Residual stress evaluation at bone implant interface using high energy x-ray diffraction

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INTRODUCTION: We developed a nano-structured hydroxyapatite (nHA) coating by spraying nanosized HA powder on titanium substrate. This nHA coating could accelerate the bone remodelling process. Being able to evaluate stress at this bone implant interface is of great interest because the loading on the implant influences the crystallites orientation. In this work the effect of stress on bone remodelling will be investigated based on high energy X-ray diffraction.

METHODS: A nano-hydroxyapatite double side coated 20x11x2mm Ti-4Al-6V plate was implanted in a sheep's tibia. The sacrifice occurred three months after implantation.

The experiment was carried out in ID15A at the ESRF (European Synchrotron Radiation Facility) using a "white beam" combined with energy dispersive diffraction.

The incident beam size is 0.1x0.1mm. The slit opening in the scattering plane is set to 0.1mm and limited by the detector window (8mm) in the perpendicular plane. We choose a fixed diffraction angle of 2θ=5º. We choose to put the bone-implant interface horizontally to get two diffraction directions x and y in the interface plane with a simple rotation around the z-axis.

We measured two diffraction directions and then we turned the sample by 90 degrees counter clockwise around the z-axis. In both configurations we had 20 measurement points symmetrically dispatched from 0 to 1 millimeter from the interfaces. We have chosen a Rietveld refinement which uses a least squares approach to refine a theoretical line profile until it matches the measured profile.

RESULTS: The calculated stresses are represented in figure 1 and figure 2. The outliers have been removed to show a coherent view of the results

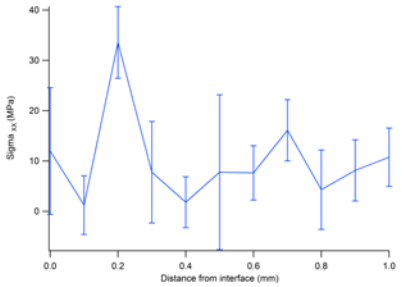

Fig. 1: Calculated stresses on the x-axis

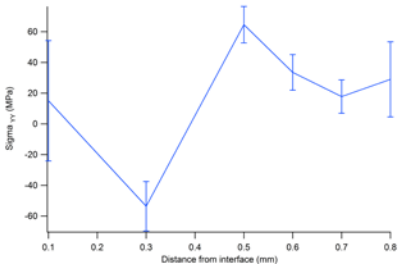

Fig. 2: Calculated stresses on the x-axisbenma

DISCUSSION: We determined that stress remains weak and vary moderately. The stress gradient must be close to the interface and the chosen spatial resolution does not allow us to observe this evolution. We note that σyy shows a stronger variation than σxx, and have much more outliers. This may be due to internal artifacts of bone,which is an inhomogeneous material.

CONCLUSION: Stress evaluation at bone implant interface using high energy X-ray diffraction appears to be an interesting method to evaluate the quality of bone regeneration if we are able to use lower size gauge. It will authorize us to compare different materials or coatings in term of bone maturation. All these measurements are realized in bulk materials, which avoid stress artifact due to cross section of samples.

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Mini-screws anchorage and mechanical stress

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INTRODUCTION: The mini-screws are a possible solution to anchoring, which is indispensable to the implementation of tooth movement during an orthodontic treatment. Stress distribution has already been studied by several authors.[1,2] The aim of our study was to analyze and compare stresses in 4 different mini-screws.

MATERIALS AND METHODS:

- 2 titanium mini-screws Medicon Aarhus Anchorage®, self drilling (ref 68.75.88 and 68.75.90)

- 2 Ti6Al4V mini-screws Dentos AbsoAnchor®, self drilling (ref SH 14-10 and SH 1514-08)

Three steps were followed: measurement, modeling and simulation. The exact size of each screw was determined using a 3D measuring machine (CEA Moronvilliers). The numerical model of each mini-screw was designed using CAD (Computer Aided Design) Rhinoceros® software (IFTS Charleville-Mezieres).

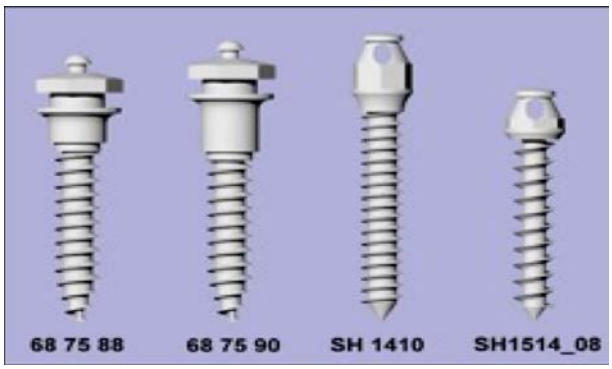

Figure 1: CAD design of the 4 mini-screws

The loading of each part was simulated using Nastran software®. We chose to apply to the head of each mini-screw a load of 3N perpendicular to the axis of the mini-screw. Meshing was conducted and calculations were performed using finite element analysis.

RESULTS: The results allow us to observe the Von Mises stresses. Stress distribution is assessed through an iso-color scale, with stress increasing from bottom to the top of the scale.

The results show a similar distribution of stresses, the latter being concentrated in the upper part of the thread.

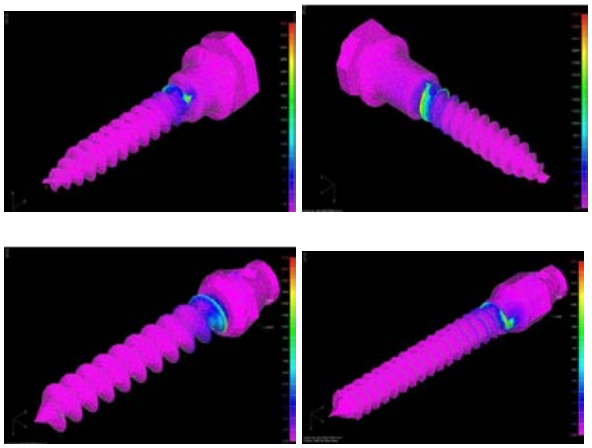

Figure 2: Stress concentration at the top of the thread after loading simulation

DISCUSSION AND CONCLUSION: The numerical simulation allows to test multiple configurations. The mechanical model used in Nastran ® considers blocking the threaded portion in 3 space directions. This simplification excludes interface between the component and its support. Our future model must incorporate the existence of this interface, as well as the properties of the surrounding bone and possible extrusion of the screw.

REFERENCES: [1] B Melsen (2006) *Indications d'ancrage squelettique en orthodontie* Rev orthop dento-fac; **40**; 41-61 [2] MMOTOYOSHI et al. (2005) *Biomechanical effect of abutment on stability of orthodontic implant, a finite element analysis* Clin oral implants res **16** (4); 480-485.

Can orthodontic adhesives alter the enamel substrate?

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INTRODUCTION: The aim of this study is to assess the changes in the enamel surface regarding chemical composition and wettability after orthodontic bonding/debonding procedures.

METHODS: 15 samples (3rd molar) underwent stages of etching, bonding of brackets with an orthodontic adhesive, followed by 3500 cycles of thermocycling, debonding of the brackets and finishing. The wettability of enamel is determined by measuring the dynamic contact angle (Digidrop GBX, France). The contact angle of a drop of water deposited on the enamel is measured for 180 s with an interval of 4 s. The wettability of the enamel surface was measured before and after stages of bonding / debonding, thermocycling and finishing.

Statistical analysis: ANOVA and the Student t test were used. Given the variability of samples (enamel), each sample was taken for his own witness.

Fourier transformed infrared spectroscopy (Mattson Genesis Thermoelectron France, Courtaboeuf, France) provided the infrared reflection spectra of the intact enamel powder, enamel after bonding / debonding from 2 samples (a superficial and the other more in depth) and the spectrum of orthodontic adhesive.

RESULTATS: Of 15 samples, one sample shows a detachment at the interface of enamel / adhesive, 5 samples at the interface adhesive / bracket, and 8 samples showed a separation both at the interfaces adhesive / bracket and level enamel / adhesive.

The contact angle measured at 180 s varies for samples before bonding of 44,1° and 77,9° (the gap is 33.8°). For samples after bonding / peeling 49.6° to 74.1° (the gap is 24,5) (Fig1).

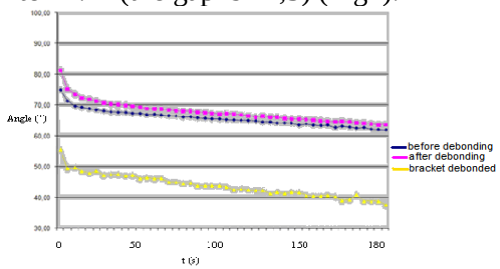

Fig1. Average values of the contact angle of water droplet deposited on the enamel over time (s).

FT-IR: On the spectrum (Fig3) of the enamel surface carbonate peaks at 871 and 1400 cm-1 have a very low intensity compared to the peaks of the carbonate untreated enamel (Fig2). This is not the case for the spectrum of deeper enamel. After bonding / debonding and finishing (Fig3), a "polymer" identified peak at 773 cm-1 was found in addition to the enamel peaks.

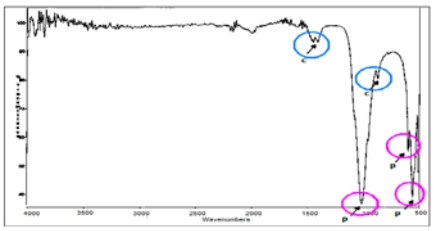

Fig2. FTIR spectrum of intact enamel with carbonate peaks (c) 871 and 1400 cm^{-1} and phosphate peaks (p) 552, 597 and 1000 cm^{-1}.

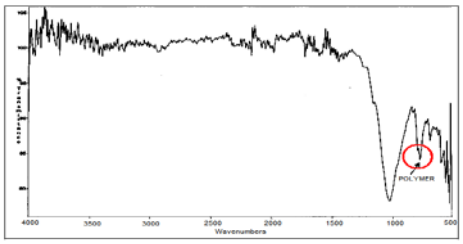

Fig3. FTIR spectrum of the enamel (from the superficial level) after bonding/debonding and finishes. Presence of the polymer peak at 773 cm^{-1}.

DISCUSSION – CONCLUSIONS: During the stages of enamel bonding / debonding of orthodontic brackets, wettability did not change significantly, but the surfaces were more homogeneous (decreased standard deviation). Infrared spectroscopy showed that etching demineralized enamel surfaces (loss of carbonates) and that the standard procedures for separation and finishing did not always eliminate the resin infiltrated enamel.

REFERENCES: [1]A HYO-BEOM et al. (2009) *Analysis of surface roughness and surface free energy characteristics of various orthodontic materials* American Journal of Orthodontics and Dentofacial Orthopedics 136:668-74. [2]F TAUBE et al. (2010) *Morphological and chemical characterization of tooth enamel exposed to alkaline agents* Journal of Dentistry 38:72-81.

Micro-sandblasting effects on adhesion of orthodontic brackets on enamel

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INTRODUCTION: The aim of this work is to study the interest of micro-sandblasting on bonding in orthodontics and more precisely compare the dry micro-sandblasting and the micro-sandblasting under irrigation.

METHODS: 200 samples of enamel from bovine incisors[1] are randomly divided into four groups.

In group 1, the orthodontic brackets (Integra® from RMO®) are bonded in the usual way (etching and bonding with composite) on the enamel.

In the other three groups, micro-sandblasting is performed prior to the same conventional bonding. Three different micro-sandblasting methods are used. In group 2 (Miniblaster®) and in group 3 (Rodoflex®), we performed a micro-sandblasting with dry powder grains of aluminum oxide with particles size of 50 micrometers. For the group 4, we used a micro-sandblasting under irrigation. The alumin-um oxide particles have, in this case, a size of 27 microns on average.

A shear test is then performed on a universal testing machine 5544 INSTRON.

RESULTS: The adhesion forces are collected. We deduce the shear stresses. The average values obtained are given in Table 1.

Table 1: Average shear bond strength

Group	(MPa)
1- Etching	10,91
2- Etching +Miniblaster ®	14,6
3- Etching + Rondoflex®	12,56
4- Etching + Prep K1®	13,44

An ARI[2] (adhesive remnant index) test was also performed, observing the amount of polymerized adhesive remaining on enamel and/or the intrados of the brackets. Results are listed in Table 2.

Table 2 : adhesive remnant index (ARI)

Score ari	1	2	3	4	5
Group 1	5	12	24	4	5
Group 2	4	25	9	1	2
Group 3	1	32	9	1	1
Group 4	10	7	16	6	4

DISCUSSION: Shear bond strength is higher when micro-sandblasting is performed prior to a traditional collage. These forces are relatively weak but nevertheless compatible with clinical use[3]. Statistically, a significant difference was observed between the groups having undergone sandblasting and others. The ARI test shows that when a micro-dry blasting is carried out prior to the traditional bonding, glue remains in 70% of the enamel, which is not the case with other treatments. This is confirmed statistically. Micro dry sanding is significantly different from other groups.

A very high intra-group variance was found in the statistical analysis. To validate our experimental protocol, the parameters responsible for such a variance should be determine in a next study. A study is now conducted that focuses on the roughness of the different surfaces before and after treatment, the homogeneity of the different particle size used and the thickness of the adhesive joint.

CONCLUSIONS: The effect of sandblasting on adhesion of orthodontic brackets is statistically demonstrated. However we cannot determine a difference between dry micro sandblasting and sandblasting under irrigation.

REFERENCES: [1] I Nakamichi et al. (1983) *Bovine teeth as possible substitutes in the adhesion test* J dent Res 62:1076-1081 [2]J Artun (1984) *Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pre-treatment* Am J Orthod 85/333-340.

In vitro study of the adhesion of orthodontic brackets to prosthetic substrates

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INTRODUCTION: The adult's orthodontic treatment leads us to modify the protocol of bonding brackets as the teeth are often restored with metallic ceramics or composites materials. In our experiment, we studied an adhesive and an adhesive composite using a shear test to evaluate the bonding quality with four different substrates: NiCr alloy (alumina sandblasted or Cojet® coated), feldspathic ceramic and acrylic resin.

METHODS: The samples were cylindrical, with a radius of curvature similar to that of the labial surface of the maxillary first premolar. They were prepared for each material and included in resin to be placed more easily in the shearing device used for our tests. Surface treatment protocols were:

-for the ceramic samples : an aluminum* oxide 50µm sandblasting followed by an etching with hydrofluoric acid at 9.6% (Porc-Etch, Reliance) and ceramic primer was applied (Porcelain Primer, Ormco).

-for resin samples: an aluminum oxide 50µm sandblasting followed by applying a primer resin (Plastic Package, Reliance).

-NiCr alloy samples were divided into two groups according to their surface treatment. For the first group, they were sandblasted with 50µm aluminum oxide. Then, we applied a metal primer (Metal Primer, Reliance).

For the second group, we used the CoJet® system (ESPE) with application of the specific silane ESPE Sil ® (ESPE).

Two brackets were glued with a dual adhesive (Excite ® DSC, Ivoclar-Vivadent) and a dual-curing luting composite (Nexus ® 2 Dual Kerr Hawe) on each sample. These were then stored in water at room temperature for a week. After different surface preparations, two samples of each treated substrate were taken to conduct a study of surface using scanning electron microscopy. All samples were tested in an Instron 5544 machine with a shear speed of 1mm/minute.

RESULTS: Our results provide the average shear stress (MPa) for each surface tested. It appears that the proposed protocols allow to obtain satisfactory adhesion values and close to adhesion values on enamel, which is our reference surface.

Table I: Shear strength results

Samples	MPa
Ceramic	16,3
Alloy-sandblasted	14,4
Alloy-Cojet®	14,1
Resin	16,6
Bovine enamel [1]	17,2

Statistical analysis did not reveal any significant difference (p = 0.278). The protocols are equivalent in terms of efficacy. The distribution of values is normal but still asymmetrical.

DISCUSSION and CONCLUSION: The results suggest an approach to the maximum adhesion necessary to this type of bonding. The proposed protocols can be validated even if it is necessary to validate them clinically.

REFERENCES: [1]A Aajaji (2007) *Etude comparative entre deux adhésifs orthodontiques* (Thèse URCA) [2] SH Chay (2007) *Effects of surface treatment and aging on the bond strength of orthodontic brackets to provisional materials* Am J Orthod Dentofacial Orthop 132:577.e7-577.e11 [3]S Karan (2007) Orthodontic bonding to several ceramic surfaces*: Are there acceptable alternatives to conventional methods?* Am J Orthod Dentofacial Orthop 132:144.e7-144.e14

State of the parietal dentin observed in a scanning electron microscope after using Hero 6.4.2 system

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INTRODUCTION: The endodontic instruments in Niti have revolutionized endodontics through the innovative concept of continuous rotation. The instruments used in the procedure of the crown-down allow formatting such difficult channels as they are in record time without abandoning the fundamental objectives of root canal preparation. Study of the surface of the parietal dentin observed scanning electron microscope after using Hero 6.4.2 system (fig. 1) in continuous rotation.

METHODS: 14 freshly extracted molars were selected. These teeth were stored in a solution of 4% formaldehyde. To allow quantification of the state of cleanliness of the canal surface instrumented shots with magnification x 500 are made, then a grid of 10 squares is superimposed on every shot and a score is assigned to each case. The results of three thirds compared by analysis of variance.

Figure 1 : Hero 6.4.2 system MM

RESULTS: Figures should use *Figure* style and have the Hero system is also interesting are active instruments that have an efficiency of better than ProFile cut. However, they are not without their drawbacks such as the phenomenon of sheathing. As for the surface, there are no significant differences recorded with ProFile.

All these teeth were radiographed according to their major axis and by their greater curvature, which has enabled us to refine our selection by checking the ductal patency by direct visualization and measuring the angle of curvature so that only the channels the angle was 20 ° and 40 °. The distribution is in the attached table. 12 channels are prepared with Hero 6.4.2 instruments according to the protocol recommended by the manufacturer. The irrigation was done with a solution of sodium hypochlorite 2.5% alternating with a chelating agent. Once all the channels prepared teeth are severed by the collar with a carborundum disk mounted on handpiece. The samples are then placed in an oven at 37 ° C in order to undergo the phenomenon of dehydration.

Finally experiencing some of these instruments to their limits use.

DISCUSSION & CONCLUSIONS: Hero 6.4.2 instruments that initially are very attractive because they are not disappointing for their cutting efficiency. The smear seems less important in the middle third. Despite the safety act and the final quality of canal preparation in terms of respect for the original path of the canal, it would seem that after the study results are a bit mixed.

REFERENCES: [1] D Martin, P Machtou, (1999) *Evolution of the concepts of root canal shaping* Rev Odont Stomat 28;13-22, [2] F Bukiet et al (2003) *Continuous rotation* Inf Dent 13:3399-407, [3] MA Baumann (1999) *Effect of experience on quality of canal preparation with ProFile rotary Files* Oral Surgery, Oral Med Pathol, Oral Radiol, Endo 88(6):714-9

State of the parietal dentin observed in a scanning electron microscope after using ProFile system

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[1] *Faculty of Medicine Oran, Department of Dentistry,* [2] *Laboratory Conservative dentistry and Endodontics, Oran, Algeria*

INTRODUCTION: The endodontic instruments in Niti have revolutionized endodontics through the innovative concept of continuous rotation. The instruments used in the procedure of the crown-down allow formatting such difficult channels as they are in record time without abandoning the fundamental objectives of root canal preparation. Study of the surface of the parietal dentin observed scanning electron microscope after using ProFile system in continuous rotation

METHODS: 11 freshly extracted teeth apex built were selected, representing a sampling of 12 channels. These teeth were stored in a solution of 4% formaldehyde. All these teeth were radiographed according to their major axis and by their greater curvature, which has enabled us to refine our selection by checking the ductal patency by direct visualization and measuring the angle of curvature so that only the channels the angle was 20 ° and 40 °. The distribution is in the table 1.

Table 1: Distribution of curvatures

	B3	**B4**	**Total**	**%**
Straight channel	5	1	6	50
Curved channel	3	2	5	41,66
Very curved channel	1	0	1	8,34
Total	9	3	12	
%	75	25		

The channels are prepared with ProFile instruments according to the protocol recommended by the manufacturer. The irrigation was done with a solution of sodium hypochlorite 2.5% alternating with a chelating agent. Once all the channels prepared teeth are severed by the collar with a carborundum disk mounted on handpiece. The samples are then placed in an oven at 37 ° C in order to undergo the phenomenon of dehydration. After drying obtained, each sample is metallic and had observed scanning electron microscope.

RESULTS: For each sample, we get a surface for each portion of the channel in terms of cleanliness of the parietal dentin. We have seen what the others who obtained the best result.

