Organ culture: a tooth model evaluation of pulpal reactions to materials

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INTRODUCTION: This study validates a full tooth organ model for evaluating the pulpal response to direct and indirect pulp capping biomaterials, under experimental conditions as close as possible to the clinical situation.

METHODS: Human immature third molars extracted for orthodontic reasons were used in this work. After preparation and obturation of cavities, the extracted teeth were cultured for 1, 14 or 28 days.

- Controls (group A) were composed of 5 dentin cavities and 5 cavities with pulpal exposure without any filling materials.
- Cavities with pulpal exposure (group B, n=8) were sealed with calcium hydroxide XR®
- Dentin cavities (group C, n=12) were sealed with Xeno III®-Quixfil®, an adhesive and resin system.

Haematoxylin-eosin staining and Masson trichrome staining were used together with immunohistochemistry.

RESULTS: In all groups of cultured teeth, haematoxylin-eosin staining and Masson trichrome staining showed an organization of histological structures similar to that in normal dental teeth: tubular dentin, predentin, odontoblasts, fibroblasts and vessels (fig. 1). The pulp viability was maintained at 28 days.

Fig 1 Histological controls at 14 days, group A pulpal cavity (A) and dentin cavity (B)-residual dentine barrier-.

Calcium hydroxide in direct capping induced the formation of mineralisation nodules. Molecular investigation showed that these nodules were characteristic of reparative dentine secreted by functional odontoblasts expressing dentin sialoprotein and nestin (fig. 2).

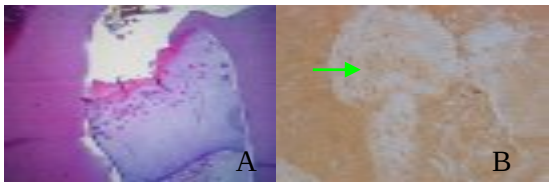

Fig. 2: At 28 days, mineralisation nodules in cavity with pulpal exposure sealed with calcium hydroxide (A) and the dentin sialoprotein-immunolabelling localised in the nodules (B).

The adhesive system in indirect capping initiated a vacuolisation, which disappeared at 28 days, and decreased the specific protein expression. Pulp viability was maintained (fig. 3).

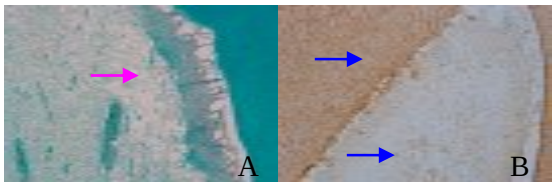

Fig. 3: At day 1, the dentin cavity group showed a vacuolisation (A). At day 28, collagen I was expressed in dental pulp cells and the vacuolisation had disappeared (B).

DISCUSSION & CONCLUSIONS: The positive nestin immuno-labelling showed the presence of functional odontoblasts and the production of reparative dentin in group B. The toxicity of resin monomers was confirmed in this study but the ability of the dentin-pulp complex to respond to a variety of pathological conditions and injury was maintained. This organ culture model, which simulated the clinical situation of direct and indirect pulp capping, showed the same results as pulp-capping *in vivo*. This model could thus be a valuable tool to examine the mechanisms involved in pulp repair and regeneration.

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Study of reparative dentin formation after pulp capping with Mineral Trioxide Aggregate in the transgenic mouse Msx1 +/-.

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Introduction

Pulpo-dentinal healing requires the recruitment of cells and their differentiation into odontoblasts. To date, the origin of the recruited cells, the process of their differentiation and the molecular reactions involved have not been clearly elucidated. Precise characterisation of the cells at the origin of the differentiation would make it possible a) to define their origin, and b) to elucidate their specific behaviour in the phenomena of repair, and thus to consider suitable therapies.

Proposals for the origin of stem cells include: a local origin (stem cells present in the pulpal mesenchym, pericytes), or remote origin (bone marrow stem cells). The presence of pluripotent cells has been established in dental pulp (Dental Pulp Stem Cells DPSCs)[1] but their eventual implication in the process of repair has not yet been shown. Stem cells are delicate to highlight *in vivo* because there is no reliable means of characterising them. The Msx1 homeobox gene is one of the transcription factors expressed by the pluripotent cells from the first brachial arch during their development[2], and confers a high level of phenotypic plasticity on the cells. It seems to characterise the early stages of cellular differentiation. Thus, Msx1 could be used as a molecular marker of the early stages of cellular recruitment in the healing process.

