	1	-	+	+	-	+	-	-
	2	-	-	+	-	+	-	+
III	2	-	+	-	+	+	-	-
	2	-	+	-	+	+	+	+
IV	1	-	+	+	+	+	-	-
total	14							

NB: all isolates were positive for *icaA, eno, clfA, clfB, sdrC* and for hemolysin encoding gene hlgB/C. All isolates were negative for the PVL encoding gene *lukL*.

DISCUSSION:

Only *agr* groups III and IV possessed the *bbp* gene. The *sdrE* gene was absent in *agr*III. These results are coherent with the observation previously reported from isolates also collected from fracture fixation devices[4]. This suggests a possible trend.

REFERENCES: [1]Arciola *et al.*,Int.J.Artif. Organs. 2005;28:1091-1100. [2]Bingham *et al.* Proc.Natl.Acad.Sci.U.S.A. 2008;105:12254-12258. [3]Tambic *et al.*, J.Clin.Microbiol. 1997 35:3092-3097. [4]Montanaro *et al.*, J.Biomed. Mater.Res.A 2010;94:825-832.

ACKNOWLEDGEMENTS: Isolates were kindly provided by Prof. Ochsner and Dr. Pranghofer, Luzern, Switzerland.

Bacterial peptides as imaging probes for functional imaging in infection diagnostics

I.Potapova, TF.Moriarty, RG.Richards

AO Research Institute, AO Foundation, Davos, CH, Musculoskeletal Infection Group

INTRODUCTION: The most promising tools for quick, non-invasive and precise diagnosis of infection are functional imaging modalities based on probes specific for bacteria. The currently available probes used in infection imaging have drawbacks that limit their clinical usefulness such as an inability to distinguish infection from sterile inflammation or Gram positive from Gram negative infections. The aim of our study is to identify new infection probes that will allow improved diagnostic imaging of infection by targeting the bacteria rather than the local inflammatory response and furthermore enabling identification of the Gram character of the bacteria. We hypothesize that bacteriocins, which are antibiotics produced by bacteria to target other bacteria, will show greater diagnostic specificity than the current alternatives.

METHODS: Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were cultured overnight in Tryptic soy broth (Oxoid, Basel, CH) at 37°C. Nisin, (Molecula, UK) which is a bacteriocin against Gram positive bacteria and Polymyxin B - PMB (Sigma, CH), which targets Gram negative bacteria were labeled with red and green dyes, respectively, using amino reactive DyeLight594 /488 labeling kits (Thermofisher, CH). Anti microbial activity of probes before and after labeling was performed by viability counts on Tryptic soy agar (Oxoid) plates and by the disc diffusion test according to EUCAST protocols. Flow cytometer (Partec PAS, DE) and fluorescence microscope (Axioplan, Carl Zeiss, DE) were employed to access specific attachment of the fluorescent probes to bacteria. BacLight Green (Invitrogen, CH) was used as the control staining of bacteria.

RESULTS: DyeLight-labeled Nisin displayed less antimicrobial activity against *S. aureus* than non-labeled Nisin. Nisin did not suppress *E. coli* either with or without labeling. Labeled and non-labeled PMB killed both bacteria.

Flow cytometry and fluorescence microscopy result that DyeLight labeled Nisin efficiently labels the Gram positive *S. aureus* as expected but does not the Gram negative *E. coli*. DyeLight labeled PMB labels both *S. aureus* and *E. coli.* The labeling of *S.aureus* alone and a co-culture of *S. aureus* and *E.coli* by Red-Nisin is shown in Fig. 1.

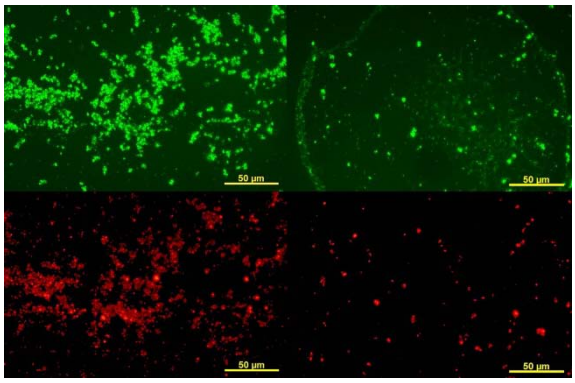


Figure 1: Fluorescence microscopy: BacLight Green staining of S. aureus (upper left) and co-culture of E. coli & S. aureus (upper right); Nisin-DyeLight594 staining S. aureus (lower left) and co-culture of S. aureus & E. coli (lower right).

DISCUSSION & CONCLUSIONS: The covalent binding of dye-carboxyls to Nisin-amines renders Nisin less antimicrobial but it still retains its specificity for the Gram positive *S. aureus.* PMB, in contrast, retains its antimicrobial activity upon labeling but displayed affinity to both Gram species. PMB therefore is not bacteria Gram specific yet remains a possible general infection probe, since the combination of Nisin and PMB probes would still allow differentiation of Gram positive from negative bacteria. Ongoing studies evaluate the probes specificity for bacteria over host cells. Subsequent studies will involve conjugation of Nisin and PMB to contrast agents enabling functional imaging.

Our preliminary results show that labeled Nisin recognizes bacteria *in vitro* and distinguishes between Gram positive and negative bacteria. Labeled PMB recognizes both species. Combination of the probes will allow diagnostics capable of specification of the bacterial Gram character.

ACKNOWLEDGEMENTS: Pamela Furlong, Christopher Sprecher, Martin Stoddard

Microemulsion Approach to Antimicrobial Nanocontainers

M. Priebe, K. M. Fromm

Chemistry Department, University of Fribourg, Switzerland

INTRODUCTION: Within recent years nanometer-sized hollow spheres, called nanocontainers, have attracted increasing interest due to their ability to enclose guest molecules inside their empty core.[1] Because of this potential, hollow nanoparticles may find numerous applications such as drug carriers, reactors, confined reaction vessels, building blocks for photonic crystals or multi-enzyme biocatalysis.[1,2]

The microemulsion approach for the production of nanocontainers is a relatively new method, which allows the preparation of nano-scale hollow spheres without using a solid template. In this case, its role is taken over by a micellar system enabling dissolution of any substance in its core. Subsequent reaction between reagents on the boundary phase between a micelle and the surrounding phase leads to the formation of a nanocontainer.[3]

The aim of this study is to encapsulate an antimicrobial silver coordination polymer inside inorganic nanocapsules (CuS, TiO_2,etc.).

METHODS: Hollow spheres are prepared by reduction in oil-in-water and water-in-oil microemulsions by a slightly modified method devised by Feldmann[3] in which the synthesis of nanocontainers is carried out on the boundary phase of the micelle. Solutions of ligand **1** and $AgNO_3$ in THF/EtOH are intended to penetrate into hollow spheres by spontaneous diffusion or under high vacuum (Fig. 1.)

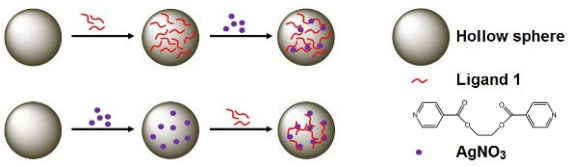


Fig. 1: A schematic illustration of the incorporation of ligand 1 and $AgNO_3$ into the hollow spheres.