DISCUSSION & CONCLUSIONS: ProFile instruments that initially are very attractive are disappointing for their cutting efficiency. The horizontal section of the radial land is probably responsible for the browning effect of smear layer on the surface and inside the dentinal tubules. In addition, they are instruments that fracture quickly mainly because of their appearance.

Despite the safety act and the final quality of canal preparation in terms of respect for the original path of the canal, it would seem that after the study results are a bit mixed and finally experiencing some of these instruments to their limits use.

REFERENCES: [1] D Martin, P Machtou, (1999) *Evolution of the concepts of root canal shaping* Rev Odont Stomat 28;13-22, [2] F Bukiet et al (2003) *Continuous rotation* Inf Dent 13:3399-407, [3] MA Baumann (1999) *Effect of experience on quality of canal preparation with ProFile rotary Files* Oral Surgery, Oral Med Pathol, Oral Radiol, Endo 88(6):714-9

Behavior of the articular disc in the TMJ using a finite element analysis model

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INTRODUCTION: Now it is accepted that the temporo-mandibular joint is a permanently stressed joint in the human body. The disc in the center of the joint is subjected to the action of different muscular forces. These forces are highest during the crushing phase of the mastication process. Its biomechanical characteristics authorize the disc to change its shape when stressed. It also plays a significant role in the absorption and redistribution of charges within the joint. The biomechanical environment of the TMJ and the mechanical properties are the key concepts for understanding the dynamics and kinematics of this joint.

METHODS: We created a 3D model as close as possible to the living and exportable to the analysis software. We use the scanner and MRI images of a healthy subject. The design method of the model requires the segmentation of each image scanner with the TGS AMIRA software. MRI images allow us to determine the limits of the disk at its segmentation on the scanner cuts.

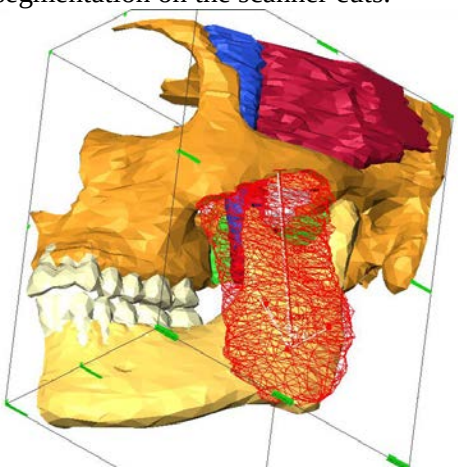


Figure 1: Full model from the segmentation of each scanner cut.

In this model, only the joint is subjected to numerical simulation. Modeling of the elevator muscles allows us, through the maximum surface section (Fig. 1), to calculate the maximum strength developed during the crushing phase.

It remains to assign to the disc a constitutive law that responds to the constitutive law of poroelastic materials. This equation can reproduce the curves of stress and strain (1)

$$\sigma = c_3 \varepsilon_{eq}^3 + c_2 \varepsilon_{eq}^2 + c_1 \varepsilon_{eq} + c_0 \quad (1)$$

RESULTS: The results were obtained using ABAQUS software by acting on the frequency and duration of loading.

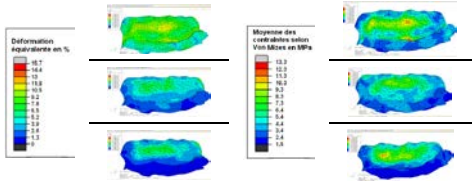
	Strains	Stresses
0,5Hz		
1Hz		
2Hz		

Figure 2: Distribution of stress and strain on the lower face of the disc after four load steps.

DISCUSSION & CONCLUSIONS: The articular disc has the characteristics of a hydraulic shock absorber. Under the action of the forces of the elevator muscles, the disc may see its thickness decreasing of 0.4 mm.

It can be seen that the frequency of 1Hz generates lower strains and stresses at the disk level and also reduced reaction forces at the base of the skull.

REFERENCES: [1] C MEYER et al (1998) *Methods proposed for the determination of external forces applied to the mandible during mastication. Initial results Rev Stomatol Chir Maxillofac. 99 1:79-85* [2] M Beek et al (2003) *Human temporomandibular joint disc cartilage as a poroelastic material* Clinical Biomechanics 18:69-76

Chlorapatite coated titanium dental implants: an alternative to hydroxyapatite

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INTRODUCTION: Calcium phosphate coatings on titanium implants have led to better clinical success rates in the long-term than uncoated implants due to the superior initial rates of osseointegration. Plasma-sprayed hydroxyapatite (HA) has been used to enhance bioactivity and mechanical anchorage of implants[1,2]. However, plasma sprayed HA-coated dental implants have been associated with clinical problems[3]. It was established that plasma sprayed HA coatings present a poor adhesion, possible delamination from the surface of titanium implant and failure at the implant coating interface. This phenomenon is attributed to the decomposition of HA into several foreign phases during plasma spray1-3. Moreover, the plasma-spray process was considered to be not very effective for coating tiny implants with complex geometries. To circumvent these disadvantages, other types of calcium phosphates have been proposed to replace HA, such as tricalcium phosphate or biphasic calcium phosphate with little improvement. Chlorapatite (ClA) however has never been tested despite its ability to melt without decomposition which could be a decisive advantage to avoid apatite decomposition and to increase coating crystallinity. This work compares physical-chemical characteristics, thermal stability, mechanical properties of ClA and HA thin coatings obtained by a novel low energy plasma spray mini-gun designed for small implants with complex geometry as dental implant screws. Cells behavior on these coatings has also been studied.

METHODS: ClA feedstock was prepared using thermal ion exchange process at 900°C between sublimated NH4Cl and commercial HA. The as-synthesized ClA powder was deposited on a titanium substrate using a novel low energy plasma mini-gun characterized by a low power range (< 13kW) and portability allowing in-situ coating deposition. Structural and microstructural properties of both ClA and HA coatings were conducted using X-ray diffraction, Fourier-transformed infrared and Raman spectroscopies. The mechanical properties of coatings were determined according to the standard test measurements ASTM C633. Thermal stability of starting powders was evaluated by thermogravimetric analyses (TGA) from 30°C to 1400°C. Osteoblastic cells were cultured on both ClA and HA coating surfaces to evaluate coating biocompatibility, and study cell adhesion, proliferation and differentiation before in vivo evaluation[4].

RESULTS AND DISCUSSION: X-ray diffraction analyses on the ClA coating indicate the presence of crystalline chlorapatite as the only crystalline phase. FTIR and Raman spectroscopies show the presence of small amounts of oxyapatite and amorphous calcium phosphate phases. However, the crystallinity ratio was 96% for ClA-coatings compared to 68% for HA coatings due to the thermal stability of ClA powder. The measurement of tensile strength indicates that the ClA coatings are adherent to substrate as the HA coatings deposited with the same process parameters. Preliminary cell culture results show that cell proliferation increases with incubation time duration whatever coating composition.

CONCLUSION: The thermal stability of ClA and its fusion without decomposition lead to biocompatible highly crystalline, stable and resistant coating including a small amount of amorphous phase. Cell proliferation test shows that chlorapatite has no adverse effect on cell growth.

ClA coatings would be a promising composition for dental implant surface treatments. The low energy plasma spray allows thin and adherent coatings in small and complex implant geometries. The yield of the present plasma spray system is higher than for conventional system.

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Neutron diffraction study of a nano-hydroxyapatite coated implant

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INTRODUCTION: Nanotechnology development in the last ten years has been significant in materials science and in the industrial sector. In orthopedic surgery, implants of titanium alloy (Ti-Al-4V) are often coated with hydroxyapatite (HAp, $Ca_{10}(PO_4)6(OH)_2$) to obtain a direct bond, stable, rapid and functional between the bone and the implant. To address some limitations, especially mechanical, we have developed a nano-hydroxyapatite coating (n-HAp). The preferred orientation of HAp crystallites in the bone-implant interface in the tibial bones of sheep was assessed using the two-axis diffractometer D20 of the Institut Max von Laue-Paul Langevin (ILL) after sacrifice and sampling at 60 days after implantation. The implant of parallelepiped shape was studied on both faces, one n-HAp coated and one HAp coated (80 micrometers particles). The samples were studied with a spatial resolution of 0.5 mm from each of the interfaces in order to evaluate the reorganization of the distribution of the crystallites of healed bone after reimplantation.

METHODS: We used in this study cortical sheep bone implanted with samples of Ti-6Al-4V (20 mm x 10 mm x 1.4 mm) located near the tibial plateau. Each implant has a face coated with n-HAp and the other coated with HAp. After two months of implantation, the bone with the implant is removed and stored in ethanol to ensure its preservation in ideal conditions without interfering with neutron diffraction. A heat treatment is absolutely necessary to remove water and the organic part of the bone to limit the incoherent neutron scattering due to the presence of hydrogen in large amount[1]. This heat treatment does not affect the preferred orientation of crystallites or the bone crystallinity. In this study, we used the two-axis high intensity diffractometer D20 with variable resolution. A sample of the sheep tibia (20×10×5mm) was mounted in the Euler cradle in order to cover all the pole figures. We followed the evolution of the texture at the interface of the implant by plotting pole figures of two reflections (111) and (002)[2]. The beam size used was 9mm×0.5mm with λ = 2.4 Å. For each 0.5 mm slice of tibia, the measurement time was 6 hours.

RESULTS: 360 diffraction spectra are recorded every 0.5 mm. To determine the crystal structure and texture, we used the MAUD software[3]. This MAUD software for Rietveld texture analysis allowed us to draw directly pole figures.

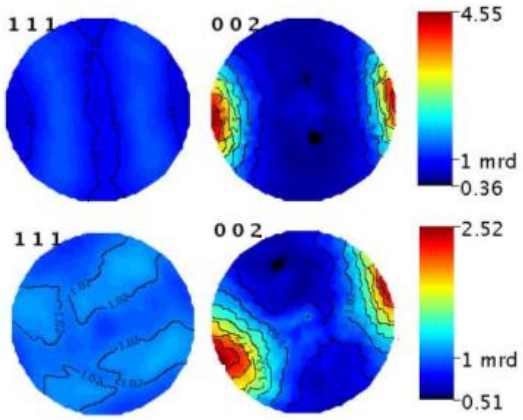

Fig. 1: Pole figures reconstructed for two reflections by Rietveld analysis using the MAUD software for all diffraction spectra at interfaces for each sample face. (above: face coated with n-HAp, below: face coated with HAp).

DISCUSSION AND CONCLUSIONS: The figures suggest that the texture of the crystallites of the cortical bone at the interface with the n-HAp is more intense (4.5) than on the coated HAp face (2.52). Thus, the n-HAp coated face would be more stable. This first series of promising measures must be confirmed by a study by high energy X-Ray diffraction method.

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Synthesis and characterization of nano-hydroxyapatite: An in vitro and in vivo study.

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INTRODUCTION: Hydroxyapatite is the major mineral component of bone. Synthetic hydroxyapatite is widely used as a biomaterial for bone repair and tissue engineering applications. The aim of this study was to synthesize a powder of nano-hydroxyapatite and to characterize it *in vitro* and *in vivo*.

METHODS:

A nano-sized cristalline hydroxyapatite powder was synthesized by wet chemical precipitation (1) and was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-Ray Diffraction (XRD) and Fourier-Transformed Infrared Spectroscopy (FTIR).

The powder was sterilized and used for *in vitro* experiments with MG63 cell line. Cell culture experiments were done using IMDM and 10% FBS (2). Live/Dead assays have been performed after 1, 3 and 8 days of cell culture. Cell proliferation has been evaluated using MTT test after 1, 3, 6 and 9 days. Finally, Alkaline Phosphatase activity has been observed after 8 days of culture. Cell morphology has been observed using SEM.

C157 Black mice (n=6) aged 27 weeks have been used for the *in vivo* study. Calvarial bone defects (3mm diameter) have been created bilaterally under general anesthesia (isofluorane) (3) and were either implanted with nHA powder or left empty. The animals have been euthanatized after 1 or 3 months then the samples were embedded in polymethyl metacrylate or paraffin, cut and stained with Light Green or Hematein-Eosin-Saffron, respectively.

RESULTS:

Concerning the physico-chemical characterizations of the powder, spectra compatible with nano-cristalline hydroxyapatite were obtained with XRD and FTIR. TEM observations have revealed needle-shaped crystals of 50 nm long.

The *in vitro* assays have confirmed that after 8 days of culture, MG63 cells were still alive and proliferating on nHA powder. SEM observations have revealed cell spreading on the material.

All the mice have survived the surgeries without any complication. Histological observations have shown a complete filling of the sites implanted with nHA particles with newly formed-bone. In the control empty defects, fibrous tissue was observed and no bone repair was present.

DISCUSSION & CONCLUSIONS:

The powder that was synthesized possesses all the physico-chemical features of nano-cristalline hydroxyapatite.

In vitro and *in vivo* results suggest that it is suitable for bone tissue engineering applications.

Nano-hydroxyapatite synthesis is the first step of a project aiming to fabricate composite materials associating nHA, hydrogels and cells for applications in Bone Tissue Engineering. More specifically, this material could be implemented in a layer-by-layer biofabrication method using laser assisted bioprinting (4).

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Nano technology: fabrication, characterization and wettability of nanostructured surfaces

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INTRODUCTION: The use of biomaterials in medicine is a common practice. These materials are used in surgery in areas such as prostheses or tissue replacement. But a number of complications like infections, inappropriate tissue responses or toxic release are sometimes associated with the use of these devices.

METHODS: In this case, systemic treatments can be used to try to reduce these complications. But sometimes these treatments are ineffective because of their low concentration and penetration in the area of maximum tissue reaction, and in some cases, the drugs used can be toxic to the patient. In this context, the idea of targeting these drugs more precisely in the immediate vicinity of the implants has been a focus of development in these therapeutic strategies. As part of medical implants, the material itself seems to be a vector of particular interest in applying these local delivery strategies to the molecules of interest.

Electrochemically engineered nanopores/ nanotube materials such as nanoporous alumina and nanotubular titania with ordered and controlled pore structures with a high aspect ratio, have attracted great attention, particularly for implantable drug delivery systems. Knowledge of the physical and chemical properties of these nanoporous materials remain essential for the devices dedicated to industrial and societal needs in the health field. The diversity of their properties is mainly due to recent advances in their fabrication, the emergence of new properties related to topography effects, the control of the stability of interfaces and that of surface functionalization.

DISCUSSION & CONCLUSIONS: In this presentation I will focuse on the concept of fabrication of the nanostructured surfaces using an anodization process, a description of their topography, structural and mechanical properties, their biocompatibility and the wetting behavior of liquids in contact with nanostructured surfaces.

Nanohydroxyapatite and bone healing: Synchrotron X-ray diffraction analysis

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INTRODUCTION: Orthopedic implants made of titanium alloy (Ti-Al-4V) are often partially coated with hydroxyapatite (HAp, Ca10(PO4)6(OH)2) in order to obtain a direct, fast, stable and functional bond between bone and implant.

In dentistry, numerous authors have rejected those coatings, for they show osseointegration failures in the medium term. To overcome such limitations, usually mechanical, we have developed a nanohydroxyapatite coating (n-HAp). In a previous communication, we demonstrated by neutron diffraction the favourite orientation of HAp crystallites in bone-implant interface in the tibial sheep bone using 2-axis diffractometer D20 of the Institut Max von Laue Paul Langevin (ILL). Our goal is to validate this method by X-ray diffraction of high energy emitted by synchrotron.

METHODS: The implant of parallelepiped shape was studied on its two faces, one coated with n-HAp and the other with HAp (80 micrometers). The results were compared with those obtained with a similar uncoated implant. These implants were placed in the tibial tuberosity of a sheep at the Branzeu Pius Center of Laparoscopic Surgery and Microsurgery (Romania).

Sacrifice and sampling were carried out three months after implantation.

The implant and the surrounding bone were removed and stored in ethanol under ideal conditions without disturbing the X-ray diffraction. Unlike the neutron diffraction, no heat treatment is required. The experiment was conducted on ID15B, which is a high-energy beamline (88.4 keV).

Scanning of the specimen was conducted 10 by 10 ° from 0 ° to 180 °. The beam size was 300µm. The X ray was monochromatic with a wavelength of $\lambda = 0.14$ Å.

The complete recording of a pole figure lasted 40 minutes.

RESULTS:

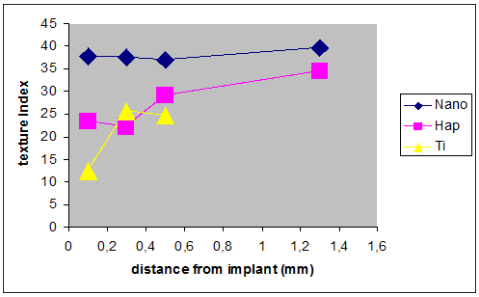

Fig. 1: Texture index for n-HAP, HAP coated and TA6V implants

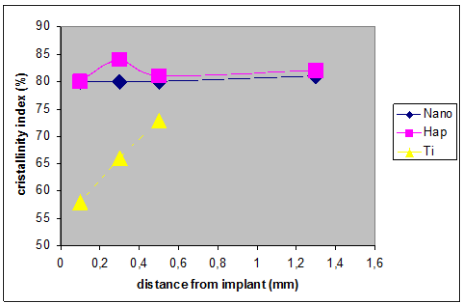

Fig. 2: Crystallinity index for n-HAP, HAP coated and TA6V implants

DISCUSSION & CONCLUSION: This non-destructive synchrotron X-ray diffraction study shows the difference in bone healing near the bone-implant interface. We did not observe any difference in X-ray diffraction between the two different coatings (HAP and n-HAP) regarding crystallinity. In contrast, nano-hydroxyapatite allows faster maturation of the bone as the texture index becomes identical to the texture of normal cortical bone at three months.

REFERENCES: [1] A Benmarouane (2004) *Heat treatment of bovine bone preceding spatially resolved texture investigation by neutron diffraction* Physica B, 350:E611-14. [2] A Benmarouane (2005) *Texture measurements of hydroxyapatite crystallites at bone-implant interfaces in sheep tibia* Solid State Phenomena 105:407. [3] HR Wenk (2001) *Rietveld texture analysis of Dabie Shan eclogite from TOF neutron diffraction spectra* J. Appl. Cryst,34:442-453

TiNOx coatings improves the early osseointegration Of titanium and cobalt-chromium implants

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INTRODUCTION: Titanium Nitride Oxide (TiNOx) coatings are well known for their great biocompatibility, hardness and high resistance to wear and corrosion, and can be easily applied by plasma vapour deposition onto a wide variety of substrates, metallic, mineral or organic

We have previously shown that Human primary osteoblasts grow 1.5 times faster on TiNOx coatings as compared to bare substrate, either rough titanium or stainless steel, while still maintaining a high degree of cell differentiation.

Therefore, dual objectives of that study were firstly to determine whether these results could translate into an improvement of TiNOx coated implants' osseointegration initial stages, and secondly to compare the effects of coatings applied onto rough titanium or CoCr, using a minipig model.

METHODS: 48 cylinders made of Ti-SLA,

Ti-SLA-TiNOx and CoCr-SLA-TiNOx (Ra = 2.49 ± 0.34µm) were implanted into the lower jawbone of 8 Gottingen minipigs. The animals were sacrificed after 1 week, 2 weeks, 1 month and 3 months. Standard morphometric techniques were applied to determine "new bone-to-implant contact".

RESULTS: All of the implants healed successfully, without any rejection or inflammation. The process of osseointegration was normal on the 3 surfaces, with signs of activity within the first week of healing. After 2 weeks (fig. 1), TiNOx coatings had increased implants' osseointegration by 1.8 times (fig.2). Furthermore, this effect was reproducible regardless the substrate coated, i.e. CoCr or Ti. These differences were no longer visible after 1 and 3 months healing.

CONCLUSIONS: TiNOx coatings improve implants' osseointegration within the first month of healing, when compared to standard SLA titanium. Furthermore, this effect is independent from the substrate, leading to similar results whether the coating is applied onto titanium or CoCr of similar roughness.

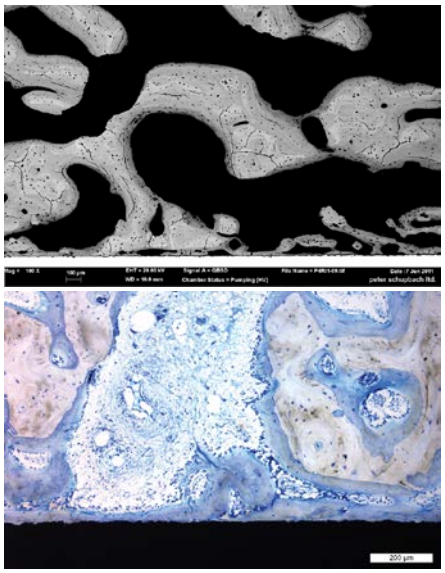

Fig. 1: High magnifications of CoCr-TiNOx periimplant zones after 2 wks healing.