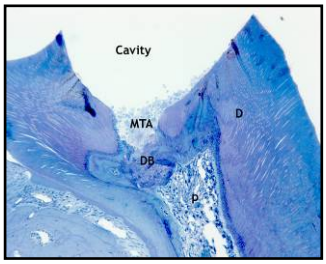
Material and method

45 wild type mice and 25 transgenic mice Msx1 +/- were used. A standard cavity was drilled on the first upper molar until the pulp was exposed, and the pulp capping was set with white Pro Root MTA®. Finally, the cavity was filled with a light cured bonded resin. The animals were sacrificed at 1, 2, 3 and 4 days, 1, 2, 3 4, 5 and 11 weeks with an intra-cardiac perfusion of 4% paraformaldehyde in PBS. The Msx1 +/- samples were secondarily incubated overnight in XGal solution at 37°C

After decalcification and embedding in paraffin, the samples were cut into 7µ slices. Routine histology was performed.

Results

Formation of the dentin bridge was systematically obtained and presented 3 layers: a first in direct contact with material presenting an increased affinity for the dyes and which seemed to correspond to a structural modification of the extracellular matrix; a second layer made of fibrodentin; and a third formed with predentin bordered by cells in full synthesis phase. On all the transgenic samples, we systematically noted the absence of β-Galactosidase revelation (β-Gal) in the pulp.



Histology at 11 weeks post-operative after pulp capping with ProRoot MTA® (MTA). The dentin bridge (DB) is in perfect continuity with the dentin wall. Staining: methyl blue/ blue Azur II. (P: pulp, D: Dentin)

Discussion:

The mouse as laboratory model

The results obtained reveal the chronological formation of the dentin bridge. Unlike the dentin bridge described after capping with calcium hydroxide, no necrotic layer was observed, nor was any persistent inflammatory zone. The dentin bridge was in perfect continuity with the predentin of the side walls of the cavity. The hiatus often described with the calcium hydroxide between the bridge and the dentinal wall was not seen.

Msx1 Expression

No expression of Msx1 was highlighted by the histo-enzymology. Two explanations can be given for these negative results (1) Msx1 is not expressed during the process of pulp healing or (2) a technical problem prevented the revelation of protein. The marking of the buccal bone area of the jawbone proved that the X-Gal staining was effective, excluding a false negative result. The immuno-histochemistry using an anti-LacZ antibody could be used for a more reliable detection of the β-Gal reporter protein.

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Cytotoxicity of Resilon and Epiphany® in a clinical model

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INTRODUCTION: Resilon associated with Epiphany, a thermoplastic synthetic polymer-based root filling material, has recently been introduced. Some of its mechanical and physical properties have already been evaluated with controversial results. In contrast, the biological properties of Resilon and Epiphany are not well documented. The results of biocompatibility studies appear in product disclosure statements and in some conference abstracts but few detailed journal articles deal with the subjects. The purpose of this study was to evaluate the cytotoxicity of Resilon + Epiphany using a clinical model[1].

METHODS: Three root canal filling materials were tested:

- Epiphany (Pentron clinical technologies, Wallingfrod, USA) + Resilon cones (Pentron clinical technologies) (batch # 123836).
- Roekoseal Automix (Coltène/Whaledent, Langenau Germany) (batch # 6503664) + gutta-percha cones (Denstply/Maillefer, Ballaigues, Swizerland) (batch # 010500).
- Sealite regular (Pierre Rolland, Merignac, France) (batch # 3895) + gutta-percha cones (Denstply/Maillefer).

1. Clinical model. Thirty monoradicular teeth were cut at the cementodentinal junction, endodontically prepared with Profile and sterilized. They were assigned to one of the three groups (n=10) and filled. The apex of the roots was dipped into 1 mL of culture medium for 30 days and the medium was renewed every day to simulate periodontal ligament clearance. The medium which remained in contact with the apex for 24 hours after 1d, 2d, 7d and 30d was called the test medium and was used to measure the cytotoxicity with the MTT assay. No dilution was made because a pilot study showed a low cytotoxicity under these conditions.

2. ISO 10-993-5. Cytotoxicity of Resilon and Epiphany was also recorded according to ISO standards after 1d, 2d, 7d and 30d aging.