The CuS nanocontainers as well as the encapsulation process are characterized using HRSEM, TEM, EDS, ICP, XRPD and FT-IR methods. In addition, the stability of nanocapsules during long-term storage is investigated.

RESULTS: HRSEM and TEM present the spherical primary nanoparticles forming aggregates. Increased electron density on the border of the particles indicates the presence of hollow spheres exhibiting an outer diameter of 41±5 nm with a wall thickness of 11±2 nm (Fig. 2).

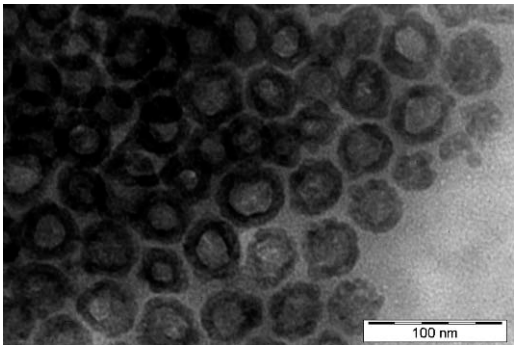


Fig. 2: TEM image of CuS hollow spheres before purification.

DISCUSSION & CONCLUSIONS: We have successfully synthesized CuS hollow spheres of nanosize with a well-defined porous shape using a water-in-oil micellar system. However, the formation of aggregates and the high sensitivity of the microemulsion to the synthesis conditions constitute a challenge. The aggregation hinders an incorporation of the ligand and $AgNO_3$ into the nanocontainers. Further investigations will improve nanoparticle synthesis and incorporation techniques. Incorporated nanocontainers will be subjected to antimicrobial and cytological tests.

REFERENCES: [1] Meier W. Chemical Society Reviews 2000, 29, 295–303. [2] Caruso F. Advanced Materials 2001, 13, 11-22. [3] Feldmann C. et al. Advanced Materials 2009, 21, 1586-1590.

ACKNOWLEDGEMENTS: We are grateful to the Swiss National Science Foundation, the NRP-62, the University of Fribourg and the FriMat for generously supporting this project.

New Evidence of Spondylitis Tuberculosis: Contaminated by Pyogenic Microorganism or Mixed Infection?

Rahyussalim[1], Andriansjah[2], Ismail[2], Andri Lubis[1], Tri Kurniawati[1]

[1]Orthopaedic & Traumatology, Cipto Mangunkusumo Hospital, Diponegoro St. 71, Jakarta, Indonesia. [2]Departement Microbiology, Faculty of Medicine, University of Indonesia.

INTRODUCTION:

The type of bacteria which causes Spondylitis is important to be recognized since it influences the therapy approach that will be done, including the rational use of antibiotics. During this time when the diagnosis of bacterial spondylitis discovered *Mycobacterium tuberculosis*, the presence of other bacteria are often overlooked, yet the discovery of mixed bacterial infections with culture method is still very possible. Bacterial culture is still difficult and impractical to determine what bacteria are involved. New strategy was developed to catch the bacteria especially the cases with chronic infection.

This paper shows the evidence of bacteria in spondylitis tuberculosis and whether they are mixed or contaminated.

METHODS:

This research was conducted at the Cipto Mangunkusumo hospital and Clinical Microbiology Laboratory; Department of Microbiology, Faculty of Medicine University of Indonesia from August to November 2010. Examining the morphology of bacteria was done by staining and culturing techniques on selective medium and differential to Spondylitis material. All tissue lesions both solid and liquid including pus, granulation tissue, sequester bone, and necrotic tissue was used as inspection raw materials.

RESULTS:

It has been successfully identified and isolate is obtained from the three bacteria, namely *Staphylococcus epidermidis (SE)*, *Staphylococcus aureus (SA)* and *Mycobacterium tuberculosis (MTB)* which was derived from solid lesions (granulation tissue, sequester and necrotic tissue) and liquid (pus), taken from four patients with Spondylitis diagnosis.

From overall 4 (four), we evaluate that there is 1 (one) case of spinal infection caused by three bacteria that cause infection.

Table 1. Bacterial culture results of Spondylitis materials samples

Patient Name	Bacteria found in the culture		
	Lesion Material	Granulation Lesion material	Pus
Mrs. HR	*S. epidermidis*	-	-
Mr. FA	-	-	-
Mrs. TSW		*M.tuberculosis*	-
Mr. RA	*S. epidermidis*	*M.tuberculosis*	*S. aureus*

DISCUSSION & CONCLUSIONS:

Culture examination should be using liquid or solid material examiner in order to obtain the whole profile of bacteria that play a role in the process of spinal infection. Bacterial culture of all substances of material lesions, cultures of material use soft needle procedures and PCR technique although each has limitations.

REFERENCES: [1]Schlossberg, D.Tuberculosis and Non tuberculous Mycobacterial Infections. Fifth Edition, McGraw-Hill 2006. [2] M.A. Ribeiro et al, PCR Identification of *M.tuberculosis* complex in a clinical sample from a patient with symptoms of tuberculous spondylodiscitis, Brazilian Journal of Medical and Biological Research (2007) 40: 1-4.[3] P. Kapeller, et al, Pyogenic Infectious Spondylitis: Clinical, Laboratory and MRI Features, European Neurologi 1997; 38; 94-98) [4] Chaudary, Saad B et al, Post Operative Spinal Wound Infections and Postprocedural Diskitis, Invited Review, J. Spinal Cord Med, 2007; 30: 440-451.

ACKNOWLEDGEMENTS:

We express our gratitude to all those who have helped this research: DRPM Universitas Indonesia which provide grant for this research

An *in vitro* Study of Bacterial Adhesion to Oxygen Plasma Treated PEEK

E.T.J. Rochford[1,2], T.F.Moriarty[1], R.G.Richards[1,2], A.H.C.Poulsson[1]

[1] *AO Research Institute, Davos, CH.* [2]*Aberystwyth University, UK.*

INTRODUCTION: Oxygen plasma treatment of polyetheretherketone (PEEK) has been shown to enhance human primary osteoblast cytocompatibility by increasing surface energy[1]. Altering surface energy causes a change in the binding and conformation of proteins, which can promote eukaryotic cell adhesion[2] and may also affect bacterial adhesion. This *in vitro* study compares the adhesion of clinically relevant bacteria to untreated PEEK versus oxygen plasma treated PEEK in both a protein free and a blood plasma preconditioned model.