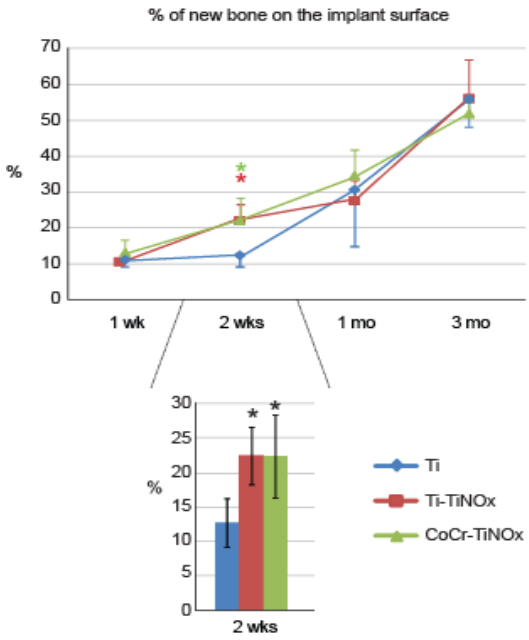

*Fig. 2: New bone-implant contact after 1 wk, 2 wks, 1 mo and 3 mos healing. Data are expressed as mean ± SE. *: significantly different ($p<0.05$) from the Ti implants at the same time.*

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Effect of surface treatments on the reliability of zirconia dental implants

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INTRODUCTION: Dental zirconia is mainly found in the form of yttria-stabilized zirconia crowns, bridges and abutments, and several companies are developing zirconia implants as an alternative to the standard biomedical grade titanium. Indeed, pure titanium and its alloys are still the materials most often used for oral implants because of their excellent biocompatibility, their favorable mechanical properties and their well-documented beneficial results. However, the grayish color of titanium may be perceived through the peri-implant mucosa causing some aesthetic drawbacks. Furthermore, metals (including titanium) may induce sensitization or allergic reactions and many patients therefore ask for completely metal-free dental reconstructions[1]. Tetragonal stabilized zirconia with addition of 3 mol% of yttria (3Y-TZP) have been shown to have several advantages than other ceramics owing to their mechanical properties, their high fracture toughness and bending strength[2] associated to a promising biocompatibility and perfect aesthetic[3]. In order to favor bone in-growth and osseointegration of zirconia implants, several strategies are now being explored to process rough and/or porous surfaces[4]. Surface treatments are elaborated, such as sandblasting, acid-etching, or using surface-structured molds or a deposition of a porous coating. If surface modification surely improves bone in-growth, it may also generate defects at the surface and changes in surface stability, leading to potential risk of slow crack growth and delayed failure and/or aging[5,6].

METHODS: The aim of the present study was to evaluate the effect of a given surface modification (coating with a porous layer) on the reliability of some zirconia dental implants. Un-coated implants presenting the same geometry were used as a reference and compared to the coated implants. The effect of the coating on resistance to failure was done by performing load to failure in bending. Stability was assessed via accelerated aging tests in autoclave and follow-up of tetragonal-monoclinic transformation by XRD. Microstructural analysis was done with SEM and FIB, enabling 3D reconstruction of the implant near the porous surface. Finally, a fractographic analysis was performed in order to investigate the origin of failure of implants after bending tests.

RESULTS: Our results clearly demonstrate that the coating, as processed by the supplier of the implants, is detrimental for the mechanical properties. A significant drop of the load to failure is observed, associated to the presence of large flaws originating from the porous surface. Flaws are in fact generated in the porous layer and at the interface with the bulk and can propagate towards the interior of the implants when stressed. LTD is not accelerated by presence of the porous coating but is likely to be observed in-vivo.

CONCLUSIONS: All these results led us to strongly advice against the use of such a porous coating and to warn the dental community about the risk of decreasing the mechanical performance of zirconia implants with uncontrolled surface modifications.

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Role of hydroxyapatite in preservation of bone and teeth ancient DNA

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INTRODUCTION: Bone and tooth remains often represent the only – but also the best – biological materials available for deoxyribonucleic acids (DNA) typing in anthropology. For T. Lindahl1, the presence of ancient DNA in samples aged over 100 000 years is explained with the hypothesis of an adsorption of DNA fragments on a solid phase, represented by bone and tooth apatitic matrix. Indeed, after death, DNA molecules undergo spontaneous hydrolytic and oxidative damages in aqueous media which are unrepaired and can degrade them completely. Persistence of DNA on geological time presupposes that those solution chemistry reactions are avoided/limited by its adsorption on a solid mineral phase. This hypothesis is not only supported by electrostatic interaction that may exist between negatively charged phosphate backbone of DNA and calcium ions from the surface of apatite but also by the exceptional reactivity of the metastable non-apatitic hydrated layer2 exhibited on the surface of apatite nanocrystals that contain labile ions which can be exchanged with ions from the surrounding solutions. Here we present the first results from an experimental model using two biomimetic apatites with different characteristics of composition and structure similar to those found in biological apatites with the goal to bring concrete and quantitative elements on the nature of DNA / apatite interactions.

METHODS: Apatites. This study focuses on non-carbonated nanocrystalline apatite and stoichiometric hydroxyapatite, prepared by coprecipitation. Their characterization was performed by FTIR spectroscopy, X-ray diffraction, measurement of specific surface, chemical and thermogravimetric analysis.

DNA. The DNA solution was quantified by UV spectrophotometry. Analysis of the size distribution of DNA fragments was performed by agarose gel electrophoresis.

Adsorptions. An adsorption isotherm was obtained for each apatite, after adsorption in deionized water at room temperature, with stirring and during a pre-determined equilibrium time.

RESULTS: The experimental points from the adsorption isotherm of (unfragmented) DNA on nanocrystalline apatite model used in this study seem to fit with a Freundlich isotherm type model, characterized by the equation:

$Qads = a \cdot C1/n$, for which the parameters a and n were estimated. A repeatable process of DNA adsorption on stoichiometric hydroxyapatite (less reactive than nanocrystalline apatites) has not been demonstrated and will require further study.

DISCUSSION: Preliminary data could be reached on DNA adsorption on apatite, but further experiments will be conducted with a model presenting more similarities to our biological study case of (average DNA fragments length of 250 bp on, carbonated apatite). Other experiments will study the effect of dilution with studying the desorption. Indeed, the possible release of ions initially located at the surface of apatite concomitantly with the adsorption mechanism will help us to make the difference between a "simple" adsorption and an adsorption involving surface ions exchange.

CONCLUSION: These initial results indicate the existence of a significant interaction between DNA and apatite analogous to bone mineral. A more detailed study is underway.

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Polyelectrolytes multilayer films on titanium and titanium alloys for biomedical applications

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INTRODUCTION: The aim of this research was to optimize titanium (Ti) and titanium alloy (NiTi) surfaces coated with Polyelectrolytes Multilayer Films (PMF)[1]. We studied different biomedical parameters related to this surface treatment in order to develop specific biomedical applications in the dental field (dental implants, endodontic instruments, orthodontic arches). Firstly, we determined if PMF had a detectable physisorbtion on Ti and NiTi. Secondly, we studied biocompatibility of poly-ethyleneimin (PEI), the precursor based-layer of PMF. Finally, sterilization tests were realized to analyze their potential impact on the physico-chemical structure of PMF.

METHODS: Two types of films were caracterized : polystyrene-sulfonate/polyallylamine hydrochloride films PEI-(PSS/PAH) and hyaluronic acid/poly-L-lysine films PEI-(HA/PLL). Physico-chemical characterization was carried out by tensiometry, atomic force microscopy (AFM), and confocal microscopy. A biological study using human fibroblasts was carried out. Cell response was observed after 0, 2, 4 and 7 days *in vitro* using morphologic criteria (scanning electron microscopy), adhesion (fluorescence microscopy image analysis), and proliferation (Methyl Tetrazolium Test). Three methods of PMF sterilization were compared: ultraviolets (UV), ethylen oxyde (ETO), and autoclave. PSS/PAH films were characterized by the same physico-chemical tests and biological studies after the sterilization process.

RESULTS: Results showed that PSS/PAH films were more biocompatible than HA/PLL films on both metallic biomaterials. The precursor based-layer study demonstrated that PEI was not biocompatible[3]. PMF sterilized by autoclave showed similar biocompatibility. Such results could not be found with UV and ETO. Nevertheless, some investigations have to be realized to prove that the structure of PMF was not perturbed after these processes.

DISCUSSION & CONCLUSION: Based on our results, we decided to use PAH as the precursor based-layer (positively charged) and to stop using PEI on biomaterials made of Ti or NiTi. We are now planning to integrate bioactive molecules between layers of the PMF. To this aim other parameters need to be studied to characterize *in vivo* potential biomedical application, such as films aging in the oral cavity, as well as in salivary or fluored environment.

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Characterization of a porous medium model a basis of natural apatite, support resinous infiltration

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INTRODUCTION: In the current concept of tissue preservation, erosion-infiltration is forseen to become a useful treatment of enamel hypomineralization. The scientific data, based on laser scanning confocal microscopy observations, show that the resin applied to the enamel surface of a lesion has the ability to permeate its entire depth (up to 500µm)[1]. But the microscopic level of resolution of this observation technique does not capture the infiltration capacity of resin at the nanoscale. Yet today's anatomopathological knowledge of hypomineralized enamel shows that their pore sizes are mainly between 15 and 300 nanometers. Before moving onward it is therefore indispensible to obtain a standard support infiltration compatible with the physicochemical characteristics of hypomineralized enamel, in order to assess the ability of three-dimensional penetration (micrometric and nanometric) of the infiltrate and the mechanical implications. As the porous volume of a healthy enamel piece is not large enough and is not representative of the damaged enamel, healthy deproteinized dentin blocks were considered. The purpose of this work is to show that it is possible to obtain, from dentin samples, a naturally porous mineral substrate carrier of a better understanding of enamel infiltration.

METHODS: Healthy dentin has a mineral composition of 70% in weight[2], close to that of hypomineralised enamel at an advanced stage. To eliminate homogeneously and non-selectively the organic phase contained in abundance in the dentin, high temperature sintering is adopted. Collagen being completely degraded at 433°C, different cooking temperatures are conducted, at 500°C, 600°C and 800°C. MEB and MET observation physically compares sintered dentin to sound dentin and hypomineralized enamel. Mercury intrusion porosimetry then quantifies pores sizes dimension and distribution. The topography of the resin infiltration is observed by MEB after EDTA localized demineralization. A numerical analysis of data transposes the level of infiltration according to the size of pores. The mechanical characteristics of porous substrate before and after infiltration are then evaluated by micro-indentation and 3-point bending tests.

RESULTS: Recrystallization that occurs after sintering reforms apatite[3]. Dentin heated at 500°C and 600°C forms a non-denaturated porous mineralized support, free of organic matrix. At 800°C a crystal melting occurs giving a globular appearance to the mineral phase. Two pore sizes are identified; one around the micron-corresponding to the tubules, and one of a few nanometers corresponding to the space between the crystals (made free by removal of the organic matrix). MEB observation after infiltration and demineralization informs us on the three-dimensional network of the resin. Mechanical tests (micro-indentations and 3-point bending) before and after infiltration directly evaluate the benefit brought by the infiltration.

CONCLUSIONS: High temperature sintering of healthy dentin allows to obtain a non-organic porous support from natural apatite, ideal for the observation and evaluation of the capillary rise of infiltration resins. Perspectives are open on understanding the infiltration phenomenon and its mechanical repercussions.

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Evaluation of the newly formed bone in irradiated areas by addition of mesenchymal stem cells to the association of biphasic calcium phosphate and total bone marrow

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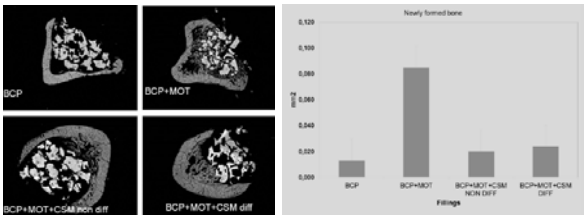
INTRODUCTION: The treatment of the squamous cell carcinomas of the upper aerodigestive tract requires large therapeutic surgical bone removal in association with external radiation therapy. The consequences are esthetic and above all functional. The standard surgical treatment of reconstruction is the use of micro-anastomosed free-flaps or autogenous bone graft. But these techniques are seldom performed because of the reduction of healing capacities of the irradiated bone. So tissue engineering using calcium phosphate biomaterials is considered a good alternative. In irradiated areas, the association of BCP and TBM provides better bone reconstruction than BCP or TBM alone[1.2.3]. And the association of BCP and MSC doesn't increase the bone reconstruction, due to the cellular and vascular poorness of the bone marrow and the irradiated bone[3]. The aim of the study is to determine if the association of BCP+TBM+MSC promotes the bone reconstruction in irradiated areas. This study is an in vivo animal study and is based on the comparison of the new bone formation after implantation of 4 filling materials.

METHODS: The study will be performed on inbred Lewis 1A rats, 24 females, and six males designated as cell graft donors. The MSC will be sampled for three non-irradiated male donors and put in culture for proliferation and differentiation. The 24 females will be irradiated at day 0, by a single dose of 20 grays. Three weeks after, critical size defects (3mm) will be created on the femurs and tibias of the females and the osseous defects will be filled with BCP, BCP+TBM, BCP+TBM+ non-differentiated MSC, or BCP+TBM+ differentiated MSC. Three weeks after implantations, implanted bone defects will be removed just after euthanasia and the non-decalcified bone specimens will be infiltrated and embedded in a glycol methacrylate resin for qualitative and quantitative analysis.

RESULTS: Histological examination: New-bone formation was greater after BCP–TBM mixture implantation than with the other filling materials. Newly formed bone was observed at the periphery of most of the osseous defects. Following BCP–TBM mixture implantation, new-bone formation was also observed towards the centre of the defect. Many newly formed blood vessels were observed in the defects filled with the BCP–TBM mixture. SEM and image analysis: New-bone formation was most dense after BCP–TBM mixture implantation, at the periphery and towards the centre of the osseous defects. After implantation of BCP–TBM-MSCs mixtures or BCP alone, only rare bone formations were observed at the periphery of the osseous defects. The rate of bone ingrowth was significantly higher after implantation of the BCP-TBM mixture than after BCP alone, and BCP-TBM-MSC differentiated or not.



1. Images of Scanning Electron Microscopy x20 and graph of the rate of newly formed bone by different filling materials

DISCUSSION AND CONCLUSION: The association of BCP+TBM+MSC doesn't increase the new bone formation. It's possible to propose some hypotheses: a competition between the TBM cells and the MSC; a higher number of implanted cells which dilute the TBM cells; and the anti-inflammatory role of the MSC could be higher than the osteoprogenitor one. The association of BCP+TBM+MSC doesn't increase the new bone formation. The association BCP-TBM is the most efficient material for bone substitution in irradiated areas. The BCP–TBM mixture may induce the angiogenesis of repair tissue and thus balance the side-effects of irradiation. On the other hand, the precise role of angiogenesis and hematopoietic cells in bone repair should be ascertained by further investigations.

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Effect of strontium on primary osteoblasts

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INTRODUCTION: Calcium phosphates are widely used as bone filling materials and for orthopaedic implant coatings. Elsewhere, strontium (Sr) is known to have the ability to modify the bone balance towards osteosynthesis and is widely used as a treatment of osteoporosis. The aim of this study is to evaluate the ability of Sr-substituted sol-gel calcium phosphate to keep the strontium activity on bone metabolism.

METHODS: Cell culture : Bone explants were obtained from the femoral heads of five patients in the orthopaedic and traumatology department of the University of Reims Hospital, France. The explants were cut into small pieces, washed extensively, digested in a solution of trypsin/EDTA and then in collagenase II. In all experiments third passage cultures were used for three culture times, 7, 14 and 21 days. Cells were cultured with HA, Sr-HA and SrCl2. A control consisted in the cell culture alone.

Proliferation: Cell viability and proliferation were assayed by the Trypan blue exclusion and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) tests, respectively in accordance with the manufacturer's instructions.

Real-Time PCR / PCR arrays: Total mRNA was extracted and cleaned with an RNeasy Micro Kit (Qiagen). RNA was quantified on a Qubit quantitation platform (Invitrogen, Cergy Pontoise, France). RNA quality was checked by 1% agarose gel electrophoresis and optical density measurement. Reverse transcription followed immediately using a high capacity reverse transcription kit (Applied Biosystems). Primers were designed and tested for specificity and efficiency for COL1A1, MMP-1, MMP-2, SERPINH1, TIMP-1 and TIMP-2. HPRT and 18S were also studied as internal controls (IC).

Cytokine Antibody Array: In order to detect differences in the synthesis of cytokines, supernatants were studied on antibodys arrays (42 cytokines were studied).

Statistics: Non-parametric exact stratified permutation tests with the P value fixed at 0.05 were carried out to determine the significance of the results (StatXact 7.0, Cytel Inc.)

RESULTS: Proliferation: After 7, 14 and 21 days cell number showed statistically significant increases in every strontium-containing condition (SrHA and SrCl2) compared with the control.

Real-Time PCR / PCR arrays : The use of strontium induce cells in an osteogenic phenotype. SrCl2 and SrHA increased the expression of type I collagen and SERPINH1 mRNA and reduced the production of matrix metalloproteinases (MMP-1 and MMP-2) without modifying the levels of the tissue inhibitors of MMPs (TIMPs). Thus strontium has a positive effect on bone formation.

Cytokine Antibody Array: 20 of the 42 cytokines studied were synthesized by the primary osteoblastic cells. Few of them demonstrated difference in their level of synthesis when strontium was used. CCL2 and MCP1 were influenced in an osteogenic way.

DISCUSSION AND CONCLUSION: We aimed to evaluate whether strontium-containing BCP particles could induce modifications in bone cell physiology. No cytotoxicity could be observed and strontium influence cells in an osteogenic way. This data is consistent with the data in the literature. We also demonstrated that strontium has a positive effect on bone formation by decreasing MMP-1 and MMP-2 production and increasing type I collagen expression.

Thus strontium ion is an interesting divalent ion to be delivered by bone substitutes in order to increase bone formation and to decrease bone degradation at the implantation site.

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Comparative behaviors of human cells on different surfaces

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INTRODUCTION: Titanium is widely used for manufacturing dental or orthopaedic implants. The understanding of the interactions between cells and surfaces is essential to optimize their adhesion, proliferation and differentiation as well as their biological integration in tissues. Some studies have already shown that surface properties like roughness, wettability and chemical composition modulate the behaviour of cells on surfaces [1, 2] and consequently, the osseointegration of implants. In this work, our aim was to characterize surface properties in order to understand the behaviour of human cells on different surfaces. For this, we decide to study the adhesion, proliferation and differentiation of human mesenchymal stem cells (MSCs), osteoblasts (hFOB) and gingival fibroblasts (HGF-1) on tissue culture polystyrene (TCPS), Glass (G) and titanium (Ti). The organization of cytoskeleton and number of focal points were investigated by immunocytochemistry and image analysis.

METHODS: TCPS, Glass and Titanium substrates were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), profilometry, wettability and zeta potential measurement. The 3 cell types MSCs, hFOB and HGF-1 were seeded and cultured on these substrates. The adhesion and proliferation were measured by counting cells after 1, 2 4, 8 hours and up to 21 days. Immunostaining for actine, vinculine and nuclei was performed. The organization of cytosqueleton and number of focal points were quantified by image analysis. Osteoblastic cell differentiation was investigated by the evaluation of Alkaline Phosphatase activity (ALP) and Red Alizarin (RA) staining at days 7, 14, 21 28 of culture. Data were statistically analyzed for significance ($p<0.05$).

RESULTS AND DISCUSSION: TCPS, G and Ti substrates had similar surface roughness (Ra=0.02 µm) but different chemistries. TCPS and Ti exhibited hydrophobic surface with a contact angle of 69° and 74°, respectively while Glass was more hydrophilic (20°). These differences disappeared when substrates were soaked in culture medium containing foetal calf serum (10% FCS) and all surfaces turned hydrophilic (~10°). Adhesion of MSC was significantly higher on TCPS than on G and Ti. A higher percentage of HGF-1 and hFOB than MSCs was also found on TCPS and G than on Ti. Cells adhesion was also observed without adding FCS in culture medium. Immunostaining allowed the time-lapse visualization of cytoskeleton and cell morphology during the adhesion of the 3 cell types on the different substrates. Image analysis applied to the pictures allowed the quantitative determination of focal points and organization of cytoskeleton. It was found that the number of nuclei was correlated to the number of counted cells. The amount of focal attachments was related to the cell area and types. The orientation of actin filament was in close relationship to the focal points. Cell growth followed the same trend than adhesion on the different substrates. hFOB appeared to proliferate more rapidly than MSCs and HGF-1. Cell differentiation as determined by ALP and RA quantification was not related to surfaces, but depended on the presence of osteogenic factors in media (dexamethasone, ascorbic acid, phosphate and BMP-2) as well as cell type.