RESULTS:

Root canal Sealer	1 day p=0.001	2 days p=0.001	7 days ns	30 days ns
Resilon + Epiphany	53 ± 2^{a}	31 ± 2^{a}	0 ± 1	0 ± 2
Roekoseal + gutta-percha	12 ± 5^{b}	6 ± 4^{b}	0 ± 4	0 ± 3
Sealite + gutta-percha	3 ± 4^{c}	1 ± 4^{b}	0 ± 4	0 ± 1

Table 1. Cytotoxicity of three root canal sealers determined in the clinical model

Root canal sealer	1 day p=0.001	2 days p=0.001	7 days p=0.001	30 days ns
Resilon	9 ± 2	0 ± 2	0 ± 2	0 ± 2
Epiphany	94 ± 7	40 ± 6	5 ± 2	0 ± 3

Table 2. Cytotoxicity of Resilon and Epiphany determined by ISO 10-993-5 standards

DISCUSSION & CONCLUSIONS:

Resilon + Epiphany was cytotoxic *in vitro* for 2 d in the clinical model. After 2d, Resilon + Epiphany was not more cytotoxic than two other filling materials used clinically with success. This temporary cytotoxicity is due to Epiphany. The material surface/medium ratio used in ISO standards is too high, making this method clinically irrelevant because cytotoxicity is a dose-dependent phenomenon. The clinical model is more clinically relevant but did not allow us to differentiate between the cytotoxicity of Resilon and that of Epiphany. Therefore, both methods were useful to determine the cytotoxicity of the root canal filling materials.

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Human dental pulp fibroblasts release angiogenic growth factors

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INTRODUCTION: After pulp amputation, complete pulp healing requires not only reparative dentin production but also fibroblast proliferation, nerve fiber growth and neoangiogenesis. We hypothesized that angiogenic growth factors may be released from pulp fibroblasts and that their release may be necessary for complete pulp healing after injury.

METHODS: Human pulp fibroblasts were prepared from immature third molars. Cells were cultured in 35-mm-diameter culture dishes (Becton Dickinson Labware, Lincoln Park, NJ) in minimum essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 UI/ml penicillin, 100µm streptomycin and 0.25 µg/ml amphotericin B (Fungizone). Injuries to fibroblasts were performed mechanically with sterile scalpels to disrupt the fibroblast monolayer. The media obtained after a contact period of 5 hours, 1, 2 and 3 days with injured or intact cells were then used for the quantification of angiogenic factors.

The growth factors investigated were platelet-derived growth factor (PDGF-AB), basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF), and were assayed using the sandwich enzyme linked immunosorbent assay (Quantikine Cytokine ELISA kit, R&D system, France).

Each experiment was done in triplicate. Error bars reflect the standard deviation and probability values were assessed using the Mann-Whitney nonparametric test. $p<0.05$ was considered significant.

RESULTS: Intact human pulp fibroblasts release angiogenic growth factors: PDGF-AB (169.69±21.89 pg/ml), FGF-2 (299.37±71.25 pg/ml) and VEGF (907.55±77.69 pg/ml). After injury, there was a significant increase in growth factor concentration (as compared to intact cells). This promotion was 150.59% for PDGF-AB; 23.35% for FGF-2 and 20.6% for VEGF. This increase was obtained 5 hours after injury and then returned to initial values. There was no significant change in expression of these factors after 1 day, 2 days and 3 days (figure 1).

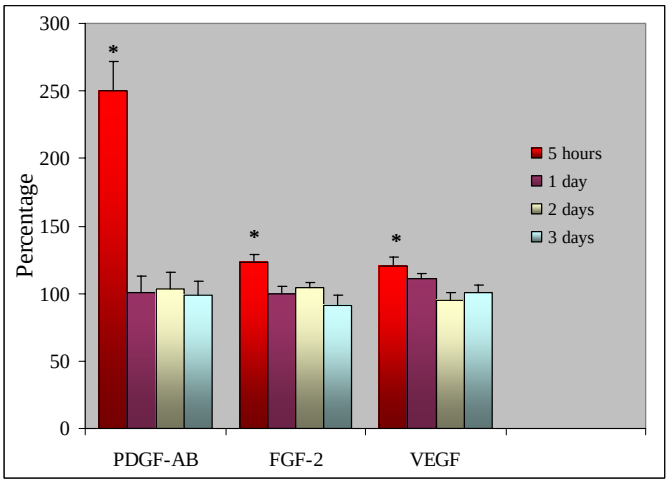

Fig. 1: Expression of angiogenic factors in dental pulp. PDGF-AB, FGF-2 and VEGF expression were up-regulated after 5 hours of injury and returned to initial values after 1, 2 and 3 days. This secretion was quantified by ELISA and was expressed as percentage of control (without injury). ***** *Significant difference ($p<0.05$).*