METHODS: Injection moulded PEEK-OPTIMA® discs (Invibio Biomaterial Solutions, UK) were oxygen plasma treated using an Emitech K1050X plasma cleaner (Quorum Tech., UK) for 900s or 1800s and compared to untreated PEEK. Wettability, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) analyses were performed on the treated and untreated samples. Adhesion of 3 strains of *Staphylococcus aureus* (V8189-94, JAR and 8325-4) were measured to the materials in triplicate, with 9 samples of each surface in each replicate. Log phase bacterial cultures were adjusted to $1x10^7$cfu ml^{-1} in PBS and incubated with the samples in a custom made adhesion chamber for 2.5h at ~37°C and 125rpm. After incubation, the bacterial suspension was replaced with fresh PBS. Following sonication and vortex mixing viable counts of adherent bacteria on the samples were performed[2]. Additionally, the adhesion of *S. aureus* JAR to treated samples exposed to human blood plasma (Schweizerisches Rotes Kreuz GR, CH, centrifuged to remove clots) was assessed using the adhesion chamber. The samples were incubated in the chamber with 50% blood plasma in PBS for 1h (37°C, 125rpm) before bacteria in PBS were added to give $1x10^7$ cfu ml^{-1}. The experiment then proceeded as described above. The data were analysed using SPSS v.16.0. One-way ANOVA and *post hoc* LSD tests compared Log_{10} transformed bacterial adhesion data (n=3,Sig:P<0.05).

RESULTS: Plasma treatment of PEEK caused an increase in wettability; an increase in atom% surface oxygen, due to an increase in oxygen functional groups; and a slight increase in surface roughness after 1800s treatment due to surface etching.

There was no significant effect on bacterial adhesion for any of the strains in PBS (Fig 1a).

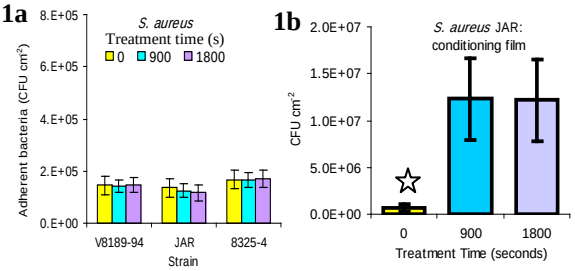


Fig. 1: Adhesion of S. aureus to the treated PEEK surfaces without PBS (1a) and adhesion of S. aureus JAR to the treated and human plasma preconditioned PEEK surfaces (1b) (n=3, ±s.e., Sig= P<0.05).

The presence of a conditioning film caused an increase in bacterial adhesion to the treated samples for *S. aureus JAR* (Fig 1b). Clotting was observed on the treated surfaces. The presence of clots provided an increased surface area which further enhanced bacterial adhesion.

DISCUSSION & CONCLUSIONS: This study shows that plasma treatment may be a viable option for increasing osseointegration, without increasing the risk of bacterial adhesion to PEEK without a blood plasma conditioning film. The increase in adherent bacteria to the treated protein preconditioned materials is likely due to a change in protein binding affinities and conformation of the proteins once bound, and should be further investigated. However, this increase in bacterial adhesion may be overcome by the positive effect previously reported for osteoblast cell adhesion and mineralisation due to altered protein binding to the treated surfaces[1].

REFERENCES: [1]Poulsson, A.H.C. (2008) *Eur Cell Mater* 16: 47-47. [2]Altankov, G.; Groth, T. *J. Mater. Sci.* Mater. Med. 1994, 5, 732–737. [3]Miles, A.A. (1938) *J Hygiene* 38, 732-749.

ACKNOWLEDGEMENTS: Financial contribution and samples from Invibio Biomaterial Solutions. Dr. Schulzki, RBSD SRK GR, for blood plasma.

Bioactive TiO_2 Nanotube Layers with Antibacterial Properties

A.Roguska[1,2], M. Pisarek[1,2], M. Lewandowska[1]. K.J. Kurzydłowski[1], M. Janik-Czachor[2]

[1]Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland, [2] Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The common problem for all biomaterials, including titanium, is the risk of an infection when they are implanted into the human body. This can be alleviated by an incorporation of Ag nanoparticles into biomaterial surface, as Ag has been reported as a powerful antibacterial agent [1]. Titanium implants are frequently coated with calcium phosphates, (primarily hydroxyapatite), to increase their bioactivity and to enhance osteointegration [2]. As a result response of bone cells to the implants and their strength/durability is strongly influenced by characteristics of the Ti substrate and the applied methods of surface engineering, in particular the calcium phosphate coating process. In this work we present a method of preparation of Ag-incorporated nanotubular titania surface and subsequent deposition of a calcium phosphate (Ca-P) coating. Such composite coatings are expected to assure both biocompatibility and antibacterial properties.

METHODS: TiO_2 nanotube arrays were fabricated via electrochemical oxidation at constant voltage in a mixture of glycerol, deionized water and NH_4F. Silver particles were deposited to the surface using sputter deposition technique in vacuum. Calcium phosphate coatings were grown on the nanotubular titania by simple immersion in Hanks' solution. To evaluate the potential use of Ag/Ca-P/TiO_2 nanotubes composite coatings for biomedical implants, protein adsorption was investigated on its surface. Bovine serum albumin (BSA) was used as a model protein in this study. SEM, XPS and FTIR surface analytical techniques were used to characterize the composite coatings before and after protein adsorption.

RESULTS: Electrochemical oxidation under optimised conditions results in the formation of TiO_2 nanotubes (hollow cylinders) arranged perpendicular to the substrate and separated from each other. The sputter-deposited Ag forms nanoparticles, with diameter varing of these particles varies from 5 to 20 nm. They are located at the top edges of the nanotubes, and on their side walls, see Fig. 1. Ag-incorporated TiO_2 nanotubes significantly stimulate apatite deposition from Hanks' solution as compared to pure Ti covered with a native oxide layer. It was revealed that the Ag/Ca-P/TiO_2 NT/Ti surface adsorbs a higher amount of protein (BSA) for a geometric surface area than does the Ti surface.

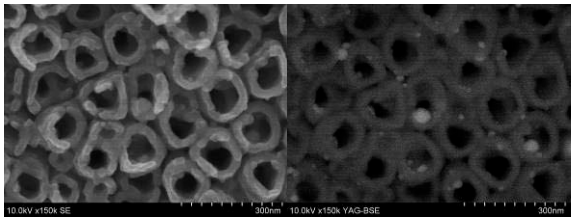


Fig. 1: SEM (SE) images of TiO_2 nanotubes covered with Ag (0.01 mg/cm^2) and immersed in Hanks' solution for 6 h (left); BSE image showing the distribution of Ag particles over the surface (right).

DISCUSSION & CONCLUSIONS: In this work, the Ag/Ca-P/TiO_2 nanotubes composite coatings were deposited on titanium substrate. Silver nanoparticles are distributed homogeneously in the coating, which is beneficial to maintain a steady antibacterial effect. High protein concentration on the Ca-P surface could enhance cell adhesion, which is favorable for achieving biocompatibility. The results imply that the Ag/Ca-P/TiO_2 nanotubes composite coatings may impart good biocompatibility and antibacterial properties, which makes them promising to be applied in hard tissue replacement against postoperative infection.

REFERENCES: [1] Rai et al,. Biotechnol Adv, 2009 Jan;27(1):76-83.[2] Paital et al., Mater Sci Eng R 2009 Aug;66(1-3):1-70.

ACKNOWLEDGEMENTS: This work was financially supported by the Polish Ministry of Science and Higher Education (grants No. IP2010 035070, N N507 355035).