CONCLUSIONS: The quantification method based on immunostaining gave new insights on the mechanisms of cell adhesion on biomaterials. Correlation between cell growth and differentiation of mesenchymal stem cells may also be pointed. Further studies will be conducted to decipher the role of nanometer-sized structured surfaces on cell behaviour.

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Zinc modulation of inflammatory response induced by biomaterials

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INTRODUCTION: Hydroxyapatite (HA) is a bioactive material widely used because of its ability to enhance the implant integration in host tissue. Such an event induces production of biomaterial particles in periprosthetic tissue leading to an inflammatory response which, if uncontrolled could lead to implant aseptic loosening. Recent study has demonstrated that HA induces activation of human polymorphonuclear cells (PMNs) and increases inflammatory mediators' secretion. Based on previous work demonstrating an anti-inflammatory effect of zinc-substituted HA, in this work we have focused on immunomodulatory effect of zinc on acute inflammatory response to HA particles.

METHODS: PMNs were collected from whole blood of healthy volunteers by using Polymorphprep®. Cells were cultured for 4 hours on basal condition or in contact with HA or zinc-substituted HA (HA5%). Cells supernatants were collected for IL-8 measurement by ELISA. Chemotactic activity of cells supernatants was also tested by modified Boyden chamber method. Cells were lysed in order to perform mRNA extraction using Epicentre masterpure RNA extraction kit. Reverse transcription was done and cDNA were used for real-time quantitative PCR. Results were expressed using the $2^{-\Delta\Delta Ct}$ method.

RESULTS: Measurements of IL-8 concentration by ELISA in cells supernatants have highlighted a 50% decrease when cells were in contact with HA5% in comparison with HA particles (Figure 1). Such a decrease in IL-8 concentration was associated with a decreased in IL-8 mRNA production as demonstrated by RT-qPCR experiments (Figure B). Modified Boyden chamber assays have also demonstrated that chemotactic activity of PMNs supernatants was decreased when cells were in contact of HA5% compared to HA particles.

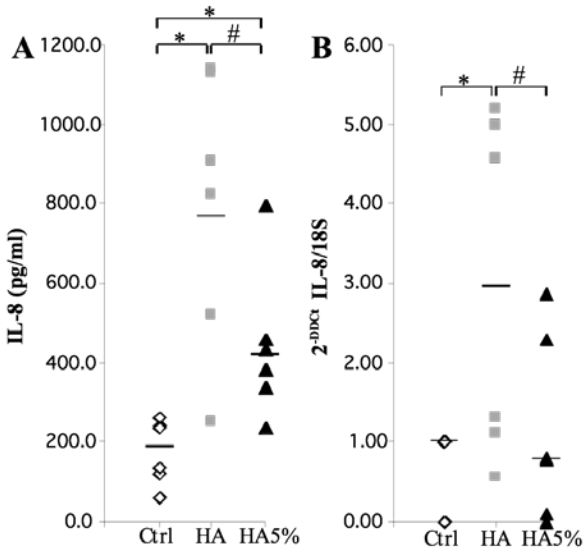

*Figure 1: Means median value. * means p < 0.05 compared to control (Ctrl) condition, # means p < 0.05 between HA and HA5%.*

DISCUSSION AND CONCLUSION: Our data clearly demonstrate that zinc-substitution of calcium-phosphate biomaterial decreases IL-8 production both at the transcription and translation levels. This leads to decreased PMNs recruitments at the inflammatory site and to a lesser activation of those immune cells. Studies are underway to determine the mechanisms and pathways involved in zinc effect. Those results are intended to provide a better comprehension of PMNs role in inflammation induced by biomaterials

Differentiation of endometrial stem cells into odontoblast cells

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INTRODUCTION: In recent years the detection of various stem cells in unerupted tooth buds, dental pulp or bone marrow has provided opportunities for their management in dentin-pulp repair. However, these cell types are limited by availability, invasiveness of extraction and in some cases limited proliferative capacity. What is currently required is a source of stem cells that overcomes these deficiencies. The purpose of this study was to evaluate the effect of dentin noncollagenous proteins (DNCPs) on the differentiation of endometrial adult stem cells into odontoblasts.

METHODS: DNCPs were extracted directly from human dentin and applied to endometrial adult stem cells. The results were evaluated through the analysis of the alkaline phosphatase (ALP) activity, immunocytochemistry to detect the presence of dentin sialo phosphoprotein (DSP) and dentine-matrix protein (DMP-1), and the formation of mineralized nodules in vitro by Alizarin red staining.

RESULTATS :

Table 1: ALP activity of different groups (OD value at 410 nm, $\overline{X}$ ± SD, n = 6).

Days	Groups	
	DNCP	Control
3 days	0.2646 ± 0.0110[a]	0.2028 ± 0.0101
7 days	0.4256 ± 0.0102[a,b]	0.2036 ± 0.0092
10 days	0.6560 ± 0.0097[a,b]	0.2066 ± 0.0066
14 days	0.8330 ± 0.0101[a,b]	0.2111 ± 0.0088

[a] P < 0.01, significant differences between the experimental group and the control group.

[b] P < 0.01, significant differences between day 7, 10 and 14.

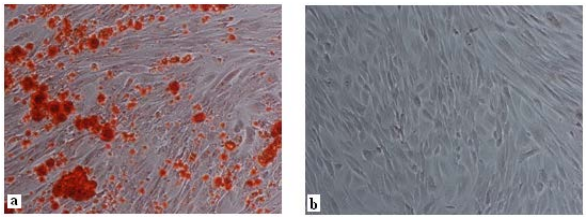

Figure 1: Staining with alizarin red: a) DNCP treated group, b) control group.

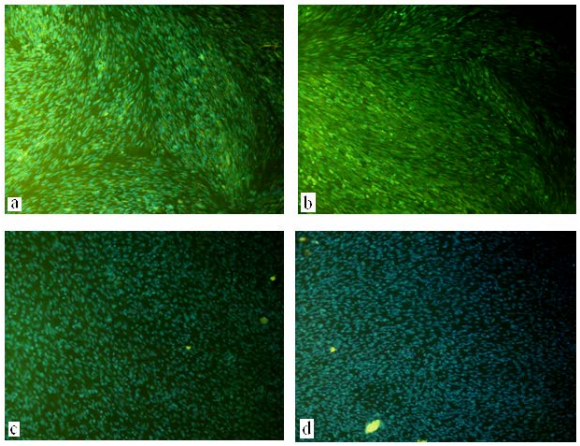

Figure 2: Positive staining in DNCP group for DSP (a) and DMP-1 antibody (b),negative staining in control group (c&d).

DISCUSSION: This study revealed -for the first time- the ability of odontoblastic differentiation of endometrial stem cells.

CONCLUSIONS: These results suggest that, dentin matrix proteins or the secreted products of odontoblasts, induced differentiation and mineralization. Endometrial stem cells appear to be able to provide a source of odontoblast-like cells. These cells will be able to be used to further study the cellular differentiation processes involved in remineralization.

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Comparison of the effects of zoledronate in two- and three-dimensional models cultures

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INTRODUCTION: Bisphosphonates (BPs) are drugs widely used to treat bone diseases in which bone resorption is in excess. Studies of action of BPs on bone have been mainly limited to their effects on osteoclast cells. However, the mechanism of bisphosphonates action on osteoblasts is not fully understood. In this report, the direct actions of Zoledronate, an amino-BPs, on human fetal osteoblasts (hFOB 1.19) were examined in two-dimensional (2D) and three-dimensional (3D) culture models. We focused on in vitro viability and cytotoxicity, spheroids morphology, and genes expression.

METHODS: Cell culture: hFOB 1.19 cells were allowed to proliferate in basic culture medium or medium containing Zoledronate at various concentrations (10-7M to 10-5M) in 2D and 3D culture models. Experiments were performed at day 3 (D3) and day 10 (D10). Viability/Cytotoxicity: The methyl-thiazolyl-tetrazolium (MTT) assay and acid phosphatase (APH) assay were applied respectively in 2D and 3D cultures to determine cell viability in various culture conditions. Spheroids morphology: To analyse spheroid diameter, phase-contrast images were collected with a camera during 21 days in various culture conditions. Spheroids were also examined using SEM.RT-PCR analysis: Total RNA was extracted from samples in 2D and 3D cultures. Reverse transcription was performed and the amplification by PCR used specific primers for the selected genes: Runx 2 and type 1 collagen.

RESULTS: High concentration of Zoledronate (10-5M) reduces cell proliferation in 2D and 3D cultures, disturbs spheroid growth and morphology. 10-6 and 10-7M Zoledronate could promoted cell proliferation and did not disrupt the growth kinetics in 3D model. Gene expression analysis shows that 3D cultures promoted osteoblastic marker genes expression. RT-PCR study did not show real changes with Zoledronate at D3 and D10.

DISCUSSION: This study is the first work to consider the effects of Zoledronate on osteoblasts in 3D cultures. Spheroid model more accurately mimics the tissue environment in vivo than 2D cultures, better reflects the physiological response pattern and promotes osteogenic differentiation. This work shows the interest of hFOB spheroid which could be useful to study mechanism of action of BPs on osteoblasts and for assessment of innovative bioactive materials in regenerative medicine. Results of this study show an anti-proliferative or cytotoxic effect of 10-5M Zoledronate; lower concentrations can enhance osteoblast proliferation in 3D model. These data are in accordance with previous studies showing the influence of Zoledronate on osteoblast proliferation in a dose-dependent manner. Future studies will highlight effects of BPs on osteoblastic marker genes expression using increased evaluation times.

CONCLUSION: This work reveals a differential effect between 2D and 3D cultures. Zoledronate has an effect on the cell proliferation-differentiation balance, which provides opportunities for application in bone tissue engineering, regenerative medicine and implantology. The hFOB 3D-spheroid culture model is an interesting tool in regeneration medicine, which may be a strong contribution to the development of 3D systems for the evaluation of bone substitutes.

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Contribution of a synthetic bone substitute in bone regeneration

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INTRODUCTION: The aim of this study is to evaluate the bone regeneration properties of an experimental calcium phosphate formed of 40% β tricalcic phosphate and 60% hydroxyapatite used in socket filling after the extraction of the mandibular premolar in the dog.

METHODS: The left and right 3rd premolars of 2 12 kg Beagle dogs were extracted under general anesthesia. The mesial part of the sockets were then left unfilled and used as controls of spontaneous healing (R3M and L3M), the distal part was filled with the biomaterial (Cross-Bone®, Biotech International, France) (R3D and L3D).

The dogs were sacrificed after 1.5 and 3 months and the mandibular premolars prepared for histological analysis. The slides referring to the central third of the alveolar sockets were computerized for bone healing histomorphometrical analysis.

RESULTS: At 1.5 months, control unfilled sockets were reduced to cortical bone with little bone trabeculae. In the experimental filled sockets, the material was found in the cervical half of the socket and the bottom was filled with a few mineralized trabeculae. At 3 months control sockets showed a few bone trabeculae between the cortical bones. On the experimental sites, particles of the material were still present and numerous. However, most of them were surrounded by new bone formation. Overall, there were more bone trabeculae than in the control group.

After 1.5 months healing, control sockets were filled with 34.82% ±6.62% bone and experimental sites with a mean 46.05% ±7.5%.

DISCUSSION: The properties of the material tested seemed similar to those observed with other calcium phosphates. Once set in place, the material rapidly recruited cells at the surface of the particles. Concerning material resorption it hardly changed in time (9.83% at 1.5 months and 10.40% at 3 months).

CONCLUSION: The material induced early cellular recruitment, rapidly followed by osteoformation due to the osteoconductive properties of the material. It increased new bone formation in the experimental sockets but the level was relatively similar to that observed in the control sockets.

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Spray-dried antibacterial microspheres to incorporate in an injectable mineral cement

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INTRODUCTION: Fast-setting calcium phosphate bone cements (CPC) have been the focus of intensive research on bone and dental restorative materials for the past few years due to their excellent biocompatibility and bioactive properties, combined with their moldability and injectability. To overcome one of their main drawbacks, i.e. their slow resorption, several original injectable calcium carbonate-based cement (CCC) compositions have been presented as promising biocompatible and resorbable self-setting pastes for bone substitution and repair due to the high proportions of CaCO3 metastable phases with higher solubility than apatite [1]. Besides, in a previous study we demonstrated the interest in incorporating degradable polymer microparticles into CPC cements [2] as they should permit to combine cohesion and resorption enhancement with controlled drug delivery. With the aim to develop an injectable and resorbable composite bone and dental substitute with sustained antibacterial activity, we first synthesized various polysaccharide microspheres loaded with silver as an antibacterial agent. Composite cement formulations with varying polymer type, polymer/ silver / mineral ratios have been prepared and characterized. The influence of polysaccharide microspheres on the composite setting injectability was studied. The drug release properties of the optimal composite formulations have then been explored.

METHODS: 1-Preparation of loaded silver microspheres: Carboxymethylcellulose (CMC), Hyaluronic acid, Carrageenan and Chitosan (Pharmaceutical Grades), and silver salt (AgNO3) were used to prepare microspheres. Polymer and silver salt were dissolved together. Solution was then spray dried using a BUCHI B290 apparatus fitted with a two-fluid nozzle (inner diameter of 1.5 mm, sol and compressed air volumic flow rates fixed at 0.34 and 357 Lh-1, respectively). The inlet temperatures were varied from 373K to 423 K.

2-Cement and composite cement preparation: Reference cement was prepared by mixing deionised water as the liquid phase with the solid phase composed of equal mass of vaterite-CaCO3 and dicalcium phosphate dihydrate (DCPD). Composite cement was obtained by adding various amounts of microspheres to the solid phase of the cement prior mixing with liquid phase.

3- Release study: Silver incorporating rate and the in vitro release of were performed in phosphate buffer (pH=7.4, at 37 °C) using dissolution in flow-through Sotax cells.

RESULTS AND DISCUSSION: Four polysaccharides types were tested to evaluate the best condition for silver encapsulation and controlled release. Spray drying conditions were optimised for each polymer type in a way to obtain reproducible 10 µm diameter loaded microspheres, in a one-pot synthesis (fig.1a and b)). Loaded polysaccharide microspheres / CCC ratios varying from 1 to 20% w/w were tested. The feasibility of incorporating spray dried microspheres into a CCC was validated (fig. 1c), as results showed that a setting reaction still occurs up to these proportions. However, microspheres swelling properties and stability over time varied according to polymer type suggesting that various controlled release profiles could be obtained by playing with polysaccharides types. Drug release studies of the optimal composite formulations showed interesting sustained release properties when compared with CCC reference. By playing with polysaccharide type and microspheres size distribution, the antibacterial agent release can be modulated and adjusted to the aim.

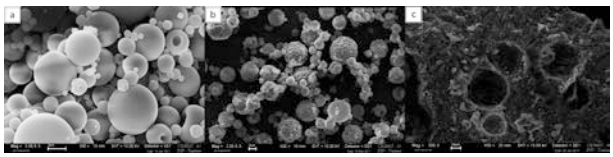

Fig. 1: Scanning electron microscopic images of; (a) CMC microspheres, (b) chitosan microspheres and (c) composites microspheres / CCC cement.

REFERENCES: [1]C Combes et al. (2006) *Preparation, physical-chemical characterisation and cytocompatibility of calcium carbonate cements* Biomaterials 27:1945-1954 [2]Girod et al (2010) *Controlled release properties and final macroporosity of a pectin microspheres-calcium phosphate composite bone cement* Acta Biomaterialia 6 : 2294–2300

Bone regeneration materials histological study

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INTRODUCTION: The aim of bone regeneration is to correct bone defects in order to increase implant osseointegration. Practitioners have the choice between GBR, autograft, allograft, xenograft and the use of synthetic materials. The aim of the study was to histologically evaluate a few bone substitutes used in peri-implant regeneration.

MATERIALS AND METHODS: Six patients (male and female) aged 40 to 69 suffering from terminal periodontitis or post-traumatic bone loss were selected. They all required alveolar bone augmentation or sinus lift before implant treatment. Materials tested were PHOENIX® graft, BIOACTYS® granules, CEROS® TCP granules, BIO-OSS® granules. Implants were placed between 4 and 9 months after bone augmentation and samples were harvested using a drill during the implant placement procedure and studied histologically and histomorphometrically.

RESULTS: In a first group, an implant was placed 4 months after socket filling with the PHOENIX® allogenic graft. Slides show low density new bone and histomorphometrical analysis demonstrated an important remodeling activity (3.3%).

In a second group, concerning the synthetic BIOACTYS® material, implants were placed 7 and 9 months after the bone graft. Slides show an incomplete resorption of the material and a remodeling activity localized around the particles. Histomorphometry measured a mean 1.9% osteoid tissue formation.

In a third group, the synthetic material CEROS® TCP was used in association with BIOACTYS® or BIO-OSS® granules. The second surgery was held 8 months later. Resorption was incomplete for all materials tested and new bone was found around the particles. The mean value of osteoid represented 2%.

Sinus lift procedures were accompanied by BIO-OSS® xenografts. Implants were placed 5 months later. Slides showed incomplete resorption of the particles and an important remodeling activity. Mean measures showed 55.5% of material and 1.4% of osteoid tissue.

DISCUSSION: All materials tested possessed the required criteria for bone augmentation: osteo-conduction, biocompatibility and relative resorption. Histological and histomorphometrical analysis showed various levels of resorption and different remodeling activities. Values measured were not always in accordance to manufactures' values. However due to the limited number of patients, these results can be considered preliminary.

CONCLUSION: Other similar studies are necessary in order to enlighten practitioners on the properties and guide them in the choice of bone augmentation materials.

REFERENCES: [1]H Tenenbaum et al. (2006). Journal of Parodontology and Implantology Oral 25 :143-151

Tissue reactions around miniscrew implants of stainless steel and Ti6Al4V : a study in pigs

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INTRODUCTION: An increasing number of patients are receiving orthodontic treatment for esthetic or functional purposes. The use of miniscrew implants, i.e., non-compliant devices temporarily fixed to the bone and removed after use, provides better clinical control of tooth movement as compared with the headgear method. For these devices, mechanical stability is needed to withstand the orthodontic forces. Osseointegration is not required, but miniscrew implant stability is nonetheless related to the amount of bone contact. The objective of the experiment was to evaluate the tissular response around two systems of miniscrew implants (of titanium alloy and of stainless steel), following immediate loading in growing pigs.

METHODS: Sixteen devices, eight of stainless steel (Leone, Firenze, Italy) and eight of Ti6Al4V (Absoanchor, Dentos, Daegu, Korea), were inserted in the mandible of four Large White pigs (three month-old), in an anterior and a posterior area. The implantation was performed in keratinized gingiva (buccal side) after mucosal incision. The devices were immediately loaded with 100g of force. After 4weeks, bone samples (containing the miniscrew implants and their surrounding tissues) were removed from the sectioned pig mandibles. Miniscrew implants were pulled out with the appropriate screwdriver before the histological analysis. The protocol was approved by the ethics committee on animal research of the National Veterinary School of Lyon in France.

RESULTS: Premilinary results showed the presence of a fibrous tissue (no osseointegration) around the miniscrew implants whatever the systems may be (figs.1 and 2).

Figure 1. Histological slide of tissue surrounding a miniscrew implant of stainless steel (Modified Goldner's Masson Trichrome – optical microscope x 1.6)

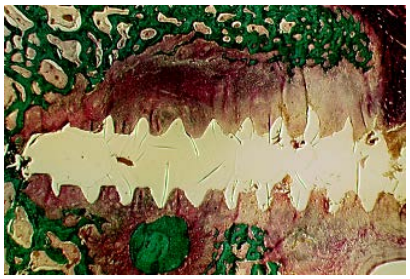

Figure 2. Histological slide of tissue surrounding a miniscrew implant of Ti6Al4V (Modified Goldner's Masson Trichrome – optical microscope x 1.6)

DISCUSSION AND CONCLUSION: Three-month-old pigs present a complete deciduous dentition (Oltramari et al., 2007) and allow for studying growing bone reaction after 4 weeks without eruption of permanent teeth near the assessed devices. In our study, no differences in bone response, in terms of osseointegration, were found between both devices. Histomorphometric analyses are planned to better understand the bone tissue response around these immediately loaded miniscrew implants.

Histological and histomorphometrical analysis of post extraction socket wound healing filled with Bioglass

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INTRODUCTION: Post-extraction bone lossis one of the major difficulties in oral implant rehabilitations[1]. Actually, alveolar bone preservation is essential in replacing the extracted tooth by an implant. Some studies show that adding bioglassin the socket could enhance bone volume preservation[2]. However, not only is maintaining the bone volume neccesary but also the quality of the bone regeneration obtained in the socket. The later being highly important for the osseointegration processes. The aim of this preliminary study was to evaluate the osteo-conduction potential of such a material in post-extraction sockets and to quantify new bone formation.