DISCUSSION & CONCLUSIONS: Our work clearly demonstrates that human pulp fibroblasts secrete PDGF-AB, FGF-2 and VEGF particularly after injury. The highest concentration was that of VEGF (907.55±77.69 pg/ml). The release of these factors is very rapid and corresponds well to the pulp physiology. After pulp injury, the migration of odontoblast progenitor cells to the injury site may require newly formed blood vessels.

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Biological effects of blue light on human gingival fibroblasts: an *in vitro* study

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INTRODUCTION: Studies of the effects of blue light on human cells report negative or positive impacts[1, 2] depending on the light sources and the type of cells used. Concerning exposure to blue light emitted by dental curing lamps, Wataha et al.[3] reported a negative impact of PAC- and QTH-light on human gingival fibroblasts (HGF). However no studies, to our knowledge, have investigated the impact of LED light on cells. Compared to QTH curing units, LEDs emit light with higher efficiency, produce less heat and have a longer lifespan. They are therefore becoming the more frequently used technology in dental practise. The aim of this study was to assess the effects of light emitted by a LED curing unit on the viability and morphology of HGF, respecting clinical procedures for use (distance, light intensity and time exposure).

METHODS: Fibroblasts were cultured from biopsies of clinically healthy human gingival tissues. The cells were irradiated at a distance of 9 mm, and the exposure durations and curing programs used were those recommended by the manufacturer to cure one layer of dental bonding and three layers of composites (Table 1), as can occur for deep restorations. The morphology of the HGF was observed by means of a scanning electron microscope. Viabilities of exposed and non-exposed cells (control group) were compared by means of the MTT assay.

	1 cycle (polymerization of bonding)	3 cycles (polymerization of dental resin)	Total duration
LED (Bluephase®, Ivoclar-Vivadent))	**Program LOP** (650 mW/cm²) 20 s	**Program HIP** (1100 mW/cm²) 3 ×20 s = 60 s	**80 s**

Table 1: Curing programs used for cell exposure.

RESULTS: Despite their exposure to blue light, microscopic analysis showed that irradiated cells presented a typical fibroblastic morphology (fig.1).

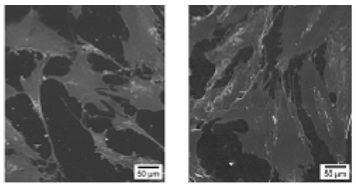

Fig.1: HGF observed by SEM (x 25: (left) control cells, (right) exposed cells.

The viability of cells exposed to the LED light was significantly greater than that of control cells 72h post-exposure (p=0.0275) (fig.2).

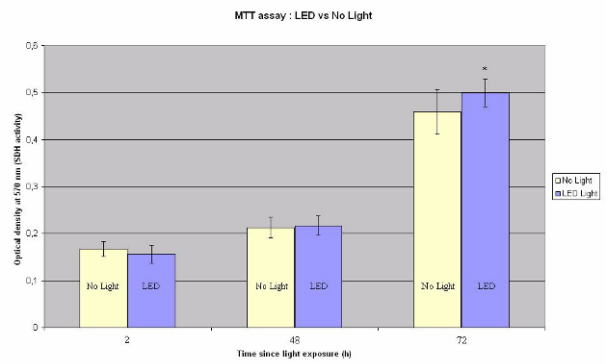

Fig.2: Effect of clinically relevant LED light exposure on HGF. Cellular mitochondrial SDH activity was measured (n=10 per condition) at 2, 48 and 72h post-exposure. Asterisk represents a significant difference between exposed and non-exposed cells (ANOVA, Tukey, α=0.05).

DISCUSSION & CONCLUSIONS: Cell morphology was unaffected, in the current study, by exposure to the LED light. Unlike Wataha et al., who found a decrease of HGF viability after exposure to PAC- and QTH light, this study showed a greater viability of exposed cells 72h post-exposure when compared to control cells. In conclusion, irradiation of HGF with a LED curing unit respecting a clinically relevant procedure did not alter cell morphology and even stimulated cell proliferation 72h post-exposure, another advantage of LED light.