Anti-infective coatings based on dispersed TiO_2 nanoparticles in polymer matrices

H. Rothe[1], D. Glöß[2], P. Frach[2], K. Rose[3], J. Kron[3], K. Liefeith[1]

[1]*Institute for Bioprocessing and Analytical Measurement Techniques, Heilbad Heiligenstadt, Germany;* [2]*Fraunhofer Institute for Electron Beam and Plasma Technology FEP, Dresden, Germany;* [3]*Fraunhofer Institut für Silicatforschung ISC, Würzburg, Germany*

INTRODUCTION: Biomaterial associated infections cause an extension of hospitalisation of 13-24 days and thus a cost overrun of up to 14.000 €/day. Infections of endoprosthesis involve additional costs of 55.000 € per infection [1]. Therefore a prevention of nosocomial infections is not only an intention in a medical point of view but also an economic factor. The aim of this work was the generation of polymer based coatings, containing photocatalytic titanium oxide nanoparticles, wich show a significant reduction of microbial colonisation.

METHODS: a) Preparation of coatings: The coatings were prepared on glass substrates using the dip coating technique. Special Ormocers® containing 20%$_{w/w}$ of TiO_2 nanoparticles were applied and subsequent hardened by plasma treatment. Pure TiO_2 coatings, deposited with magnetron sputtering were utilised as the active reference. Uncoated glass substrates were used as inactive reference.
b) Surface characterisation: Prior to biological testing, the surface free energy components (acid-, base-, v.d.Waals) were calculated using contact angle measurements. To exclude that topographical distinctions may influence the adhesion of microorganism atomic force microscopy measurements were performed and roughness parameter were calculated.
c) Efficacy testing: The photocatalytic activity was observed via degradation measurements of methylene blue. The samples were prepared in a fluidic chamber and overflowed with a 10mM methylene blue solution for 24h.
During the incubation one sample of each coating was exposed to UV-A irradiation (365nm, 1mW/cm²) while a reference was kept dark. The degradation of methylene blue was measured photometric. According to this experimental setup bacterial adhesion tests were performed. Samples were overflowed by a mixed bacterial suspension of *Staphylococcus aureus* and *Staphylococcus epidermidis* with a final concentration of $1*10^8$ bacteria/ml for 48h. Analysis of bacterial colonisation was performed using vital staining (BacLight™) and confocal laser scanning microscopy.

RESULTS: Figure 1 illustrates representative images from the bacterial tests. A reduction of adhered bacteria due to the illumination with UV-A is clearly detectable. Reference samples without any photocatalytic activity did not show any significant effect. The reduction was about 83,0% for Ak_Qe coatings and 76,6% for G_QmTi coatings, pure TiO_2 surfaces showed a reduction of 93%. A significant degradation of methylene blue was observable at all tested coatings but not at the uncoated reference.

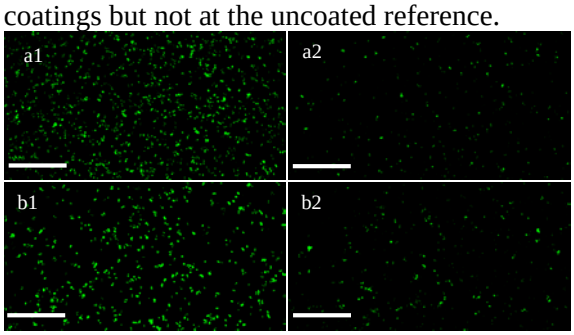


Fig. 1.: Photocatalytic effect to the microbial colonisation; a - Ak_Qe +20%$_{w/w}$ TiO_2; b – G_QmTi + 20%$_{w/w}$ TiO_2; left images are without irradiation, right images with UV-A exposure. Scale bars are 30 µm.

DISCUSSION & CONCLUSIONS:
Coatings based on dispersed TiO_2 nanoparticles in special Ormocer® matrices show a high potential for anti-infective surfaces. Reductions of around 80% in bacterial colonisation could be observed. Due to the polymer matrix and tuneable plasma treatment conditions the coatings can be adapted to a wide range of substrates and requirements.

REFERENCES: [1]Hoffmann, G.O. 2004, Infektionen der Knochen und Gelenke, Elsevier

ACKNOWLEDGEMENTS: The authors would like to thank the BMBF (FK 13N9311 - 13N9319) for financial support.

Biodegradable Nano Apatites for Improved Delivery of Antibiotics

T. S. Sampath Kumar[1]

[1]*Medical Materials Laboratory, Department of Metallurgical and Materials Engineering, Indian Institute of Technology Madras, Chennai 600036, India*

INTRODUCTION: Nanocrystalline calcium-deficient hydroxyapatites (CDHA) are ideal bone substitutes due to the similarity in size and composition with the mineral component of hard tissues. Bioresorbable CDHA nanoparticle based local delivery system with desired sustained drug release profiles seems to be an efficient way for the treatment of many bacterial bone infections such as periodontitits [1], osteomylitis, etc. Doxycycline, a long acting form of tetracycline, has both antibiotic properties and ability to block the action of colleagenase enzyme. Cephalexin is a first-generation cephalosporin antibiotic widely used in localized bone and joint infections. In the present study, enhanced and extended delivery of doxycycline and cephalexin by CDHA nanocarriers have been evaluated.

METHODS: The CDHA nanocarriers with different Ca/P ratio were synthesized through a microwave accelerated wet chemical reaction. The amount of reactants was calculated based on the Ca/P molar ratio of 1.56, 1.61 and 1.64 with the compositions to form CDHAs covering the properties from stable hydroxyapatite phase to degradable tri-calcium phosphate phase and the samples have been coded as CD156, CD161 and CD164 respectively. The ceramic nanocarriers were well characterized by physio-chemical and microscopic techniques. The drug loading and release profiles on CDHA nanocarriers were evaluated with an ultraviolet visible spectrometer. The effect of pH on the encapsulation of drug into nanocarriers was initially carried out in phosphate buffer to determine the suitable pH for loading maximum amount of the drug. Drugs loading as well as the release profiles were obtained by measuring the absorbance values of the drug.

RESULTS: The amount of doxycycline uptake by CDHAs with varying pH showed a maximum amount at the physiological pH of 7.4. The drug loading seems to depend on its surface area values than the Ca/P ratio for the different CDHAs. However, the amount of drug loading was greater in CD161 (82%) than on CD156 (66%) and CD164 (72%).

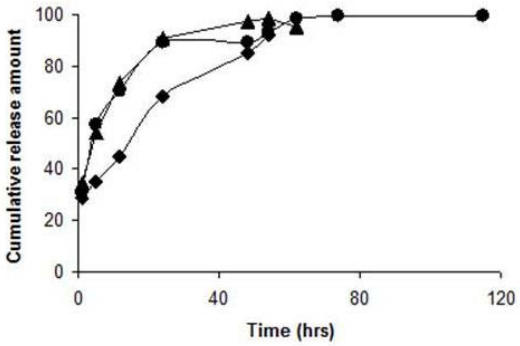


Figure.1. In-vitro release profiles of doxycycline loaded CD161 (●), CD156 (♦) and CD164 (▲) nanocarriers

The nanocarriers exhibit both single and two stage release profiles of doxycycline depending on the Ca/P ratio of CDHA as shown in Fig.1. The initial burst release is due to desorption of doxycycline from the CDHA surface, followed by a slow release as a result of the dissolution of the doxycycline-CDHA complex. The CD161 with Ca/P ratio similar to bone releases 80% doxycycline in 112 h. But, the loading and release profile of cephalexin using CD161 exhibits around 46% and 16% respectively at physiological conditions. However, an interaction between cefalexin and CDHA was also observed

DISCUSSION & CONCLUSIONS: The antibiotic release by CDHA nanocarriers seems to have a stronger dependence on the Ca/P ratio and can be tailored for sustained release from 3 to 5 days for effective treatment of bacterial bone infections.