METHODS:

1- Patient inclusion criteria: five patients: 3 male and 2 female were included in the study. 7 teeth were extracted and the 7 sockets were filled with bioglass. 7 bone samples were collected after a healing period of 6 to 9 months.

2- Bone samples preparations: Each sample was fixed in 70° alcohol then dehydrated and embedded in a polyester resin. Resin blocs were then cut using a microtome into consecutive slides. The most central slide was stained and analyzed under light microscope. A computer image analyzing software was used to isolate and quantify the different parts of the bone biopsy samples.

RESULTS: As shown in other studies, traces of intra-particular mineralization were found as well as particles of bioglass directly in contact with the bone. Material resorption was limited and an important quantity of particles remained embedded in the non-mineralized connective tissue. The quantity of new bone formed varied between samples but never exceeded 20%.

CONCLUSION: The Bioglass tested in this study and used as a bone substitute revealed very low osteo-conductive properties.

Figure 1: Computerized image for histomorphometrical analysis of a sample after Masson-Goldnerstainning

REFERENCES: [1]Araujo (2005) J Clin Perio 32:645-652 [2]Stavropoulos (2011) Clin Oral Impl Res 19

A study of the adhesive properties between the prosthetic teeth in SR Vivodent PE® resin and four types of thermo- and auto-polymerisable resins of prosthetic bases

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INTRODUCTION: It has been estimated that between 22 and 33% of the repairs of removable prosthesis concern teeth splitting, mainly in the anterior area. The fact that these splitting prevail in this part can be correlated to the reduced surface of contact between the denture base and the prosthetic teeth, and to the direction of the stress during the masticatory function. The aim of this study is to evaluate the quality of the chemical bonding occurring between prosthetic teeth and denture base polymers.

METHODS: Four systems of resin are tested. The Perform resin by Coltène Whaleadent©, the Ivobase High Impact® and Ivobase Hybrid® resins by Ivoclar/ Vivadent© are all auto-polymerisable resins. The Probase Hot® resin by Ivoclar/Vivadent©, is thermopolymerisable. The tests methods followed correspond to the international standard ISO 22112. Five moulds of six incisors (n=30) are made for each type of tested resin. Each sample is set on a tensile testing machine of the INSTRON type and each tooth undergoes a tensile test (with 4mm/mn stroke speed).

RESULTS: Comparison of the modes of fractures observed between resin teeth and denture base polymer: the bond passes the test if the fracture mode is cohesive within the tooth or the denture base polymer. Whatever the resin, the majority of the recorded fractures are of the cohesive mode within the tooth-denture base polymer. The Ivobase High Impact® and Ivobase Hybrid® resins obtain 100% of cohesive fractures within the tooth-denture base polymer, versus 93.3% for the PERform® resin and 89.5% for the Probase Hot® resin.

Comparison of the ultimate tensile stresses: They correspond to the stresses of the prosthetic teeth, not to those of the resin bases. It is the reason why there are no significant differences between the different values obtained. This is confirmed by the analysis of variance ANOVA tests.

Young's moduli recorded: the most rigid resins are the Probase Hot® and the PERform® resins.

Resins	Prob.	Perform	Ivob. High Imp.	Ivob. Hybrid
Mean (MPa)	471	526	441	469
Standard dev.	139	139	50	79

Table 1: Average and standard deviation of Young modulus recorded (MPa)

DISCUSSION: All the cohesive fractures we have obtained are situated within the prosthetic teeth. It can be deduced that this is the "weakest link" of the chain 'prosthetic tooth / interface / denture base resin. The results that have been obtained set forward the weakness of the mechanical resistance of the tooth compared to that of the interface. Except for the Probase Hot®, whose behaviour can be directly reliable to the operators' skill, the three other systems of resins are entirely managed by the machine.

CONCLUSION: Forces measuring the masticatory pressures recorded with totally edentulous patients who wear a dental appliance, have been estimated between 70N and 80N. Thus, all these resins bases should be sufficiently resistant. The results of this study points out the very interesting mechanical properties of Ivobase High Impact® and Ivobase Hybrid® Ivobase resins, that may be superior to the other systems. These new autopolymerisable systems, are to be on the public market soon.

REFERENCES : [1]JL Cunningham (2000) *Shear bond strength of resin teeth to heat-cured and light-cured denture base resin* Journal of Oral Rehabilitation 27: 312–316 [2]AM Fletcher et al. (1985) *A method of improving the bonding between artificial teeth and PMMA* J Dent 13:102-108 [3]H Thean et al (1996) *Shear bond strength of denture teeth to base: A comparative study* Quintessence Int 27:425-428

In vitro comparison of machinable materials for cad-cam inlays manufacturing

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INTRODUCTION: Inlay/onlay dental restorations can be obtained from various machinable materials through CAD-CAM manufacturing.[1] Choosing the right material in a clinical situation can sometimes be hard for a dentist. Clinical studies would give the best arguments to guide this choice. However, only one comparative clinical study has been published in this field and does not provide decisive conclusions.[2] Some reviews give ideas,[3,4] but clinical trials are missing. An upcoming trial (CECOIA) shall compare ceramic and composite inlays but, in the meantime, in vitro arguments are the only ones available. The aim of this study was to compare the most common materials available in vitro and to try and correlate the results later with the clinical results.

METHODS: The materials compared in this study were: one feldspar ceramic (Mark II, Vita), two leucite-reinforced glass-ceramics (ProCAD and EmpressCAD, Ivoclar-Vivadent), one lithium-disilicate reinforced glass-ceramic (eMax CAD, Ivoclar-Vivadent) and one resin composite (Lava Ultimate, 3M Espe).

Specimens of each material were obtained by cutting14L-sized blocks for Cerec using a diamond disc in a precision cutting machine.

Vickers hardness (HV): A slice of each material was gold sputtered and Vickers hardness was measured.

Measurement of flexural strength and flexural modulus: 10 bar-shaped specimens of each material (1 mm x 4 mm x 14 mm) were fabricated. Flexural strength and modulus of the materials were measured according to ISO 6872, modified to accommodate bar sizes that could be sectioned from commercially available mill blocks. The three-point bending test was performed using the universal testing machine at a cross-head speed of 1 mm/min with a 10mm span.

Measurement of fracture toughness (SEVNB): 10 specimens of size 3 mm × 4 mm × 14 mm were fabricated for each material, ground, polished and a 1.5mm deep notch was cut using the V-notch option. Fracture toughness of the materials was measured according to ISO 6872 (modified to accommodate blocks size). A three-point bending fracture test was performed at a cross-head speed of 0.5 mm/min with a 10 mm span. The critical stress intensity factor or KIC (Pa.m1/2) was calculated.

Scanning electron microscopy (SEM): Some fractured specimens – either polished or treated with their respective surface treatment – or their fracture surfaces were gold sputtered in a sputtering device and analyzed under the SEM at 15 kV to observe the materials' structure and the topographical changes of treated surfaces.

Atomic force microscopy (AFM) imaging was carried out, using non-contact mode and silicon tips, under ambient conditions, to study the surface topography and roughness of the specimens after their surface treatment.

Statistical analysis: A statistical software (Stata 12) was used to compute the means and standard deviations and to perform a 1, 2 or 3-way analysis of variance, followed by post hoc Tukey' HSD tests at α=0.05.

RESULTS: upcoming

REFERENCES: [1]DJ Fasbinder (2012) *Chairside CAD/CAM: an overview of restorative material options* Compend Contin Educ Dent 33:50-8 [2]DJ Fasbinder et al (2005) *The clinical performance of CAD/CAM-generated composite inlays* J Am Dent Assoc 136:1714-23 [3]R Hickel et al (2001) *Longevity of restorations in posterior teeth and reasons for failure* J Adhes Dent 3:45-64 [4]Wittneben (2009) *A systematic review of the clinical performance of CAD/CAM single-tooth restorations* Int J Prosthodont 22:466-71

DENTICAST, a new centrifugal casting machine: analysis of performance

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INTRODUCTION: The objective is to study the mechanical and physical properties of dental casted alloys with a new prototype of universal casting machine DENTICAST. This prototype, combining induction melting, injection by high rate centrifugation in a secondary vacuum allows casting all dental alloys such as NiCr, CoCr, Titanium and TA6V. The samples casted with DENTICAST are compared to samples obtained with various casting systems on the market (centrifugation, pressure/vacuum).

METHODS: Hardness tests, Tensile strength tests and evaluation of residual stress by neutron diffraction method are performed on cast samples. For this study, we selected three different non-precious dental alloys (NiCr and CrCo alloys and commercially pure titanium). Different shapes of samples were tested: parallelepipeds (hardness, method of small-angle neutron scattering), tensile samples and MBS model (neutron diffraction).

After locating the zones of highest stresses by finite element, we experimentally validated this simulation using a non-destructive technique using a neutron diffraction method. The measurements are performed on line G5.2 (Léon Brillouin Laboratory - Saclay, F). The measurement conditions are: $\lambda = 0.332$ nm Ti {101}, a = 0.295 nm, c = 0.4686 nm; $2\theta = 95.85$ °. Reference: powder method. Mechanical conditions: $\sigma ZZ = 0$, σXX, σYY

RESULTS: Mechanical tests: Tables 1 and 2 presents the results from samples cast by DENTICAST, compared with two other casting systems: ERSCEM® and DORAMATIC®. For all the alloys tested, DENTICAST system gives superior results.

Neutron diffraction: the level of residual stress is very low and generally homogeneous below 200 MPa for the parallelepiped samples and about 50 MPa for the MBS disk.

DISCUSSION AND CONCLUSIONS: DENTICAST prototype is an original approach to casting alloys. It produces good quality samples, validated by the results of mechanical testing and measurement of residual stresses by a non-destructive technique of neutron diffraction for the various alloys used in dentistry and especially for titanium.

Table 1: Hardness tests (ERSCEM, DORAMATIC, DENTICAST

Hardness [HV]	ERSC.	DORA.	DENTI.
NiCr	163	179	216
CrCoW	334	354	369
CrCoM	283	299	392
Ticp	184	194	302

yield strength [MPa]	ERSC.	DORA.	DENTI.
NiCr	270	244	287
CrCoW	385	328	392
CrCoM	362	370	492

Table 2: Yield strength tests (ERSCEM, DORAMATIC, DENTICAST

REFERENCES: [1]RC Atwood et al. (2005) *Multiscale modeling of titanium investment cast dental prostheses* Materials Science and Engineering C 25 255-262. [2] K Liu (2005) *A single step centrifugal casting TiAl automotive valves* Intermetallics, 13:9:925-928 [3]Lu J. (1996) *Handbook of Measurement of residual Stresses* Fairmont Press: 238p. [4]I. Lopez (2008) *Mechanical and Physical Analysis of Alloys Samples Produced with a New High Centrifugal Rate Machine* Materials Science Forum 571-572:33-38

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Development of a residual stress measurement method in veneering ceramic layered on metal and ceramic frameworks

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INTRODUCTION: Excessive chipping of the veneering ceramic in zirconia-based restorations compared to porcelain-fused-to-metal (PFM) systems is still a misunderstood problematic1. In another hand residual stress developed during manufacturing process constitutes a predicting factor of the mechanical behavior of restorations and can explain failures. To author's knowledge no measurement method of residual stress profile through the veneer thickness has been described in the literature until now.

METHODS: Residual stress measurement was performed using strain gages and the Hole Drilling Method (ASTM E 837). Strain gages were bonded on the surface of disk samples (2cm diameter) composed of a 1 mm thick Co-Cr alloy and a 2 mm thick veneering ceramic layer (Vita VM 13). The discs were manufactured following manufacturer recommendations. The hole drilling (0.1mm incremental steps) in the veneering ceramic induces residual stresses relaxation and then material deformation. This deformation was registered in surface by the electrical strain gages. The gages deformations values were then registered and encoded in HD Drill\ software (Vishay\), which calculates the residual stress profile.

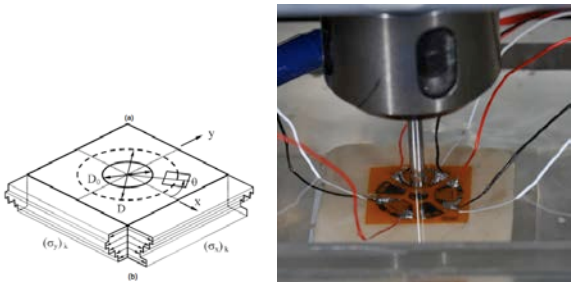

Figure 1: Strain gages and Hole Drilling Method

RESULTS: Because of the high sensitivity needed in comparison with industrial applications, a high sensitivity electrical measurement chain was developed (10 nanoVolts).

The changes brought to the original method allowed stress measurement at a 0.01 MPa sensitivity level. Compressive stress around 20 MPa was measured in surface of the veneering ceramic. This stress was found to decrease continuously with depth towards the framework.

DISCUSSION AND CONCLUSIONS: Even if time-consuming (several hours by sample), the hole drilling method was shown be a practical tool for measuring residual stress in veneering ceramics. It offers new perspectives in terms of biomechanical behavior analysis of restorations, notably about the influence of manufacturing process. The presence of compressive stress can be explained by thermal phenomenon's, which occur during cooling2. Future experiments should focus on zirconia-based samples in order to contribute to failures explanation.

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Influence of cooling rate on residual stress profile in veneering ceramic : measurement by hole drilling

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INTRODUCTION: The manufacture of dental crowns and bridges generates residual stresses within the veneering ceramic and framework during the cooling process. Residual stress is an important factor that control the mechanical behaviour of restorations. Knowing the stress distribution within the veneering ceramic as a function of depth can help the understanding of failures, particularly chipping, a well-known problem with Yttria-tetragonal-zirconia-polycrystal based fixed partial dentures. The objective of this study is to investigate the cooling rate dependence of the stress profile in veneering ceramic layered on metal and zirconia frameworks.

METHODS: The hole-drilling method, often used for engineering measurements, was adapted for use with veneering ceramic 1. The stress profile was measured in bilayered disc samples 20 mm in diameter, with a 0.7 mm thick metal or Yttria-tetragonal-zirconia-polycrystal framework and a 1.5 mm thick veneering ceramic. Three different cooling procedures were investigated.

RESULTS: The magnitude of the stresses in the surface of the veneering ceramic was found to increase with cooling rate, while the interior stresses decreased. At the surface, compressive stresses were observed in all samples. In the interior, compressive stresses were observed in metal samples and tensile in zirconia samples.

DISCUSSION AND CONCLUSIONS: Cooling rate influences the magnitude of residual stresses. These can significantly influence the mechanical behaviour of metal-and zirconia-based bilayered systems. The framework material influenced the nature of the interior stresses, with zirconia samples showing a less favourable stress profile than metal. The hypothesis of phase transformation of zirconia is introduced to explain this phenomenon.

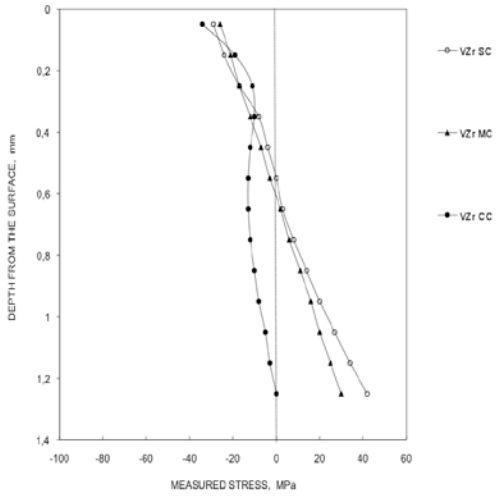

Fig 1: Residual stress vs. depth profiles in the veneering ceramic layer of VZr samples. CC= Classic Cooling, MC= Modified Cooling, SC= Slow Cooling.

REFERENCES: [1]A Mainjot et al. (2011) *Residual stress measurement in veneering ceramic by hole-drilling* Dental Materials 27: 437-444.

Attachment fatigue after clinical use: an electron microscopy and infrared spectroscopy study

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INTRODUCTION: Implant retained overdenture has been shown to provide a simple and cost-effective treatment to edentulous patient, but wear of attachment components were responsible for a decrease in retention. Patrix or matrix with plastic components or polymeric inserts were considered as the weakest link of these attachments and responsible for a short term maintenance after implant overdenture delivery.

The aim of this study was to analyse morphological and chemical degradations of the nylon surface of patrix attachment components after different periods of clinical use.

METHODS: The nylon inserts (DuPont Zytel 101L NC-10 Nylon 66) of 12 Locator patrix attachments (Zest Anchors, Escondido, CA, USA) were investigated after different periods of clinical use ranging from 1 to 6 months. 4 unused inserts were added and analyzed as a control group. Half of these specimens (8 nylon inserts) were examined by Scanning Electron Microscopy (SEM) (5 Kev, 10-10 A; JSM 6400, Jeol, Osaka, Japon) to assess the morphological changes of surface. A backscattered electron analysis has completed the observation of surfaces examined. After ultrasonic cleaning, two areas of 1 mm^2 located on the inner part of the remaining inserts were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) in attenuated total reflectance mode (Thermo Nicolet, Madison, WI, USA) to determine the surface chemical changes. The spectral range was between 4000 and 650 cm^{-1}.

RESULTS: The observations in SEM of control specimens showed a homogeneous material without presence of fillers or fibers. Changes in morphology and surface alterations were clearly very important, particularly visible for nylon inserts worn for 3 months. Plastic deformations and tears were present on all surfaces observed. No contamination by metal matrix has been found in the used nylon inserts. After background correction, FT-IR spectra of nylon components have demonstrated a significant difference in intensities and frequencies for amid bonds I and II in the polymeric chain of nylon (1700 cm-1 and 1500 cm-1) after clinical wear. Hydrothermal degradation of the material with mechanical wear has been revealed by a broad peak located between 3500 and 3000 cm^{-1} (O-H and NH stretches).

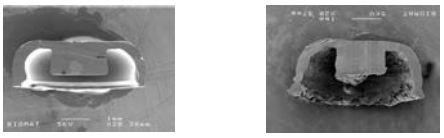

Fig 1: Vertical section of nylon inserts: Unused and after 3 months of clinical use (x20)

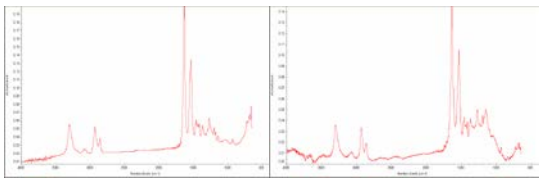

Fig 2: Infrared spectra of nylon insert: Unused and after 3 months of clinical use

DISCUSSION AND CONCLUSION: The results of this study show the low resistance of these unreinforced polyamide nylon inserts against hydrothermal and mechanical degradation. The hydrolysis and plasticizing of the material result in a decrease in the mechanical behavior of this polymer [1,2]. This present findings seem to be consistent with the poor biomechanical behavior of nylon inserts reported in the literature[3].

The surface of the nylon inserts analyzed show dramatic morphological and physico chemical changes despite a limited period of clinical use.

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A study of in-depth evolution of 3Y-TZP transformation

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INTRODUCTION: Zirconia high strength and fracture toughness aroused a general interest for this material in dental prosthodontics. Its properties come from the phase transformation induced by temperature and mechanical stresses. Partially stabilized zirconia, metastable at room temperature is used. Under mechanical stress, zirconia grains experience a tetragonal to monoclinic transformation. Hydrothermal conditions also trigger this transformation; this phenomenon is usually referred to as Low-Temperature Degradation. LTD is well studied in surface in 3Y-TZP. It leads to the apparition of surface roughness and microcracks in the first microns in depth[1]. LTD effects on zirconia dentures longevity are yet unknown[2]. It is of interest to know the in-depth transformation kinetics induced by water diffusion, an information unavailable with XRD (X-Ray Diffraction).

The aim of this study is to measure zirconia transformation rate after ageing under hydrothermal conditions, in depth, with Raman spectroscopy.