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Microtomography: a method to assess root canal geometry and changes after endodontic preparation

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INTRODUCTION: Medical imaging has progressed greatly in the last 30 years. In endodontic studies *in vitro*, new technologies and techniques have been explored to assess root canal geometry and anatomical changes after canal preparation. X-ray microtomography is one of these techniques, which allows three-dimensional images of canal geometry to be obtained before and after an endodontic treatment [1]. This non-destructive imaging has been in use for a decade [2,3]. In this preliminary study, the objective is to propose a modified version of methodology described previously by Peters and Paqué [1,3].

METHODS: Two steps were used to obtain root canal images: image acquisition and three-dimensional (3-D) reconstruction.

Acquisition: The specimens (freshly extracted human maxillary molars) were scanned before and after root canal shaping with HeroShaper instruments (Micro-Mega, Besançon, France) following the manufactures' guidelines. Acquisitions were performed using a Skyscan1072 high resolution microtomography scanner (Micro Photonics Inc, Allentown, PA, USA). Each acquisition contained 470 slices with a thickness of 38.57µm.

3-D Reconstruction: Three major steps were involved in this generic task: 1) data filtering; 2) delineation and registration of the 3-D shape of the ROI (regions of interest), and 3) mapping measurements over these shapes. The second step, i.e. 3-D image segmentation and registration, was performed using the Amira commercial software, (Indeed Visual Concepts, Berlin, Germany). The other steps, i.e. filtering and measurement, were carried out through a research platform developed in our laboratory. Anatomical changes were assessed using the mathematical method of cylindrical representation.

RESULTS: Three-dimensional images of the root canal were obtained (Fig. 1), and the anatomical changes (volumes, surfaces areas, etc.) assessed. The volume and surface area, cylindrical representations, and the canal axis for each canal before and after preparation were also calculated (Fig. 2). Curvilinear abscissas were calculated for each canal before and after canal shaping with the endodontic instruments (Fig. 3).

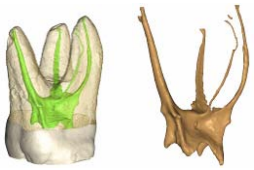

Fig. 1: 3-D dental reconstruction: several tissues (left) vs. pulp structures (right).

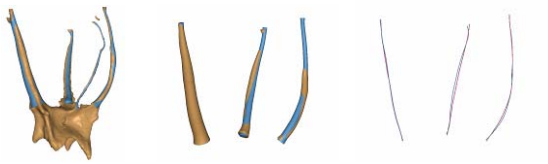

Fig.2: 3-D representation of the root canals and canal axes before (brown or red) and after (blue).

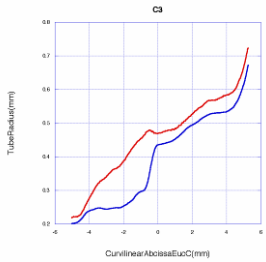

Fig.3: Curvilinear abscissa variation for a canal before (red) and after (blue) preparation.

DISCUSSION & CONCLUSIONS: This methodology seems to be a very adequate *in vitro* endodontic methodology for exploring root canal geometry and assessing anatomical changes. Using a mathematical method, i.e. cylindrical representation, allows the anatomical changes to be better evaluated.

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Students' Practical training in endodontics: root canal shaping and NiTi rotary system.

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INTRODUCTION: The use of Rotary Nickel-Titanium systems for root canal preparation is taught during university studies. In our dental school this knowledge is dealt with after conventional hand instrumentation.
The aim of the present survey was to investigate root-canal shaping with manual and rotary Ni-Ti files performed by undergraduate students**.**

METHOD: For this survey, students beginning their third-year (D1), who had not received any information on endodontics, were chosen. They were randomly divided into two equal groups of thirty students: equality was determined using the reports of the past year practical tests in the prosthesis and restorative options.
A three-hour course on general endodontics was delivered to the two groups. Then each received specific training for 2 hours: on the rotary Ni-Ti files system for group1, and on a manual technique for group 2.

After this training, each student had to treat 4 teeth of increasing difficulty: first 2 single-root teeth and next 2 molars.
The dental crown was removed to make canal access easier. A pre-operative X-ray and a technical card were provided for each tooth. The card mentioned which canal was to be treated, its working ledge and the recommended operative sequence.
In group 1 (using rotary files) students used the HEROShaper® system: one difficult sequence for curved canals 6% ∅20, 4% ∅20, 4% ∅25, 4% ∅30 and a medium one for straight canals: 6% ∅25, 4% ∅25, 4% ∅30.
Group 2 used a set of HELIFILE® files (sizes 15/100 to 35/100).