REFERENCES: [1]Sunita et al., J Biomed Nanotech. 2008, 4, 1-8.

ACKNOWLEDGEMENTS: This work was supported by Department of Biotechnology, Government of India.

Using Premanufactured Solid β-TCP Scaffolds for Clindamicin Release

M.Seidenstuecker[1], Y. Mrestani[2], R.Neubert[2], A.Bernstein[1], H.O.Mayr[1]

[1]*Department of Orthopaedics and Trauma Surgery, University of Freiburg Medical Center, Freiburg, D,* [2]*Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther-University, Halle, D.*

INTRODUCTION: Polymethyl methacrylate (PMMA) bone cements are frequently used to eliminate osteochondral defects. In order to prevent infections and associated inflammations of the bone tissue, the PMMA cements are combined with antibiotics, notably Gentamicin sulphate (GS), Vancomycin (VAN) or Clindamicin (CLI). Because PMMA prevents external diffusion, the drug is released only from the material's surface. Moreover, PMMA is not biodegradable. Beta-tricalcium phosphates (β-TCP) has proved as suitable material concerning mechanically solidity and biodegradability [1]. Scaffolds made of this material show a progressive resorption and a replacement by bone [2]. The objective of this study was using industrially prefabricated β-TCP scaffolds with a porosity of 40%, interconnected micro cavities and a 5 µm median pore diameter and coat them with antibiotics.

METHODS: Two methods to prepare the scaffolds were used: the drop-coating and the dip-coating method with 150 mg mL^{-1} CLI - solution. After drying for 72 h at 37°C, 2 mL pure water was added to the CLI loaded scaffolds, followed by incubation at 37°C. All tests were realized at a pH-value of 7.4. After 1, 2, 3, 6, 9, 12 and 14 days, the liquid was completely extant and replaced with 2ml pure water. The obtained solutions were stored at -4°C for the examinations using dedicated Capillary Zone Electrophoresis (CZE) method [3] and the agar diffusion method.

RESULTS: The drop-coated scaffolds showed smaller CLI release during the first 24 h because of the smaller quantity of CLI available: 22.3 ± 2.84 mg was transferred to the scaffolds and 20.2 ± 2.9 mg was released within 24 h. In contrast, the dip-coated scaffolds were loaded with 23.5 ± 2.9 mg CLI and released 14.3 ± 0.8 mg CLI within 24 h. In both cases CLI was released rapidly: 90.4 ± 1.5% within 24 h. CLI release was completed after 6 days. All samples taken at later times had a CLI concentration below the detection limit of 4 µg ml^{-1}

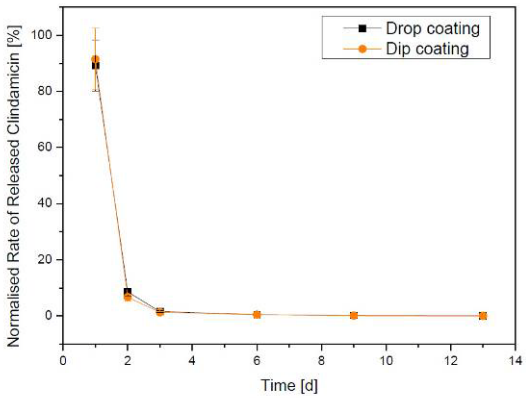


Fig. 1: Release kinetics of CLI coated β-TCP scaffold (N=10) .

DISCUSSION & CONCLUSIONS: The ratio of coated and released VAN was in all cases the same with 90.4 ± 1.5 % within 24 hours and a complete release after 6 days. Similar experiments described in further literature using GS or VAN [4] confirms theses results. The released amount of CLI on the first three days was characterized by an antimicrobial activity using *Staphylococcus aureus* ATCC 25923. The specimens of the following days show amounts of CLI below the detection limit and below the MIC. Thereby the coating of β-TCP scaffolds using an aqueous CLI solution allows a temporary release of CLI from the scaffolds. Further optimization of the drug release system is necessary in order to prolong significantly the release of antibiotics from the scaffolds.

REFERENCES: [1] A. Bernstein, et al., J Biomed Mater Res B Appl Biomater **84** (2), 452-462 (2008).[2] H. O. Mayr, et al., Arthroscopy **25** (9), 996-1005 (2009).[3] M. Seidenstuecker, Diploma thesis, Martin Luther University, 2010. [4] M. V. Cabanas, et al., Eur J Pharm Sci **37** (3-4), 249-256 (2009).

Sequential release kinetics of two substances from one-component polymeric coating on implants

[1]Strobel, C; [2]Bormann, N; [1]Kadow-Romacker, A; [3]Schmidmaier, G; [1,2]Wildemann, B

[1]Julius Wolff Institut, [2]Berlin Brandenburg Center for Regenerative Therapies, Charité - Universitätsmedizin Berlin,
[3]Department Orthopädie, Unfallchirurgie und Paraplegiologie, Universitätsklinikum Heidelberg

Introduction: Due to the disturbed vascularisation systemically applied substances might not reach the fracture side in an effective concentration. The use of scaffolds for drug delivery is possible in situations with larger bone defects, but the application of foreign material should be reduced to the minimum. Therefore, the present study aimed in the improvement of a local drug delivery system based on an implant coating. For early infection prophylaxis an immediate antibiotic release should occur followed by a more sustained release of an osteoinductive growth factor to stimulate bone healing.

Materials and Methods: As a carrier system Poly(D,L-lactide) (PDLLA) was chosen to coat titanium Kirschner-wires by a dipping technique. The release kinetics of incorporated factors was modified by changing the ratio of the PDLLA/Solvent/Drug (Tab. 1, Fig. 1). Ethyl acetate (EA) was used as solvent.

Tab. 1: Coating design: ratios of PDLLA, solvent and drug

	PDLLA/EA	Substance
Layer 1	300mg/1.5ml	3% w/w BMP-2
Layer 2	100mg/1.5ml	10% w/w gentamicin

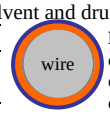


Fig. 1: 2-Layer coating scheme on the k-wire cross section

For control, single coatings as well as a substance free PDLLA were also analyzed.

The coatings were analysed by SEM.
Released BMP-2 was quantified by enzyme-linked immunosorbent assay and gentamicin by cloned enzyme donor immunoassay. In addition, the activity of the released growth factor and gentamicin was investigated *in vitro*. BMP-2 coated wires were placed via transwells into 24-well-plates seeded with the myoblast cell line C2C12 (culture medium: DMEM+1%FCS+1% Pen/Strep). After 3 days cell activity was measured with Alamar blue and BMP-2 induced osteogenic differentiation with Alkaline Phosphatase assay (ALP). Subsequently the transwells with the wires were placed onto freshly seeded cells and the procedure repeated weekly for 8 weeks in total. To detect gentamicin activity, the elution samples were applied on discs, which were placed on Mueller-Hinton-agar spiked with Staphyloccocus aureus. Agar plates were incubated over night and the zones of inhibition were documented.