METHODS: Two XRD controlled pure tetragonal samples of 3Y-TZP (VITA®) were exposed to in vitro aging at 130°C in Ringer solution under a pressure of 6 bar for 10, 15, 25 and 90h. The monoclinic volume (Vm) was measured using Raman spectroscopy (HeNe laser, λ=632nm). The spectroscope is coupled with a confocal microscope. Objectives x10 and x80 are used to reach spatial resolutions at the micrometer scale (around 3 µm and 1 µm respectively). Confocal pinhole aperture is gradually enlarged to deepen laser penetration.[3]

RESULTS: Transformation rates were measured until a 70µm depth, with the x10 objective. The x80 objective enables a more accurate study of the 5 first microns

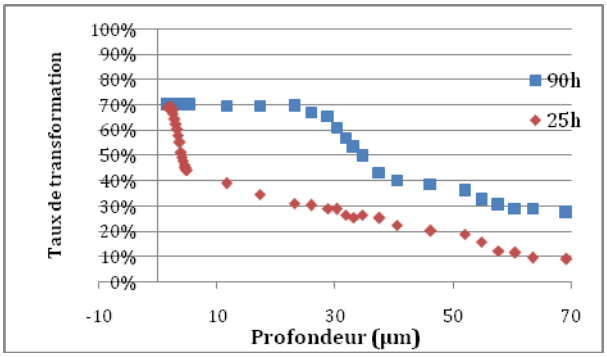

Fig 1: Transformation rates evolution between 1 and 70µm (obj x10)

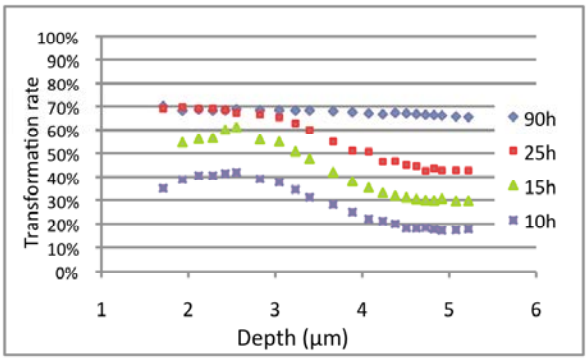

Fig 2: Transformation rates evolution between 1,5 and 5µm (obj x80)

DISCUSSION & CONCLUSION: Transformation rate reaches a maximum around 70%[4]. Vm decreases following an exponential law, as water diffusion triggers transformation. Transformation study after 10h and 15h ageing shows that Vm is maximal at a 2,5µm depth. Surface grains "pull-out", that is responsible for stress relaxation in surface, could explain that the monoclinic content is less important in the first two microns.

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Fit of unitary zirconia substructures on dental abutments: comparison between a cad/cam process and a mechanized manufacturing process

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INTRODUCTION: The fit of a restoration influences its biological and periodontal integration, its longevity, its optical properties, cement integrity, and health of the underlying abutment. Processes used for zirconia substructures manufacturing are usually CAD/CAM processes. A non-CAD/CAM system allowing the manufacture of zirconia substructures in laboratory appeared recently: the Ceramill® pantograph (Amann Girrbach). It's a copy-milling machine intended to manufacture ceramic restorations. The principle of this system is to copy resin wax-up in the prosthetic material by using homothetic properties. Nowadays, no study compared this system with a CAD/CAM process. The objective of this study is to compare internal and marginal fit of unitary zirconia substructures manufactured with Procera® (Nobel Biocare) and Ceramill® processes. To test the operator influence on the final substructures fit, two different laboratories were compared.

METHODS: 20 models of abutments prepared in vivo for all-ceram crowns were selected. Dies of prepared tooth were scanned and replicated in epoxy resin with the Procera system. Procera and Ceramill substructures were manufactured and evaluated on these replicas. To compare both processes, the fit evaluation was realized on couples of replicas coming from the same preparation. These replicas were set in a parallel way in a block of acrylic resin and substructures were cemented on their respective dies with Clearfil Esthtic Cement® (Kuraray). Replicas-substructures sets were sectioned centrally from buccal to lingual using a diamond saw (Accutom-50-®, Struers). 7 measures of internal gap (IG) were realized by substructure. Marginal fit was assessed in buccal and lingual position with two measures : the marginal gap (MG) and the absolute marginal discrepancy (AMD). Over- and under-extended contours were also estimated.

RESULTS: Means and standard deviations of IG, MG and AMD are illustrated in Figure 1. No significant difference of IG was found among coping groups ($p=0.13$). However significant differences were found for MG ($P<0.005$) and AMD ($P<0.0001$) between coping groups. Procera copings had significantly better MG and AMD than Ceramill copings. The percentage of over-extended margins for Ceramill copings was of 71% while thus for Procera copings was of 43%. Whether it is for IG, MG or AMD, no statistically significant influence of laboratory was demonstrated.

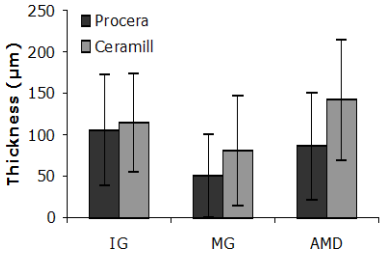

Fig 1. Means and Sds of IG, MG and AMD

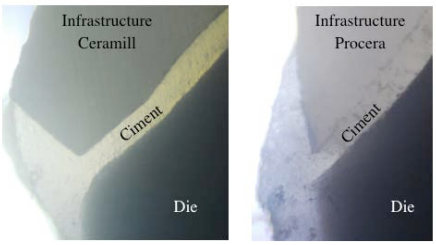

Fig 2. Procera and Ceramill substructures

DISCUSSION AND CONCLUSIONS: Marginal fit of both types of substructures is situated, according to the literature, in the clinically acceptable limits (<100µm).1,2 However, marginal fit of Procera substructures is significantly superior to Ceramill substructures. Furthermore, Ceramill copings present a high percentage of over-extended margins (Figure 2), what could have a detrimental effect on periodontal tissues.3,4 In the limits of this study conditions, the Ceramill mechanized process does not seem operator-dependent.

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A new class of resin composites via thermo-polymerization under high pressure

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INTRODUCTION: The evolution of restorative materials has resulted in biomaterials with improved mechanical, biological, and optical properties, such as ceramics and composites, responding to increased aesthetic demand from patients[1]. Furthermore, the emergence of CAD / CAM lead to improved consistency in quality and avoidance of laboratory associated errors. Composites are easy to machine and can be easily repaired; however, their mechanical properties are inferior to those of ceramics. The aim of this study was to obtain a new class of dental composite blocs, for CAD/CAM, by thermo-polymerisation under high pressure of four commercially available composites.

METHODS: Four dental composites: Gradia (GC Corporation), Vita VM LC (Zahnfabrik Vita, Germany), Grandio (VOCO, Germany), were selected for this study. The samples were made according to the ISO 4049 specification following two polymerization protocols: the control protocol was by photo-polymerization and the other one by thermo-polymerization under high pressure at 250 MPa.

The following mechanical properties were then evaluated: a) flexural strength and flexural modulus by three point bending tests, using a Lloyd LRX (Lloyd, UK) universal testing machine, with a cross-head speed of 1 mm/min and the NextGen® software; b) Vickers hardness (VHN) with a Microhardness tester (Metkon Microhardness MH3); c) fracture toughness[2] (KIC), by the notchless triangular prism (NTP) specimen method, using an Instron (model 4301, Instron Canada Inc.) at a cross-head speed of 0.01 mm/min. The density of the composites was determined using a method based on Archimede's principle. For each composite pair, the results were analyzed with student t-tests. .Weibull moduli were calculated for the flexural strength. Fracture surfaces were characterized by SEM.

RESULTS: Flexural strength, HVN, and density of samples obtained by thermo-polymerisation under high pressure were significantly higher than photo-polymerized ones for all the composites. Regarding KIC, only the thermo-polymerized under high pressure version of Grandio had a significantly higher value. Weibull moduli suggested that the new composites were more homogeneous.

DISCUSSION AND CONCLUSIONS: The results of this study suggestes thermo-polymerization under high pressure could introduce a new class of dental resin composites with superior mechanical properties, suitable for use by CAD/CAM. These findings may also open a whole new field of research.

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Fixatives for complete denture: An experimental study

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INTRODUCTION: The denture fixatives are still widely used and millions are sold each year. It is therefore the dental surgeon's duty to know how they should be used. However, few studies have been conducted on these materials. The major concern of patients with denture prosthesis is that it "holds" during oral functions[1]. Dental surgeons look for fixatives improving prosthetic retention, the best dosage form provoking a limited compression without iatrogenic effect to the supporting tissues. It is necessary to follow the design concepts of the denture for a suitable prosthesis.

METHODS: Two denture fixatives were tested: Neutral Fixodent ® Pro Care gel and Corega Ultra ® powder. Five compression tests were carried out:

1. without adhesive

2. with artificial saliva kind Artisial ®

3. Fixodent ® with 120 minutes immersed in Artisial ® with a pre-load of 20 N,

4. with Corega Artisial ®

5. Corega ® with immersed in Artisial ® 120 minutes with a pre-load of 20 N.

A resin disk, simulating the denture was pressed on a resin tank, representing the supporting tissues with the Instron 5544 machine. Tests 1, 2, and 4 were carried out directly with a preload of 1 N to 50 N, with a speed of 1 mm / min. Tests 3 and 5 were carried out in the same way, but after a preload of 120 N ² 20 minutes (Fig. 1).

RESULTS: This study shows that in term of compression the most favorable results are obtained with Corega ® Ultra powder form, when compressed 120 minutes with a pre-load of 20 N.

DISCUSSION AND CONCLUSIONS: Denture fixatives used according to the manufacturer's instructions increase the retention of dentures. The adhesive gel with a high viscosity may cause increased bone resorption and a lack of adaptation. The powder then offers a limited but sufficient retention for a suitable prosthesis.

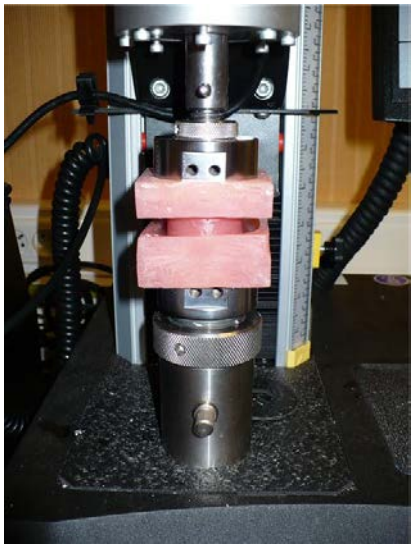

Figure 1: Test model on Instron 5544.

REFERENCES: [1]AJ Coates (2000) *Usage of denture adhesive*s J Dent 2:137-140. [2]R Koppang (1995) *A method for testing denture adhesives* J Prothest Dent 73(5):486-491

Record base in thermoplastic baseplate: Study of stability and stiffness

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INTRODUCTION: The success of a removable partial denture is related to its occlusal integration. The accuracy of registration of the intermaxillary relationship is related to the quality of baseplates. These thermoplastic baseplates (fig 1) reinforced by a metallic wire are commonly used to record the intermaxillary relationship. However, clinical experience shows sometimes a lack of resistance on the part of these baseplates (fractures, lack of rigidity, stability and accuracy...).

The objective of this work was to study different baseplates to determine which were more stable and more rigid.

METHODS: Different record bases stabilized with impression paste Eugenol ZnO (Table 1) were tested using compressive test.

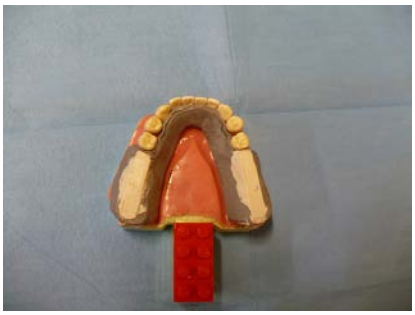

Fig. 1: thermoplastic record base n°4 on the silicon model.

Table 1: Different record bases tested.

Record base n°1	Single layer	Without metallic wire
Record base n°2	Single layer	With metallic wire
Record base n°3	Double layer	Without metallic wire
Record base n°4	Double layer	With metallic wire

Compressive tests (universal testing machine, Instron ® 5544) were performed on these models at a speed of 1 mm / min with a load of 5N. The vertical displacement (depression) and the horizontal deformation of the record bases were measured (extensometer Instron ® 2620-602).

RESULTS: The lowest vertical displacement is obtained with the record base n°4 with a double layer baseplate and a metallic wire. The record base n°1 with a single layer baseplate and no metallic wire is the one that sinks the most.
The model showing the lowest horizontal deformation is the record base n°3 with a double layer baseplate and no metallic wire, the record base n°1 with a single layer baseplate deforms the most.

DISCUSSION AND CONCLUSION: Record bases made with thermoplastic baseplate are not rigid enough and should always be strengthened. However, the addition of a reinforcing metallic wire does not bring the expected results. The use of a second baseplate layer seems to increase the rigidity of the base. Our study shows that the lowest deformation and displacement are finally obtained with the record base with a double layer baseplate and no metallic wire. A finite element analysis will be conducted to improve our experimental model.

REFERENCES : [1]GN Graser *Completed bases for removable dentures* J Prosthet Dent 1978; 39:232-236 [2]A Harrison et al *Some physical and mechanical properties of shellac dental baseplate materia*l J Oral Rehabil 1995; 22:509-513 [3]A Azouka et al. *The production of shellac and its general and dental uses: a review* J Oral Rehabil 1993; 20:393-400.

Implanto-prosthodontic vinylpolysiloxane and polyether open-tray impression in vitro: a three dimensional preliminary study

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INTRODUCTION: The passive insertion of prosthetic elements is mainly conditioned by the reliability of the impression and is particularly important for the clinical success of treatments involving implant-supported prosthesis1,2. Regarding of numerous techniques and materials used for impression in implantology, we have developped an experimental procedure designed for the evaluation of their dimensional reliability. In this preliminary study, a vinylpolysiloxane (Aquasil Ultra Monophase® DECA) was compared with a polyether (Impregum Penta Soft®), on the scope of open-tray impressions with coping system.

METHODS: 30 Aquasil® impressions and 30 Impregum® impressions were performed on a steel reference model composed of three implant analogs (3.4 mm in diameter, distanced by 10, 20 et 22 mm). Impressions derived stone casts and the steel reference model were both measured by a video measuring system (Nikon® Inexiv VMA-2520) (fig.1). 12 variables (distances, angles, heights) were measured on each models in 3D. In order to evaluate and to compare the dimensional reliability of the 2 impression materials, each stone casts group was compared to each other and with the steel reference model.

Fig.1 : Evaluation of the dimensional reliability with the video measuring system Nikon® Inexiv VMA-2520.

Statistical analysis was assessed by a Student's t distribution test ($\alpha = 0.05$)

RESULTS: The standard error is less than one micrometer for the heights (z axis) and is comprised between 2 and 3µm for the distances (x and y axis). The standard error is of the same magnitude than the measurement accuracy (1µm), which goes to show the reliability and the repeatability of the measurement method. Concerning the variables measured, there is a lot of significant differences between the steel reference model and the 2 groups of stone casts : 10 of the 12 variables measured are significantly different for Aquasil® and 9 of 12 are significantly different for Impregum® ($p<0.05$). On the other hand there is few significant differences between the 2 groups of stone casts: 2 of 12 variables ($p<0.05$).

DISCUSSION: It appears that even with in vitro conditions, it is impossible to reproduce exactly the initial situation. But interestingly, Aquasil® can produce stone casts slightly similar to those produced by Impregum®.

CONCLUSIONS: On the scope of open-tray impressions with coping system, the clinician will make his choice between Aquasil Ultra Monophase® and Impregum Penta Soft® according to another criteria than the dimensional reliability. The thixotropy, the hydrophily or the dimensional stability will be taken into account. The experimental procedure designed in this study will be used to evaluate the dimensionnal reliability of other materials or techniques used in implantology.

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Electrodeposition of zinc, copper, aluminium and their alloys from ionic liquids

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INTRODUCTION: Zinc (Zn), copper (Cu), aluminium (Al) and their alloys have attracted much interest owing to their several current or potential applications in many fields: aeronautical, automotive, household appliances, building trade and biomedicine.

New solvents for metal electrodeposition : air- and water-stable ionic liquids have received interest for their : low melting points, good conductivity, extremely low vapour pressure, low toxicity, non volatility, high electrochemical stability, wide electrochemical window (4-6 V) and nonaqueous nature. At room temperature they allow the synthesis of good quality metallic deposits and the deposition of highly reactive metals such as aluminium, magnesium, titanium and tantalum.

METHODS: The present work reports on the electrodeposition or the attempt of electrodeposition at room temperature of zinc, copper, aluminium and copper-zinc and copper-zinc-aluminium shape memory alloys on various substrates in the second generation ionic liquid 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)amide [BMP]NTf2, air- and water- stable considered, in which the metallic salts Zn(NTf2)2, Cu(NTf2)2 and/or Al(NTf2)3 were dissolved.

To the best of our knowledge, we report also the first try to obtain shape memory alloys Cu-Zn and Cu-Zn-Al nanowires by means of electrodeposition on polycarbonate membranes. Recently, nanowires have received much interest for their unique properties. They increase the specific surface-area of the material; they can also increase the properties of many composite materials by their integration inside; their study should help us to better understand the materials properties at nano-, mico- and macroscopic scales.

In this work, the electrochemical study was performed by means of cyclic voltammetry. The morphology and chemical analysis were investigated by scanning electron microscopy combined with energy dispersive X-ray analysis (SEM/EDX); the crystallographic characterization was performed with X-ray diffraction (XRD)

RESULTS: The studies showed that water and oxygen presence in [BMP]NTf2 ionic liquid seemed to accelerate the decomposition of this solvent. Many decomposition products were found in the deposits and at deposition potentials were smaller than the reduction potential of the solvent.

Most of Zn, Cu and Cu-Zn deposits were uniform, dense, well adherent and crystalline. The morphology and the composition of the deposits were influenced by many parameters: presence of degassing of the ionic liquid before deposition (pumping under vaccum during 48 hours), voltage applied value and deposition duration.

DISCUSSION AND CONCLUSION: We encountered many difficulties obtaining aluminium deposits. Those were always poor in aluminium, rich in monoxide suggesting a formation of alumina, presented many cracks and were discontinuous.

In XRD patterns, no peaks characteristic of aluminium were noted. Contradictions of composition between Cu-Zn flat deposits and Cu-Zn nanowires could be explained by a probable competition of adsorption on the substrate between Zn(II) and Cu(II). We succeeded in realizing Cu-Zn and Cu-Zn-Al deposits and nanowires by electrodeposition but no alloy was detected. Besides, the nanowires composition was inhomogeneous.

The properties of the dentine/self-etching adhesive/resin-modifed glass ionomer interphase

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INTRODUCTION: Resin-modified glass ionomers (RMGI) have a number of advantages: they are hydrophilic, have an intrinsic adhesion to dental structure because of chemical interactions[1] and a long term release of fluoride[2]. However their dentin bond strength is low compared to the couple adhesive/composite resin. This is the reason why it was suggested to combine self-etching adhesive (SEA) with RMGI. The dentin bond strength is then increased from 50 to 130% in vitro[3,4]. During bonding procedure in the oral cavity, moisture and salivary contaminations can affect the quality of the dentin/adhesive interface. RMGI are considered less susceptible to moisture contaminations, but no study has analyzed the dentin bond strength of the combination SEA/RMGI in presence of moisture or saliva. The aim of this study was: - to compare the dentin shear bond strength of RMGI, SEA/RMGI and SEA/composite resin - to test the effect of moisture and salivary contaminations on the dentin bond strength of the association SEA/RMGI.

METHODS: Ninety freshly human teeth were collected, cleaned of soft tissue, stored in a solution of 1% chloramine-T at 4°C and used within three months. The teeth had been extracted for reasons unrelated to the objectives of this study and with the patients' informed consent. Their roots were removed and the buccal surface of the crowns abraded on water-cooled sandpaper using a polishing machine, to expose a flat dentin surface (>7 mm2), onto which a cylinder of RMGIC could be formed and bonded. The residual crowns were embedded in self-cured acrylic resin in plastic mold with the flat dentin surface exposed. The samples were randomly divided into 9 groups of 10. Materials: - as SEA: I Bond® self-etch (Heraeus) - as RMGI: Fuji II® LC (GC) - as composite resin: Z100® (3M Espe) - as polyacrylic acid: Cavity conditioner® (GC). The following experimental groups were tested: - no dentin treatment, RMGI - polyacrylic acid, rinsing, drying, RMGI - SEA, drying, light curing, RMGI - SEA, drying, light curing, composite - salivary, SEA, drying, light curing, RMGI - SEA, drying, light curing, salivary, RMGI - water, SEA, drying, light curing, RMGI - SEA, drying, light curing, water, RMGI. According to these experimental groups, cylinders of composite resin or RMGI were placed onto the prepared dentin surfaces. The samples were stored in 37°C water and sheared after 24h.The failure types were noted. For each tested series, an additional sample was prepared and cut vertically in order to obtain a cut as close to a diameter of the cylinder. The interfaces obtained were polished and the samples prepared for electron microscopic scanning. The bond strength values were analyzed with an ANOVA test, followed by a PLSD Fisher test.

RESULTS: Means and standard deviations of shear bond strengths (in MPa) for the various groups tested are summarized in the table 1.