Four specifically trained examiners observed the post-operative X-ray and each apical foramen with a microscope (X 16). Afterwards, a size 15 K-type file was introduced into each root canal to establish patency. The parameters noted were instrumental fracture visible on X-ray, lost operative length, canal ledge and dentinal plug, over-instrumentation and apical zips.

The data were analyzed statistically using analysis of variance and fisher's PLSD test (all the statistical tests were interpreted at the α=5% significance level) performed by the Statview system (Brain-Power Inc. Agoura Hills, CA, USA).

RESULTS :

Group	Canals	Fracture	Lost length	Over shaping	Zips
NiTi	**104**	**8**	**11**	**34**	**21,2**
Manual	**104**	**0**	**13**	**28,3**	**14,2**
		ns	ns	ns	ns

All the parameters appeared in both groups without statistically significant differences.
Instrumental fracture occurred only in the rotative group (2).

DISCUSSION & CONCLUSION:
The students involved in this survey had received limited training. Thorough initial knowledge should lead to better results, in particular knowledge of the correct technique for the rotary Ni-Ti system, and for applying light apical pressure.
There is a national consensus on the need for teaching undergraduates about rotary Ni-Ti systems in France[3].
Prior experience with a hand preparation technique was not reflected in improved quality of the subsequent rotary preparation. Rotary Nickel Titanium files for root canal shaping could be taught earlier in the D1.

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Influence of the quality of coronal restoration on periapical health

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INTRODUCTION: Many teeth presenting poorly filled and probably poorly prepared root canals remain clinically and radiographically asymptomatic for years. Therefore, factors other than the endodontic treatment quality determine the clinical success. Many laboratory studies have shown that coronal leakage may lead to the contamination of the periapical area by bacteria originating from the oral cavity. Some clinical studies have evaluated the influence of the coronal seal on periapical health but, apart from Hommez et al.'s work, they suffer from a severe bias because the quality of coronal restoration is only based on a radiographic examination. The aim of the present work was to confirm previous results, combining a clinical and a radiographic investigation, to evaluate the relative influence of coronal restoration on periapical health.

METHODS: A radiographic investigation, evaluating the endodontic treatment quality and the presence of periapical radiolucency, was performed upon 592 teeth with root canal treatment performed at least 4 years before the examination. Three factors were recorded. Factor 1: periapical health was recorded according to the PAI index[2]. Factor 2: the endodontic treatments were classified into “good endodontic treatment" i.e. dense filling material within 2 mm of the apex and “poor endodontic treatment” i.e. other cases. Factor 3: the quality of the coronal restoration was then recorded according to the USPHS criteria [3]. A chi[2] test was performed, at the 95% confidence level, to evaluate the influence of quality of endodontic treatment and quality of coronal restoration on the presence of periapical radiolucency.

RESULTS:

Table 1: Number of teeth showing periapical radiolucency according to quality of root canal filling (n=592)

p=0.004	With periapical radiolucency	Without pericapical radiolucency
Good endodontics	16	201
Poor endodontics	117	258

Table 2: Number of teeth showing periapical radiolucency according to quality of coronal restoration (n=592)

p=0.04	With periapical radiolucency	Without pericapical radiolucency
Good restoration	70	302
Poor restoration	63	157

Table 3: Number of teeth showing periapical radiolucency according to quality of root canal filling and quality of coronal restoration (n=133)

	Good restoration	Poor restoration	Total
Good endodontics	5	11	16
Poor endodontics	65	52	117
Total	70	63	133

CONCLUSIONS: Both factors influence periapical health . A good coronal restoration increases periapical health by 10% regardless of the quality of root canal filling.

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Microleakage of three filling materials for furcation perforation

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INTRODUCTION: Success of endodontic treatment can be compromised when furcal-floor or radicular perforation occurs[1]. When an iatrogenic or pathological perforation exists, this communication must be removed by a hermetic and biocompatible filling. The aim of the study was to evaluate and compare the sealing ability of three biomaterials used for furcal-floor perforation obturation: IRM® (Intermediate restorative material from Detrey Caulk,), MTA (Pro-root®, from Detrey Maillefer, Ballaigues, Switzerland) and an ordinary commercial version of Portland cement (abbreviation: PC).