Results: SEM pictures show a smooth homogenous coating before elution (Fig. 2a), whereas after elution all coatings were swollen and porous (Fig. 2b).

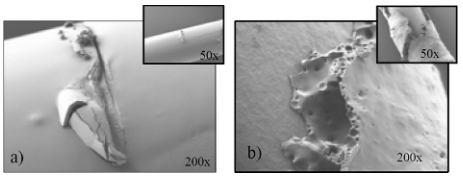


Fig. 2 SEM, artificial damage of the coating a) before elution b) after 10 weeks of cell culture

The release kinetics revealed an initial burst release of gentamicin and a slower and more sustained release of BMP-2 from the coated wires (Fig. 3). The different release kinetic is reflected by the *in vitro* activity of the two factors. Gentamicin showed an immediate inhibitory activity (Fig. 4) whereas BMP-2 stimulated the ALP-activity of C2C12 cells with a maximum after 2 weeks (Fig. 5).

Discussion:
The present study showed the realizations of different release kinetics from one simple polymer coating of implants by only changing the Polymer/Solvent/Drug ratio.
The initial release of gentamicin will prevent the colonisation of bacteria on the implant. The delayed release of BMP-2 might be beneficial to induce bone formation as shown in animal models, where the initial burst BMP release was less effective [1-3]. The next step is to use this combined coated wires to treat rats with an open osteotomy showing delayed healing [4]. This animal model shows an infection rate comparable to the clinical situation and is therefore suitable to prove the efficacy of this double coating.

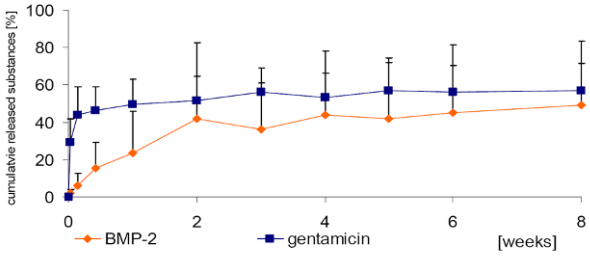


Fig. 3 Release kinetics of gentamicin and BMP-2 combined coated on one wire (n=6 per group)

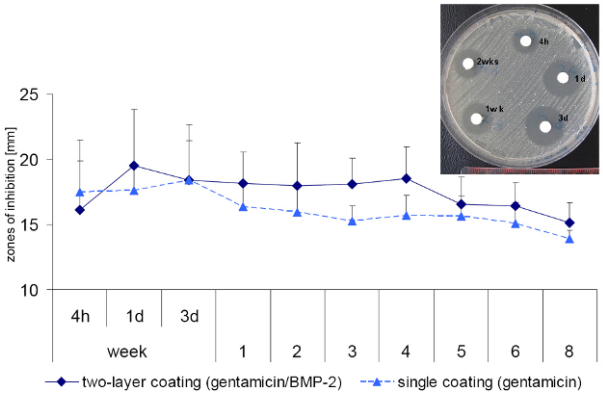


Fig. 4 Zone if Inhibition (n=6 per group)

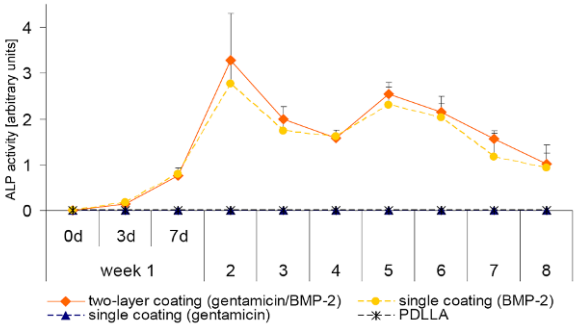


Fig. 5 ALP activity of C2C12 cells (n=6 per group). The ALP activity measured in optical density [OD] was normalized to 10^4 cells [arbitrary units]

References:
1. Betz OB, et al. Gene Ther. 2007, 14(13):1039-44;
2. Kempen DH, et al. Biomaterials. 2008, 29(22):3245-52;
3. Wu G. et al. Biomaterials. 2010, 31(29):7485-93;
4. Kratzel C, et al. BMC Musculoskelet Disord. 2008, 8;9:135.

Acknowledgement: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, SFB 760)

Polylactide : Antimicrobial modifications

Marina TURALIJA[1], Sabyasachi GAAN[2], Sandra BISCHOF VUKUŠIĆ[1]

[1]University of Zagreb Faculty of Textile Technology, Department of Textile Chemistry & Ecology, Zagreb, Croatia [2]Laboratory of Advanced Fibers, EMPA Swiss Federal Laboratories for Materials science and technology, St Gallen, Switzerland

INTRODUCTION: Biocomposite materials are among the most promising material for the production of environmentally-friendly biodegradable packaging materials in food and health application. In this research we have carried out bulk modification of PLA to achieve antimicrobial functionality. Antimicrobial PLA would prevent the formation of biofilm and improve its utility especially in biomedical and food packaging applications.

METHODS: Films of PLA (20-60 microns) were prepared from solutions of dichloromethane (25%) using film making instrument: Coatmaster 509 MC-I, supplied by Erichsen Testing GmbH and Co. Testing Equipment. Bulk modification of PLA was done with addition of Sanitized®BC A 21-61. Sanitized®BC A 21-61 is an antimicrobial additive based on a nature active ingredient in form of silver encapsulated in a patented glass ceramic material. Furthermore, glycerol (GA) or polyethylene glycol (PEG) were used as additives, to achieve hydrophilic PLA surfaces and facilitate contact with bacteria. Antimicrobial activity of PLA films were measured against Gram-positive bacteria *Staphylococcus aureus* (ATCC6538) and Gram-negative bacteria *Escherichia coli* (ATCC 8739), using a standard test[1] ISO 22196 : 2007, based on the viable cell count method.

RESULTS: Addition of glycerol (GA) and polyethylene glycol (PEG) significantly reduced the contact angles of modified PLA films from 70° for unmodified PLA to 40° for modified PLA. Antimicrobial agents are those that kill or inhibit the growth of micro-organisms. To effectively inhibit microorganism growth, the antimicrobial agent must interrupt the growth cycle. The two tested bacteria applied in the study showed different sensitivity to the bulk modified PLA films: *Staphylococcus aureus* was sensitive with PLA/GA/0.6SBCA films > 4 log reduction *(Fig.1)* whereas *Escherichia coli* was very sensitive with PLA/PEG/0.6SBCA and PLA/GA/0.6SBCA films > 5 log reduction *(Fig.2).*