Table 1 Means and standard deviations of shear bond strengths

Experimental groups tested	Dentin shear bond strength (MPa)
RMGI	$5\pm1,3^{a}$
Polyac Ac+RMGI	$8\pm1,6^{b}$
SEA+RMGI	$15\pm2,4^{d}$
SEA+composite	$18\pm8,2^{d}$
sal+SEA+RMGI	20 ± 7^{d}
SEA+sal+RMGI	19 ± 6^{d}
water+SEA+RMGI	16 ± 2^{d}
SEA+water+RMGI	21 ± 4^{d}

Values with the same online small capital letter are not significantly different at p=0.05.

CONCLUSION: (1) The association SEA+RMGI triples the dentin bond strength of RMGI and shows no significant difference with the association SEA+composite**.** (2) The association SEA+RMGI could tolerate moisture**.** (3) The association SEA+RMGI could tolerate the salivary contamination**.** Clinical studies are needed to confirm these in vitro results.

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The adhesion/decalcification mechanism of selected self-etch adhesives

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INTRODUCTION: Following the adhesion/ decalcification (A/D) concept, acid monomers attach to the calcium in hydroxylapatite HA crystallites in a first step, then dissolve and carry away the calcium ions in a second decalcification step. We wanted to clarify whether all acid monomers reacted this way.

METHODS: Four self-etch adhesives (Optibond All-In-One, Kerr, Orange, CA, USA (Oa); Adper Easy Bond, 3M ESPE, StPaul,MN,USA (Ae); XenoV (Dentsply, De Trey, Konstanz, Germany (Xv); AdheSE One, Ivoclar Vivadent, Schaan, Liechtenstein, (A1).) were tested for acid strength, concentration and calcium dissolution potency on dentine powder1. Potentiometric determinations (Metrohm 827 pH meter) were carried out to monitor acidity, and the presence of organic and mineral components was analysed by infrared spectroscopy (Mattson genesis) and thermogravimetric analysis (SDQ600, TA Instruments, Courtaboeuf, Fr.)

RESULTS: Two products (Ae and Oa) had low acid concentrations (0.99 and 1.24 mmol/g), the other two (Xv and A1) having twice as much acid (2.07 and 2.28 mmol/g).

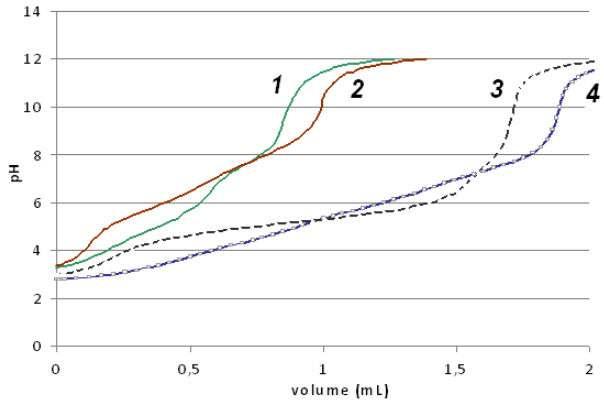

Fig.1. Titration curves of the self-etch adhesives studied, 1= Oa; 2= Ae; 3= Xv; 4= A1.

Titrations curves showed inflexion points with corresponding pKa values between 3.0 and 7.8.

Table 1: pKa values and acid concentrations of the adhesives studied.

	Ae	Oa	Xv	A1
pK1	3.7	4.5	3.2	3.0
pK2	6.2	7.8	5.2	5.0
[H+]	0.99	1.24	2.07	2.28

Upon contact with dentine powder, the acids were buffered by the dentine HA, and the Organic/mineral ratios changed. Xv was the adhesive that released the most calcium ions into solution whereas Oa released the least. Oa showed strong residual adhesive infrared absorptions after reacting on dentine whereas Xv left only small traces. The organic/mineral ratios increased significantly for Ae and Oa.

DISCUSSION AND CONCLUSIONS:
The weaker acid dissolved less calcium but increased more the organic/mineral ratio. This indicates deposition of organic adhesive components on the dentine surfaces[2]. The acid monomers all react with the HA phase in dentine (the adhesive step), but only the stronger acids proceed to the decalcification step (debonding) by dissolving the calcium salt. Regardless of their pKa values, both weak and strong acids bind to the HA calcium ions. The extent of demineralisation then depends on the solubility of the calcium salts formed. Solvent compositions and polymer hydrophobicity[3] will also contribute to long term stability of the interface in the hybrid layer. Less acid left over might preserve the collagen on the long term.

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Hydrolytically stable acidic monomers used in two-steps self etching dental adhesives

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INTRODUCTION: Two-step self-etching adhesives system (SEA-2) combine etching and priming into one step which is usually achieved by using derivatives of methacrylic acid monomers. Under the necessary acidic conditions required to achieve etching, most of the monomers employed undergo hydrolysis during storage resulting in a loss of their bonding ability1. In this context, we developed SEA-2 systems comprising methacrylic acid monomers and novel acrylamidophosphonic acid monomers to investigate their stability after prolonged storage.

Our objective was the assessment of the impact of storage on monomer degradation and shear bond strength on dentin.

METHODS: Experimental SEA-2 where composed of experimental self-etching primer (SEP) made with either (methacroyloxy) phosphonic acid (EMM) or (acrylamide) phosphonic acid monomers (EAM) used with a commercial bonding resin (AdheSE, IvoclarVivadent, Schaan, Liechtenstein). Each synthesized product was characterized by 1H-NMR, 13C-NMR, 31P-NMR and by High Resolution Mass Spectroscopy.

Experimental groups were constituted as follows: fresh EMM based SEP (MF), fresh EAM based SEP (AF), 18 months 4°C stored EMM based SEP (M18), 18 months 4°C stored EAM based SEP (A18).

Storage stability for each monomer and each experimental group was assessed by detection of degradation byproducts with31P-NMR and HPLC-MS while adhesive properties were measured by Shear Bond Strength tests (SBS) on batches of 10 samples for each experimental group. Statistical analysis was done with a Wilcoxon test for time of storage and Mann-Whitney test for monomer at a significant level of .05.

RESULTS: 31P-NMR coupled with HPLC-MS showed no degradation for acrylamide based monomers and SEP while methacrylate based monomers and SEP had a degradation ratio approaching 80%. SBS results are given in table 1.

	Mean SBS ± SD (MPa)	
storage	none	18 mo. 4°C
EAM	16.2±3.0[a,c] (AF)	18.0±3.5[a,d] (A18)
EMM	15.1±2.4[b,c] (MF)	16.0±6.4[b,d] (M18)

Table 1: Results of SBS tests ± SD (Given in MPa). Results with same letter in superscript are not significantly different.

DISCUSSION: Our results correlate with current data where degradation of methacrylate based monomers in aqueous acid solution is a proven phenomenon. However, its influence on adhesion isn't established in our study. As no significant difference was found between a stable SEP and degradated SEP, we proposed a different adhesion mechanism. Given the type of degradation by-products of methacrylate based monomers1, an adhesive layer based on ionic interactions instead of covalent liaisons obtained after polymerization could be envisioned2,3.

CONCLUSIONS: Acrylamidophosphonic acid based monomers appear as potent candidate for use in dental adhesives as they are equivalent to their homologous hydrolytically degradable methacrylate based counterpart in terms of bond strength and resist hydrolysis in acidic aqueous conditions. Concerning the degradation of methacrylates, further investigation should be conducted to establish their behavior towards aging.

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Evaluation of leakage and degradation mechanisms of modern self-etching adhesives: an in vitro study

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INTRODUCTION: The sealing of a restoration is one of the most important factors for clinical success of our adhesive restorations. The review of literature shows that the use of self etching adhesives leads to degradation mechanisms at the interfaces in terms of leakage. The purpose of this study was to evaluate two degradation causes of recently commercialized self etching adhesives by two different methodologies analyzing microleakage and nanoleakage.

METHODS: Materials: Adper Easy Bond® ADP (3M), G-aenial Bond® GB (GC) et OptiBond XTR® OPB (Kerr), Clearfil SE Bond® SEB (Kuraray)). Method 1: Microleakage Evaluation : preparation of Class V cavities, obturation with SAM and Z100, thermocycling, silver nitrate dye solution, sections, optic microscope observations, enamel and dentinal microleakage quantification (%), statistical analysis. Method 2: Nanoleakage Evaluation: realization of flat dentinal surfaces, obturation with SAM and Z100, thermocycling, ammoniacal silver nitrate dye solution, sections, MEB observation.

RESULTS & DISCUSSION:

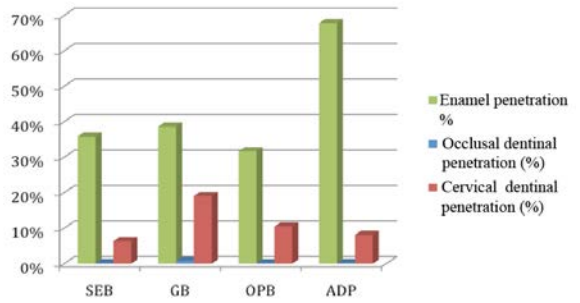

Fig. 1: percolation bar graphs (%)

SEB presents good enamel and dentinal results: the 10-MDP is able to create chemical bond with the hydroxyapaptite. At the enamel margin, ADP presents the most severe leakage with significant differences with the three other systems. At the cervical dentinal margin, the worst results are obtained with GB, SEB presents significant differences with OPB and GB.

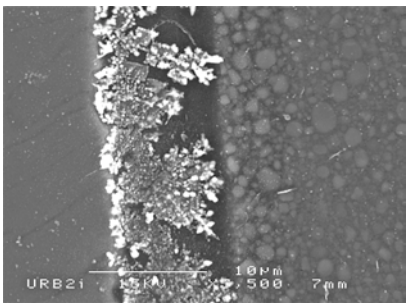

Figure 2 : MEB observation of the GB/dentine interface : presence of water trees

Our MEB observations show that, whatever the adhesive system, degradation mechanisms are observed at the adhesive interface but the extent and the quantity are different. This phenomena is called «water trees » due to movements of water. The origins are the separation of phase, the incomplete polymerizaton of the adhesive resin layer or the formation of HEMA hydrogels.

CONCLUSION: This in vitro study shows that modern self etching adhesives are able to present in terms of leakage some results comparable to classic system adhesives but significant differences between them may exist. SEB seems to be always a gold standard.

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Study of the compatibility of 5 self etching adhesives associated with two composite cementation materials

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INTRODUCTION: The arrival of the new self etching bonding systems (SEBS) revealed a problem of incompatibility due to the new systems initiators of the polymerization of resin luting cements (RLC). The aim of this study was to assess the compatibility between five SEBS and two RLC (a dual curing RLC and a self curing RLC) by in vitro dentin-RLC-titanium SBS determinations. The idea is to observe the influence of the bonding associated on the one step SEBS (SEBS1) compatibility.

METHODS: The RLC tested was Variolink II® (dual cure) and Multilink® (self curing). The SEBS associated were 2 one step SEBS (SEBS1) : One Up Bond F® (OUBF) and Xeno III®(X3) and three two steps SEBS (SEBS2) (Clearfil SE Bond®(CSEB), Unifil Bond®(UB), and one experimental association of the one-step Xeno III® as a primer, followed by the application of the bonding resin of Clearfil SE Bond®, (X3+bCSEB). 150 molars were tested. Ten experimental groups were carried out according to the nature of both luting and adhesive materials procedure employed. The specimens were stored in 100% RH, at 37°C for 7 days, then were tested in shear mode.

RESULTS: The bond strength results were significantly influenced by the adhesive type whatever the coupled RLC dual or chemical curing. SEBS2 were compatible with RLC wathever the polymerization mode. SEBS1 (X3 and OUBF) revealed incompatibility when it coupled with chemical curing RLC. The experimental coupling X3CSEB for Multilink and CSEB and OUBF for Variolink exhibites the highest adherence values. There is a linear correlation between pH and adherence values.

DISCUSSION: The shear bond strength of the Multilink/X3CSEB adhesive interface is higher than that of Multilink/X3. Multilink tertiary amine was probably protected from the X3 acidity by the resin layer of CSEB.

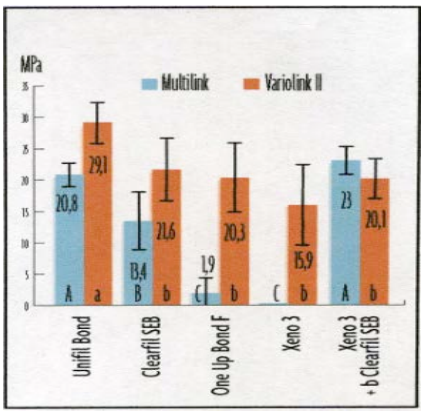

Figure 1 Means of shear bond strengh results (MPa).

However, the pH of bCSEB is lower than X3 and the RLC polymerization seems not affected to its contact. This is an element which lets supposes that the pH is not the only parameter to be considered in the interface quality. We can note for Multilink, the better performance of UB (+28%) or the test group X3CSEB (+42%) compared to its manufacturer adhesive. The fact that UB contains 4-MET may explain these results

CONCLUSION: SEBS1 are less reliable than SEBS2 under our experimental conditions. The incompatibility of SEBS1 and self-curing RLC is not only due to pH and inactivation of the tertiary amine. It also depends on the individual composition of adhesives, drying, water diffusion intrinsic to the dentin adhesive interface.

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Degree of conversion of composite resins and dental adhesives using raman spectroscopy: application to the measurement of oxygen-inhibited layer

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INTRODUCTION: Raman spectroscopy is a non-destructive technique, based on the detection of inelastically scattered photons, following interaction of the sample with a beam of monochromatic light. The frequency difference between laser photon and scattered photon provides information on the chemical nature of the molecule responsible for scattering. The Raman spectroscopy is used for some years to measure the degree of conversion of dental composite resins (Gauthier et al, 2005a) The purpose of this study was to quantify the degree of conversion of different dental composite resins and characterize the oxygen inhibited layer (Truffier-Boutry et al, 2003; Gauthier et al, 2005b).

METHODS: Six materials were selected for this study with different types of composites and adhesives, either: two restorative composites resins (Tetric EvoCeram and Grandioso), two flowable composites (Supreme XTE Flow and SDR Flow), two enamel-dentin adhesives (Scotchbond Multipurpose and Easy Bond). Raman spectra were obtained through a Confocal Raman Microscope WiTec ® Alpha 300R (Witec GmbH, Ulm, Germany). The optical resolution is limited to 200 nm laterally and 500 nm vertically. The spectral resolution is less than 4 cm-1. The excitation source is a He-Ne laser with a 632.8 nm wavelength and an output power of 35mW. The pinhole diameter is 50 micrometers. An x20 objective with a numerical aperture of 0.46 was used. The used grating had 600 lines per millimeter. The calculation of the degree of conversion was determined by the formula:

$$DC\% = 100 - \frac{\left[Peak1640cm^{-1}/Peak1610cm^{-1}\right]polymerized}{\left[Peak1640cm^{-1}/Peak1610cm^{-1}\right]notpolymerized} x100 \quad (1)$$

The degree of conversion was determined for each material, and then the thickness and the degree of conversion was quantified for Oxygen –inhibited layer.

RESULTS: The degree of conversion was calculated, with single point spectrum, for each tested materials on the surface polymerized in air. Fluorescence is a varying parameter which can disturb the measurements. For the degree of conversion, there is variation between different materials of the same class, but especially between the 'adhesive systems' class and the 'restorative composite resins' class. Oxygen-inhibited layer thickness and conversion gradient was quantified with line spectrum and 2D scan mode with and without cluster analysis.

DISCUSSION AND CONCLUSIONS: Raman spectroscopy is a very powerful technique suitable for the analysis of the degree of conversion of dental composite resins and adhesives and quantification of the oxygen-inhibited layer for methacrylate resins. The management of fluorescence is an essential parameter during the analysis of dental resin composites by Raman spectroscopy. 2D representation coupled with cluster analysis provides more precise information on the chemical mapping of the oxygen-inhibited layer.

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Are pit and fissure sealants toxic?

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INTRODUCTION: Pit and fissure sealants (PFS) indicated to seal deep pits and fissures[2] in high caries risk patients on their premolars and permanent molars, have been considered by the media with suspicion because they may contain BPA or its derivates a toxic agent[1] and endocrine disruptor. Questions are asked concerning the safety of those materials[3].

METHODS: This presentation resumes scientific knowledge about pit and fissure sealants (PFS) that may contain BPA or its derivates.

RESULTATS: The fear of a possible toxicity of the PFS arises mainly from works, which, from 1996, mentioned the release of BPA from PFS[4, 5]. The ADA takes a clear position minimizing the risk related to BPA in pits and fissure sealants6.

DISCUSSION: To reduce the possible toxic potential of the PFS[7], the practitioner should use, after placing the PFS, a light abrasive on the surface of the polymerized product to eliminate the monomers possibly remaining on the surface and, for older children, to rinse their mouth for 30 seconds with tepid water, or to wash the surface with tepid water with the water-air syringe for 30 seconds, with aspiration of the water and debris from the child's mouth.

CONCLUSION: The pit and fissure sealants do not present any danger for young patients. A possible exposure to BPA could only happen in the first hours following the placement A cleaning of the sealed surface, as described above, eliminates the possible traces of BPA.

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The danger due to the release of components by composite resins

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INTRODUCTION: Composite resins, dental adhesives and pit and fissure sealants (PFS) release components in service, and some among them like TEGDMA, are recognized as being dangerous for the body. Recent studies from the Walther Straub Institute for Toxicology and Pharmacology in Munich have shown without doubt that all composite resins, dental adhesives and PFS do release components whose nature and quantity vary greatly from one product to the other. The objective of this presentation is to describe these studies and to evaluate through them the possible consequences on the human health.

The release of components is obtained by immersion in water, methanol or ethanol. The analysis of the eluted products is mainly done by gas chromatography1. The evaluation of the cytotoxicity is obtained by the XTT test2, or by impedance (xCELLigence system)3, and the genotoxicity by the COMET test4.

The tested products release various types of components in various quantities. Among the main components, triethyleneglycol-dimethacrylate (TEGDMA) and hydroxyethyl-dimethacrylate (HEMA) are the most frequently detected. No trace of bisphenol A (BPA) has been found.

HEMA is the less harmful component for the cells, but all the released components have shown some cytotoxic effect. The COMET assays have shown a slight genotoxic effect of the released components.

Concerning TEGDMA, it has a well known sensitizing effect.

One PSF in particular is known for having released traces of BPA by salivary degradation of one of its components: the bisphenol-dimethacrylate (Bis-DMA). Most of the recent PFS, however, do not contain Bis-DMA anymore, and thus this potential danger is eliminated.

Most of the tested products have shown a more or less important release of components, and the tests for biological evaluation could not totally exclude some risk, except for the products which do not contain co-monomers. The best possible protection is therefore to use products (composite resin, dental adhesive and PFS) which do not contain TEGDMA or HEMA. Such products exist and, despite the absence of co-monomers, they offer very good clinical properties associated with a quasi-elimination of biological risks.

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Resorbable and antibacterial cement for dental applications

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INTRODUCTION: For some decades, bone substitutes were developed for small bone defects filling. Among these substitutes, phosphocalcic cements are biocompatible and osteoconductive materials.[1] Moreover, their moldability allows a perfect filling of geometrically complex cavities found in dental surgery. However their rate of resorption needs to be enhanced.

The aim of the present study was to demonstrate the feasibility of an antibacterial and resorbable cement by addition of silver in the paste. The material we focused here on is a cement including a calcium phosphate (apatite analogous to bone mineral) and a metastable calcium carbonate (vaterite). The metastability of the latter should lead to a faster cement resorption.[2] In addition, since Ag+ ions were found to have an antibacterial activity, particularly in some apatitic coatings[3], silver salts were introduced in the cement in order to prevent or to treat possible post-operative infections which could be associated with implant and implantation. The cement injectability associated with its resorbability and its antibacterial or even bactericide properties could also find an application in the surgical treatment of bone lysis, for example due to pathologies such as periodontitis.

METHODS: The cement was prepared by mixing a mineral solid phase with a liquid phase (deionised water). In the reference cement, the solid phase was made by mixing equal weights of dicalcium phosphate dihydrate (DCPD; $CaHPO_4$, $2H_2O$) and vaterite $CaCO_3$. Otherwise, different silver salts - $AgNO_3$, Ag_3PO_4, Ag_2CO_3, Ag_2SO_4 – were introduced into the solid phase to obtain 5 wt% Ag-loaded cement pastes. The structure and the composition of the cements were determined by FTIR spectroscopy, X-Ray diffraction and scanning electron microscopy analyses at different times during the setting and hardening. The setting time was determined by the Gillmore needles standardized method. The antibacterial activity of the different silver salts was first evaluated by determining MIC and MBC values against referenced strains of S. aureus and S. epidermidis. Then, the antibacterial properties of silver-loaded cements (Ag_3PO_4 in the solid phase or $AgNO_3$ in the liquid phase) were studied.