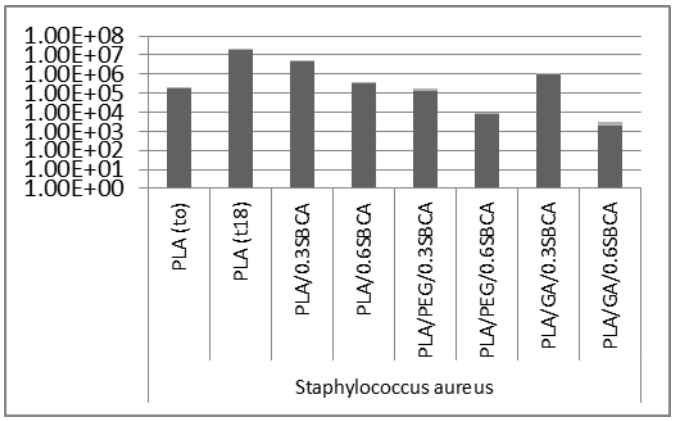


Fig. 1: Antimicrobial activity of PLA materials with bulk modification

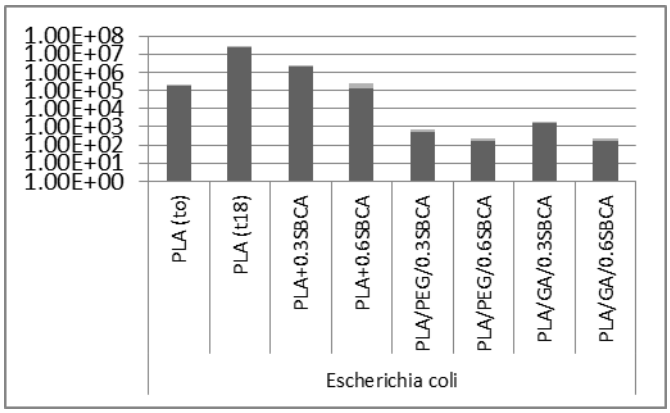


Fig. 2: Antimicrobial activity of PLA materials with bulk

DISCUSSION & CONCLUSIONS: Silver (Ag) is an efficacious and useful antibacterial agent. Small amounts of silver already show antibacterial properties. The bulk modified PLA films with hydrophilic additives were particularly efficient against *Staphylococcus aureus and Escherichia coli* which are opportunistic pathogens and these bulk modified PLA films might find future application in biomedical and food packaging areas.

REFERENCES: *[1]Gupta,B.; Revagade,N.; Hilborn, J.: Poly(lactic acid) fiber: An overview, Prog. Polymer Sci., **32** (2007), pp. 455-482*

[2]ISO 22196:2007, Measurement of antibacterial activity on plastics surfaces

Bioactive and Antibacterial PMMA-based Bone Cement

E. Vernè[1,2], M. Miola[1,2], G.Fucale[3], G.Maina[4]

[1]Politecnico di Torino, DISMIC, ITALY, [2]BionicaTech s.r.l. Torino, ITALY, [3]CTO A.O. Maria Adelaide, Chemical, Clinical and Microbiological Analyses Department, Turin, Italy, [4]University of Turin, Traumatology Orthopaedics and Occupational Medicine Department, CTO, Turin, Italy

INTRODUCTION: PMMA-base bone cement is golden standard for the anchoring of implants or as bone substitute since 1945. However PMMA bone cement do not promote tissue integration and most of all it has elevated risk of infection when implanted into the human body. In order to avoid this problem, antibiotics-loaded bone cements are widely used, but their effectiveness is controversial. In this study innovative bioactive and antibacterial bone cement have been carried out by incorporating a silver containing bioactive glass in the PMMA matrix [1]. Silver ions, well known antimicrobial agents, are introduced in the glass by means of a patented procedure [2].

METHODS: a glass belonging to the system SiO_2-P_2O_5-CaO-Na_2O-B_2O_3-$Al_2O_3$was prepared through melting and quenching technique; glass was milled and sieved to obtain powders $\leq$ 20μm. Powders were subjected to a ion-exchange process in a silver aqueous solution to incorporate Ag ions in the glass structure. Process parameters were optimized to obtain a controlled Ag concentration and release. A maximum amount of 30%wt of Ag-doped glass was added to commercial bone cements (Palacos®, Simplex P®) with different viscosity. Composite cements were analysed by means SEM-EDS techniques, *in vitro* reactivity in SBF solution and mechanical test. Ag ions release tests in the SBF solution were carried out using a GFAAS, in order to verify the amount and kinetics of silver ions release. To evaluate the antibacterial behaviour two tests (in accordance to NCCLS standards [3,4]) on *S. aureus* were performed: the count of colonies forming units (CFU) and the evaluation of inhibition zone. Biocompatibility MTT test (EN ISO 109935: 2009) have been performed on each kind of composite cement.

RESULTS: samples characterizations demonstrate that glass powders are well incorporated in the polymer matrix. Bioactivity test reveals the hydroxyapatite precipitation on sample surface after 7 days. Mechanical test demonstrate a good compression strength of composite cements. Silver ions are gradually release in the solution: the higher amount of silver was released during the first days of immersion, the most critical for infections development, while a subsequent slowly Ag release can be important in case of latent infections. The composite cements created a significant inhibition halo and they are able to limit both the adhesion and proliferation around the samples of bacterial colonies. Moreover, the silver doped cements did not reveal any toxic behavior.

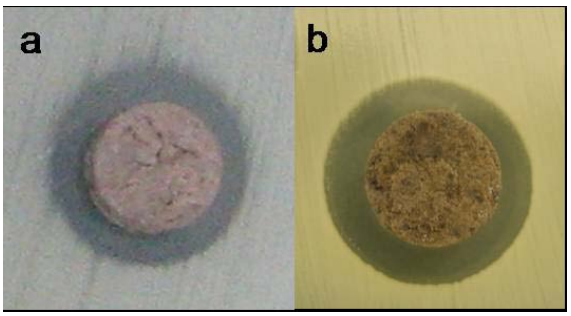


Fig. 1: Inhibition halo of bioactive and antibacterial PMMA-based bone cement low (a) and high (b) viscosity.

DISCUSSION & CONCLUSIONS: The realized composite cements were characterized by good homogenization, mechanical and bioactive properties. Silver was released in SBF in critical time for the development of infections. Biological tests demonstrate the good antibacterial effect of the PMMA-based composites towards *S. aureus* stock.

REFERENCES: [1]Verné E et al., patent PCT/,IB2010/053181. [2]Vernè et al., N.EURO-PCT 05823528.4. [3]NCCLS M2-A9. [4]NCCLS M7-A6

ACKNOWLEDGEMENTS: The authors would like to acknowledge Nobil Bio ricerche s.r.l for the *in vitro* test.

Morphological Effects of Quaternary Ammonium Compounds on Staphylococci

A.Zerdoum[1], M.Crismaru[2], J.A. Loontjens,[2] H.C.van der Mei[2], H.J.Busscher[2], M. Libera[1]
[1] Dept. of Chemical Engineering & Materials Science, Stevens Inst. of Tech., Hoboken, NJ, U.S.
[2] Dept. of BioMedical Engineering, University Medical Center Groningen, Groningen, NL

INTRODUCTION: A wide range of antimicrobial agents are being explored to control bacterial colonization of surfaces and biofilm formation. Among these are quaternary ammonium compounds (QACs). We are interested in exploring the mechanism of action that QACs have on killing staphylococcal bacteria commonly implicated in biomaterials-associated infection. Here we use take advantage of recent developments in scanning electron microscopy (SEM) to image *S. epidermidis* before and after exposure to a particular QAC. These images indicate that the QACs disrupt the cell wall.