RESULTS AND DISCUSSION: Whatever the salt initially introduced, Ag_3PO_4 was always formed in the hardened cement. It indicates that in all cases silver did not take part significantly into the setting reaction. However some differences were observed in the setting kinetics and the setting time in the physical properties as well as the chemical reaction leading to apatite formation. On the other hand, 10 ppm of Ag^+ was found to be an inhibitory and lethal concentration for the strains, which were tested. Finally, the in vitro colonisation of the staphylococcus was inhibited when in contact with Ag-loaded cements.

CONCLUSION: Calcium carbonate - phosphate mixed cements doped with silver seem to be promising candidates as resorbable and antibacterial bone substitutes for dental applications. We can expect a prolonged release of the antibacterial agent (Ag) controlled by the cement resorption. Finally, silver seems to be an interesting alternative of the widespread use of antibiotics, which can induce some bacterial resistance.

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Choosing the allocation method for clinical trials in restorative dentistry

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INTRODUCTION: Treatment allocation in restorative trials is generally defined by pre-set random tables. However, this method does not account for prognostic factors of the restorations and does not respect allocation concealment, so that treatment groups are not comparable and conclusions are biased, all the more since the trials are small-sized. Comparable groups are obtained by optimizing balance between groups and limiting allocation predictability. Two methods could be used in restorative dentistry to achieve comparability: stratified blocked randomization and minimization. Stratified randomization consists in using a separate randomization list for each prognostic group. Minimization is an adaptive method that minimizes the imbalance between the number of patients in each treatment group over a number of prognostic factors. The objective of this study was to compare stratified randomization and minimization in terms of predictability and balance, with the example of a planned trial in restorative dentistry intended to compare ceramic and composite CAD-CAM inlays.

METHODS: A Visual Basic for Applications program was computed. 1000 sets of 350 patients were simulated according to the proportions of patients expected for each of the four main predictive factors (inlay/onlay, premolar/molar, vital/non vital tooth, operator). These patients were allocated by minimization with a varying random element and stratification with blocks of 2 and 4. Allocation methods were compared in terms of predictability and balance.

RESULTS: The balance obtained with blocked randomization was better than expected, although minimization proved to better account for balance and predictability at the same time. Minimization with a random element of 30% achieved the lowest imbalance (0.51% of the sample size) and predictability (52.68% when operator remembered his three last allocations).

DISCUSSION: There are not many limitations to this study, except its generalizability: the results correspond to a given situation and simulations need to be tailored to each trial (number of prognostic groups and classes, size of the trial).

Although minimization can be a useful tool for small and open trials since it can deal with a number of prognostic factors while maintaining balance and non-predictability, it has never been used in dental trials.

This is probably due mainly to the difficulty in implementing a minimization.

CONCLUSIONS: Minimization with a 30% random element allowed to account for four prognostic factors in the planned clinical trial and achieve excellent group comparability. Simulations can help decide which allocation method is best for a clinical trial in restorative dentistry.

Minimization could be an interesting alternative in dental trials.

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Microleakage of an experimental cement used as dentin substitute

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INTRODUCTION: The aim of this study was to compare and to observe the microleakage of an experimental cement (RD94), at present marketed (Biodentin, Septodont) used as dentin substitute in the " opened sandwich " technic for the restoration of cavities of class II with regard to that of a resin modified glass ionomer cement (RMGIC, Fuji II LC, GC).

METHODS: 84 class II were realized on mesial and distal faces of every 42 extracted third molars. Cavities were randomized in 6 groups. The dentin substitutes were used as the RD94 (Septodont) to fill the first third of the cavity, excepted for groups 1 and 2 having been totally filled:

G1: RD94; G2: Fuji II LC; G3: RD94 + Optibond Solo Plus® + silane + Filtex Z250®; G 4: same as group 3, without silane; G5: RD94 + Septobond® + Filtek Z250®; G6: Fuji II LC® + Optibond Solo Plus® + Filtek Z250®.

Teeth were thermocycled 2500 times in two baths of water (5 and 55°C, dwell time of 20s). The tracer used for the microleakage evaluation was the silver nitrate (Ag NO3); 50 % in weight). Teeth were then embedded in a resin (Scandiplast®) and three sections were realized through the restorations (6 measures by interface for every restoration). The microleakage was estimated by ordinal scores.

All the measures were realized at the level of the interface composite enamel / resin, resin composite / dentin substitute (dentin bonding agent), dentin substitute / dentin (cervical wall). The penetration was evaluated separately by two examiners.

The maximum score found among the 6 evaluated surfaces per restoration was selected to summarize the information.

RESULTS:

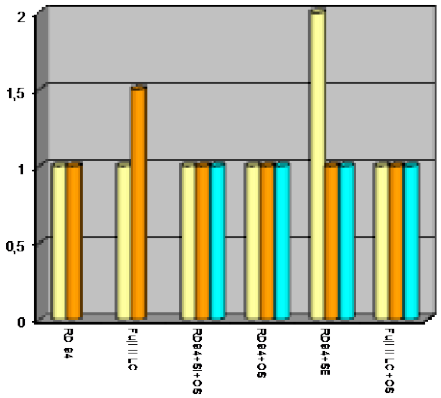

Figure 1: Median of the maximum scores of microleakage at the enamel (yellow), dentin (orange) margins and at the interface dentin substitute/ composite resin (blue)

No statistical significant difference was registered between every group, excepted for the group 5 at the enamel limit.

DISCUSSION AND CONCLUSION: The RD94 had a behavior similar to the RMGIC (Fuji II LC®) with biological (no cytotoxicity by induction of pulp fibroblasts in odontoblasts cells) and mechanics (better compression resistance) properties, superior to the RMGIC as well as an easier implementation (no cavity conditioning walls). The RD94 is also more tolerant with the humidity but is more time consumer.

Liquid diffusion in dental composite resins by impedance spectroscopy

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INTRODUCTION: The technic of electrochemical impedance spectroscopy is applied to the characterization of diffusive and absorption properties of water in dental composite resins.

OBJECTIVES: Following water immersion, assessment using impedance spectroscopy allowed estimation of diffusion rates and water uptake. In parallel, classic gravimetric experiments were undertaken on free films of the same material. Results of the two techniques are compared.

METHODS: A typical electrochemical impedance spectroscopy experimental set-up consists of an electrochemical cell, a potentiostat and a frequency response analyser, which also supplies the initial voltage. The frequency response analyser applies a voltage sine wave and analyses the response of the system to determine the impedance of the system.

A layer of polymer was applied to a thin, annealed aluminium sheet. Cylindrical impedance cells were pressed onto the surface with a surrounding O-ring joint and a clamp to ensure good sealing during the experiment. The cylinder was partially filled with the electrolyte (deionised water), and electrodes attached to the aluminium plate and immersed in the water at a depth of 6cm.

RESULTS: Agreement between the results obtained with the two techniques was found to be good, and indicates that impedance spectroscopy could be developed as a new tool for in situ assessment of dental composite resins.

CONCLUSION: Measurements of changes in the impedance of dental resins immersed in water over time, provide a very suitable and a quantitative method of comparing the stability in an aqueous environment.

KEYWORDS: dental composite resins, ageing, diffusion, durability, hydrophilicity, impedance

Water uptake of a silorane based composite

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INTRODUCTION: The polymerisation contraction of resin-based composites (RBCs) used for coronary filling is a central problem in odontology. Here we present a new Silorane-based composite (SBC) with oxirane monomers and siloxane. Using ring-opening oxirane monomer causes less polymerisation contraction1. It is well known that the mechanical properties of the RBCs -and thus their durability- are influenced by water absorption 2-3. We therefore attempted to quantify in vitro the long-term water uptake of this new SBC, as compared with a conventional RBC.

METHODS: Filtek® Silorane (FS, 3M) is the SBC, Grandio® (G, Voco) is the RBC. 15 samples of FS and G were prepared. The material was placed in a cylindrical mould before polymerisation (halogen lamp, 2x40s on each face). Each sample was weighed (initial weight, i.w.). Samples were stored in a steam room at 37°C, 5 in bi-distilled water (wat, experimental group 1), 5 in water-saturated atmosphere (sat, experimental group 2) and 5 in dried air (47% hygrometry, dry, control group). They were then weighed with a scale of 10-4g accuracy (Sartorius® BP221S), every hour during the 1st week, then once a week for the next 18 months. The measured increase of the weight of the samples is due to the absorption of water. The results presented are the mean (±SD) weight changes expressed as a percentage of the initial weight. The statistical analysis was performed using a Multiple Range Test.

RESULTS: absorption's curves are presented in figure 1. Values are: FSwat (+0.99%±0.01), Gwat (+0.34%±0.03), FSsat (+0.98%±0.01), Gsat (+0.63%±0.02), FSdry (+0.35%±0.03), Gdry (+0.06%±0.01). There are significant statistical differences ($p<0.05$) for FSwat – Gwat, FSsat – Gsat, FSdry – Gdry, and Gsat –Gwat. We found no statistically-significant difference for FSwat-FSsat ($p>0.05$).

DISCUSSION: the higher water absorption of FS in the two experimental media can be explained by the different load, which is 76 wt% for FS and 84 wt% for G; the less the material is loaded, the higher the proportion of water-absorbing resin4. If the difference between sat and wat is taken as the solubility value5(s), we find that G is solubilised (s=0.15%) but not FS (s=0). This is probably due to the ring-opening oxirane monomer polymerisation process6. In FS, the presence of very hydrophilic oxirane7-8 could explain the high moisture sensitivity seen in the control group.

CONCLUSIONS: in this in vitro study, 18-month water uptake for FS is significantly higher than for G when they are stored in water and water-saturated atmosphere. FS takes up water in dried air, probably because of the presence of oxirane in its formulation. FS has a zero solubility.

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Tribological behaviour of two types of glass ionomer cements

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INTRODUCTION: Regarding the literature, wear processes of dental materials and particularly of glass ionomer cements have been poorly studied excepted for "material ranking". The purpose of this study was to characterize the wear kinetic of two types of glass ionomer cements after the achievement of the setting process.

METHODS: The materials used are a high viscosity conventional glass ionomer cement Fuji IX GP Fast® (GC, Tokyo, Japon) and a resin modifies glass ionomer cement Fuji II LC® (GC). Vickers hardness experiments have been made at 1 day, 7 days, 14 d, 21 d, 28 d, 35 d, 105 d et 189 d. The tribological experiements have been made after 28 days using a pin-on-disc movement. The sliding wear is applied by an alumina cylinder pin with spherical design on end (diameter: 200mm). The normal load is 5N, the radius of the wear track is 2.5 mm and 7500 cycles are made at the frequency of 2 Hz. Silicon replicas (Aquasil® Ultra LV Regular Set, Dentsply DeTrey Gmbh, Konstanz, Germany) are made at 0, 2500, 5000 and 7500 cycles. They are then metallized, observed in a profilometer (probe: 310 µm) (Fig. 1) and analyzed with a specific software (Mountains Map Universal) (Fig. 2).

The statistical analysis was made using a t-test of Student ($p<0.05$).

RESULTS: For both materials, after day 28, hardness data are no statistical significant differences between the results ($p>0.05$). However, data obtained for Fuji IX® are superior than for Fuji II LC® with a significant difference ($p<0,05$). The kinetic of wear shows an increase of the wear correlated to the number of cycles ($p<0,05$). This evaluation shows a linear progression between 2500 and 7500 cycles with similar wear velocity for each material. There are statistical significant differences between the 2 materials at each step of measurements ($p<0.05$) with inferior measurements for le Fuji IX®.
The statistical analysis was made using a t-test of Student ($p<0.05$).

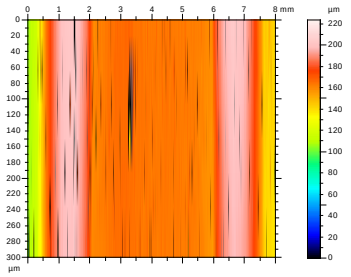

Fig. 1: View under profilometer of a tribological wear

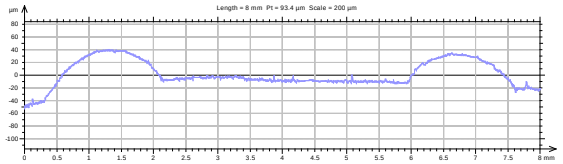

Fig. 2: Example of profile

DISCUSSION & CONCLUSIONS: Hardness results are in accordance with values observed in the literature1-3 and are an indicator of the maturity of the setting reaction. The kinetic wear of glass ionomer cements is not homogeneous during the experiment: the velocity before 2500 cycles is higher than between 2500 and 7500 cycles. However, after 2500 cycles, it appears that the wear kinetics is constant. The wear's law of GICs does not follow the Archard's law which characterizes a linear and homogeneous wear during the whole tribological process. Results are in accordance with values obtained by Yap et al.3 The literature review shows that the evolution of wear kinetic in concrete4 has a similar behaviour than for glass ionomer cements.

This work needs further investigations in different fields: behaviour of the materials before 2500 cycles to characterize the superficial stratum, incidence of the maturation media, characterization of the superficial stratum (roughness, cohesion between fillers and matrix…).

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C. albicans biofilm formation on restorative materials, and its modulation by fluorure

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INTRODUCTION: The use of Biomaterials in restorative dentistry became routine with the improvement of bonding and polymerization systems, and mechanical and physical properties of the resin systems1. However, dental restorative materials are prone to biofilm formation, affecting oral health. Oral microorganisms adhere to and accumulate bioflims on these restorative materials2. Oral biofilms mostly consist of multiple bacterial strains, but Candida species are found on acrylic dentures. Biofilms on gold and amalgam in vivo are thick and fully covering, but barely viable. Biofilms on ceramics are thin and highly viable. Biofilms on composites and glass-ionomer cements cause surface deterioration, which enhances biofilm formation again3.

METHODS: In this study we investigated the adhesion and growth of C. albicans following culture in the presence of resin composite, diamond D, Ivocap and glass ionomer. Adhesion and growth were analyzed4 using scanning electron microscopy and MTT assay, respectively. Since, Glass ionomer is fluorine restorative material and since fluoride plays an active role against bacterial growth, we also investigated the effect of exogenous fluoride on C. albicans growth and morphological changes.

RESULTS: Scanning electron microscopy showed that C. albicans adhered to all tested restorative material. However, adhesion was greater with Diamond D and Ivocap then on Composite resin and on glass ionomer. C. albicans growth using MTT assay confirmed this observation. Indeed, C. albicans growth, after 1 to 4 days, was two folds high with Diamond D and Ivocap as compared to composite resin and Glass ionomer. It is also interesting to note that the lowest adhesion and growth were obtained with Glass ionomer that could be through fluoride release. To confirm such hypothesis we investigated Fluoride effect on C. albicans growth and transition showing that fluoride at 50 and 100 ppm significantly inhibits C. albicans growth and its morphological changes from blastospore to hyphae forms.

DISCUSSION: Based on the results of the present study, all tested restorative materials are favourable surfaces to C. albicans adhesion, growth and biofilm formation confirming those previously reported studies1,3. Of great interest is the difference of C. albicans adhesion and biofilm formation on each tested materials. This suggests the need of selecting the appropriate material for clinical application based on their affinity to microorganism adhesion and growth. Furthermore, integration of anti-microbial molecules in the restorative materials could be a great improvement5. Indeed, compared to other material glass ionomer could be the appropriate material because it may inhibit C. albicans adhesion and growth through fluoride release. This is supported by the present study showing that exogenous fluoride inhibits C. albicans growth and morphological changes.

CONCLUSIONS: This study clearly demonstrated that restorative materials a prone to C. albicans adhesion and biofilm formation. However, this was specific to each material. This study also demonstrated that fluoride release from glass ionomer controlled C. albicans adhesion and growth. Finally, we demonstrated for the first time that exogenous fluoride down regulated C. albicans growth and transition from blastospore to hyphae forms.

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A resin composite (SDR, Dentsply) and a RMGIC (Fuji II LC, GC) microleakage evaluation as dentin substitutes

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INTRODUCTION: The purpose of this study was to test the microleakage of Class II cavities, in opened-sandwich technique, filled with a flow composite resin (SDR, Dentsply) or a resin modified glass ionomer cement (RMGIC; Fuji II LC, GC).

METHODS: 20 class II were realized under the cemento-enamel junction (CEJ) of mesial and distal faces of 10 extracted third molars. Cavities were restored in 2 groups:

G1: SDR (Dentsply) + Bonding XP Bond (Dentsply) (XPb) + composite resin Ceram X Mono (Dentsply) (CXM); G2: Fuji II LC (GC) (F) + XPb + CXM.

After thermocycling (2500 cycles of 15 seconds between 5°C and 55°C), the marginal microleakage was estimated with silver nitrate. For each cavity, 6 measures were taken in the interface tooth / material and 6 in the interface dentin substitute / CXM. The evaluation of the infiltration was realized with scores from 0 to 3. The statistical analysis was realized with a Mann-Whitney test and with a Wilcoxon test ($p < 0,05$).

RESULTS: A statistically significant difference was observed for the dentin margin microleakage: the microkeakage was more important for G1 (Fig.1) ; the dentin margin microleakage was statistically more important than enamel margin microleakage for G1 only. No statistically significant difference was registered between enamel microkeakage of the two experimental groups (Fig. 2). No statistically significant difference was registered between the two filling materials at the interface of this material and the composite resin (CXM) (Fig. 3).

DISCUSSION: Studies[1, 2] showed that RMGIC (Fuji II LC ®) used as dentin substitute under the CEJ remain the more suitable material in this type of situation and in term of longevity in vivo[3, 4].

CONCLUSION: The use of SDR as dentin substitute in Class II cavities under the CEJ showed more microleakage than Fuji II LC®.

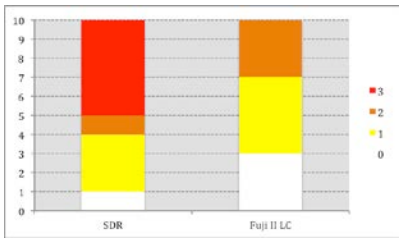

Figure 1: cumulative absolute frequencies of scores for dentin microleakage.

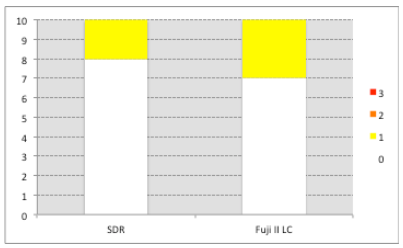

Figure 2: cumulative absolute frequencies of scores for enamel microleakage.

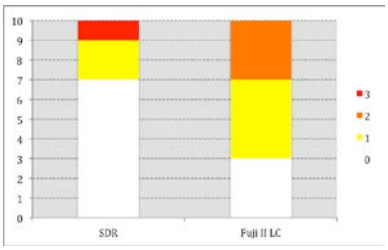

Figure 3: cumulative absolute frequencies of scores for interface microleakage at dentin substitute/composite resin interface.

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Effects of dental alkaline or acid cements on some representative bacterial strains

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INTRODUCTION: Dental cements are potentially bioactive due to their ion transporters properties. The setting reaction of these cements, whether they belong to the family of polyalkenoate or Portland and derivatives, induce either acidification or alkalinization of the surrounding medium. The aim of this study was to evaluate the influence of these variations on the growth of bacterial strains representative of the oral cavity.

METHODS: Pellets of glass ionomer cements ((Ionofil Molar AC Quick®, Ketac Cem Aplicaps®, Ketac Fil Plus Aplicaps®), ProRootMTA® et de Biodentine™) were prepared. A total of five bacteria strains provided by Pasteur Institute (Enterococcus faecalis, Streptococcus mutans, Fusobacterium nucleatum, Lactobacillus casei and Actinomyces naeslundii commonly isolated from carious dentine and infected root canals were selected. The antibacterial activity was assessed by testing growth inhibition in agar and liquid medium. In the first procedure the chip is placed in an agar and the diameter of inhibition around the materials is recorded after 24 to 72 hours of incubation at 37 ° C. In the second procedure, the wafer is placed in a bacterial suspension; then samples are taken at 4 and 24 and planted in agar plates, for counting the "colony forming units".

RESULTS: The inhibition in agar showed that all these cements are active on A. naeslundii, however the CVI are effective against F. nucleatum, while calcium cements are effective against L. casei. No effect on E. faecalis and S. mutans were revealed. Tests of growth inhibition in liquid medium showed that tested cements have an effect on the growth of all bacterial strains for 4 h, but the effect was not extended to 24 hours except for L. casei . no reliable data was reported for F. nucleatum.

DISCUSSION AND CONCLUSION: Preliminary results showed effect dependent pH for some bacterial strains. Further studies are underway in a liquid medium to potentiate bacterial growth and ionic exchanges at the expense of the pH effect. These results should be integrated into a more comprehensive study of the interactions between biomaterials and tissues, whether dental or bone.

KEYWORDS: Cements, pH, bacteria