METHODS: Staphylococcal biofilms were grown in 35 x 10 mm non-coated bacterial suspension dishes for 24 hrs. The biofilms were then washed with phosphate buffer. An amount corresponding to the miminum (planktonic) bactericidal concentration (1xMBC) of Ethoquad ® C/25 (Cocoalkylmethyl [polyoxyethylene (15)] ammonium chloride) QAC (150 µg/mL) was then added to the culture medium and it was incubated for 12 hrs. The biofilms were then fixed using 2% glutaraldehyde and stained with OsO_4.

Leaving each biofilm in its original polystyrene culture well, the fixed and stained biofilms were embedded in epoxy using standard electron-microscopy protocols. A Leica Ultracut UCT microtome was then used to uniformly expose the biofilm/polystyrene interface. SEM imaging and focused-ion-beam (FIB) machining were performed using a Zeiss Auriga FIB-SEM using 1.90 keV electrons and an in-lens backscattered detector (EsB). FIB milling was done using 30 keV Ga ions at a current of 600 pA.

RESULTS: Figure 1 shows typical results. A considerable morphological difference was noticed between the control and QAC-treated biofilms. The cell walls of the treated bacteria are lysed in several cases. 3-D images constructed by rendering FIB serial sections of 400 images sliced at 20 nm intervals indicate that such morphology is common in the QAC-exposed cells and absent in the untreated control specimens. Work is now ongoing to explore such specimens in the unfixed, unstained, frozen-hydrated state using cryo-SEM techniques.

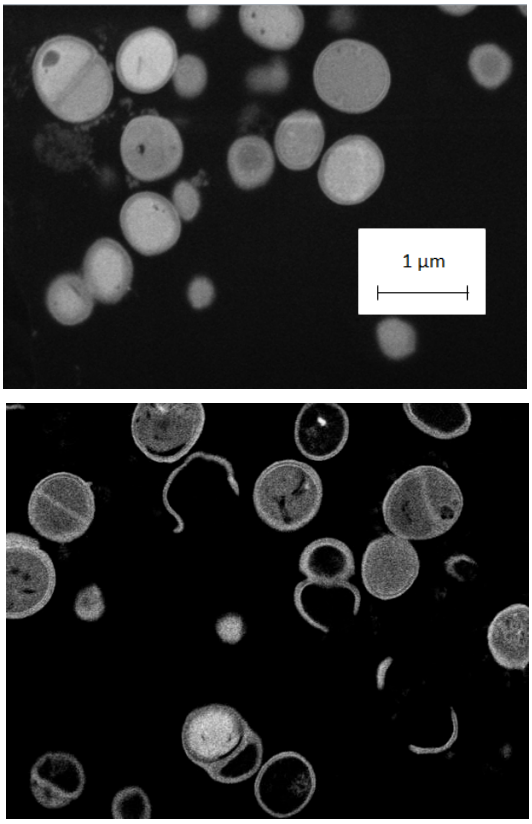


Fig. 1: Backscattered SEM Images of *S. epidermidis* biofilms with (lower image) and without (upper image) exposure to 1xMBC of QAC during growth.

ACKNOWLEDGEMENTS: This project is supported by U.S. National Science Foundation grant #CBET-0708379 and Dutch Technology Foundation grant STW grant# GPC 7844.

Evaluation of cytotoxicity in vitro of biodegradable polylactide fibers with spin finishes

B. Żywicka[1], E. Zaczyńska[2], A. Czarny[2], K. Twarowska-Schmidt[3] (bzywicka@heksp.am.wroc.pl)

[1]Medical University, Wrocław, Poland, [2]Institute of Immunology and Experimental Therapy, Wrocław, Poland, [3] Institute of Biopolymers and Chemical Fibres, Lodz, Poland

INTRODUCTION: Biodegradable fibers with controlled properties may meet the requirements for medical applications. Poly(lactic acid) (PLA) is a biodegradable linear aliphatic thermoplastic polyester. There were prepared five type of PLA fiber with five type spin finishes. PLA fibers were assayed for in vitro cytotoxic activities.

METHODS: The PLA fibres were prepared by a two-step melt-spinning process. The PLA Polymer 6201D, fiber grade polymer with nominal MFI=15-30 g/10min, a NatureWorks LLC product was used. During spinning PLA fibers were coated with 5 types of spin finishes:

PLA sample	Spin finish	
	type	oil pick up %
24	Glicerol Ph Eur (Pharma Cosmetic)	2,4
25	Lurol PL 801 (Goulston Techn)	0,40
26	Stantex 6457 (Pulcrachem)	0,61
27	Lurol PT-L216 (Goulston Techn)	0,36
28	**Estesol PF 790 (Bozzetto GmbH.)**	**0.62**

After drawing and cutting process the fibres with linear density 2,2 – 4,8 dtex, tenacity 35 – 39 cN/tex, elongation ~50 % were obtained.

METHODS: To determine whether biodegradable fibers with spin finishes can affect cells, line cultures L929 (ATCC CCL1) was used for the cytotoxicity study in vitro. The mouse fibroblast-like cell line was maintained in Eagle's medium. The cells were seeded in the tubes, 1 ml of $2x10^6$ cells/ml in the culture medium Eagle'a and were incubated with fibres for 24h, 48h and 72h at 37°C in the atm. of 5% CO_2 in air. Cell growth, morphology and viability (Trypan Blue Staining) were determined.

RESULTS:

Table 2. Cytotoxicity activity of PLA fibers on L929 cells after 72hours, in vitro

Material	Mean cell count		level of toxic.
	Alive %	Dead %	
PLA 24	62	38	2
PLA 25	0	100	3
PLA 26	0	100	3
PLA 27	0	100	3
PLA 28	99	1	**0**
Fenol	6	94	3
L929	97	3	**0**

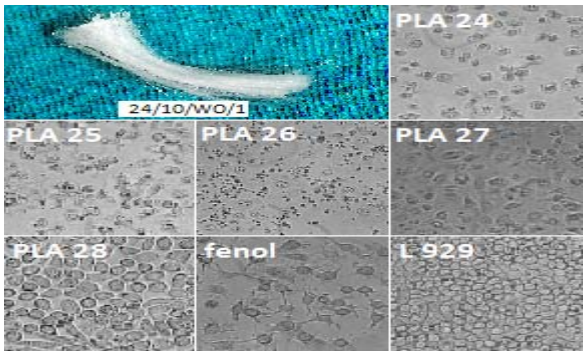


Fig.1. Macroscopic view one of PLA fibres and Morphology of L 929 cell line culture after contact with PLA fibers

DISCUSSION & CONCLUSIONS:

Fibroblast cultures after contact with four of five tested PLA fibres with lubricants showed cytotoxity effects. The cells had been dead or had changed morphologies and had showed lower proliferation. The result of the testing of PLA fibers with spin finish with Estesol did not show any cytotoxicity effects and may be promising candidate for medical applications.

Financial support by the project "Biodegradable fibrous products", POIG.01.03.01-00-007-/08-00 and EU in the frame of IE OP financed from the ERDF, is gratefully acknowledged.