METHODS: 40 lower molars were selected. Easy access to the furcation area was a criterion of selection. Three groups of twelve teeth each were randomly formed: a group of 4 teeth was used as a control group (2 negative and 2 positive controls). Classical Access cavities were created. Standardised perforations were made using ISO # 006 round burs. Filling material was applied according to the manufacturer's protocol, using a Messin gun and a small plugger. Cotton pellets moistened with sterile distilled water were placed, and the access cavity was filled with IRM. The teeth were placed on a wet support for 24 hours. All surfaces of the teeth except the furcation area were coated with two layers of nail varnish. The microleakage test was carried out using a passive dye penetration method (1% basic fushin solution). After embedding in epoxy resin and cutting exactly at the furcation point, each sample was placed under a light microscope connected to a video camera and a computer. A qualitative analysis (score method) and a quantitative analysis (measurements of dye penetration length) were performed. For qualitative analysis, the Fisher's modified Chi squared test was used and, for quantitative analysis, the Kruskal and Wallis test and the Mann and Whitney U-test were used.

RESULTS: All the perforations sealed with IRM were infiltrated by the dye up to the pulp chamber. The modified Chi^2 test showed that the scores were dependent on the filling materials. The differences between IRM and MTA, and between IRM and Portland cement were statistically significant ($p<0.005$) but there was no statistically significant difference between MTA and Portland cement. The comparison of mean dye penetration lengths was statistically significant ($p<0.001$).The Mann and Whitney U-test showed a statistically significant difference between IRM (105.93 µm ± 27.23) and MTA (31.85 µm ± 34.90), and IRM and Portland cement (34.90 µm ± 38.88), but no statistically significant difference between MTA and Portland cement.

Fig 1: dye penetration for IRM, MTA, and PC

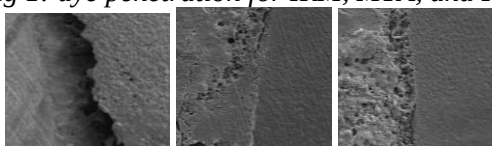

Fig 2: SEM Observation(x1000) for IRM (gap), similarity for MTA and PC (no gap)

DISCUSSION & CONCLUSIONS: Many other studies have been carried out concerning microleakage of MTA and PC, using different evaluation methods. They all conclude that both have a better sealing ability than other cements[2]. MTA and PC used to repair furcation perforation seem to have the best sealing ability according to the conditions of this experiment. MTA and PC show comparable properties[3] when evaluated in vitro and in vivo[4]. The results suggest that PC has the potential to be used as a less expensive root-end filling material.

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GAS PERMEABILITY FOR ENDODONTIC LEAKAGE

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INTRODUCTION: Watertight filling of the root canal system following intracanal preparation is a major objective of endodontic treatment. Various methods such as dye penetration, radioactive isotope test, bacteria or bacterial metabolites leakage test, electrochemical technique, and fluid filtration have been used to evaluate the sealing properties of endodontic cements[1]. All of them are dependent on wetting and the capillarity properties of water. Therefore we propose a gas permeability method for quantifying endodontic leakage which is currently used for measuring aerogel porosity.

METHODS: To evaluate this new method, 18 roots of freshly extracted anterior teeth were prepared (Protaper file F3, Maillefer, Switzerland) and filled using one of 3 methods; 6 roots with zinc-oxide eugenol based cement and master cone 6% (MC-ZnO), 6 with zinc-oxide eugenol based cement and system B (SB-ZnO), and 6 with gutta flow (Coltene Whaledent) and master cone 6% (MC-GF). All roots were glued to glass tubes (araldite).

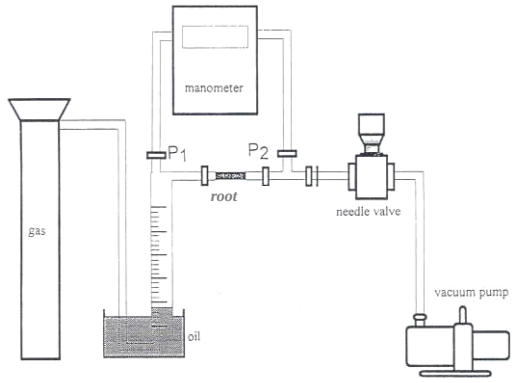

Figure 1: experimental schema.

Figure 1 shows the experimental setup. The graduated tube on the left of the sample was filled with nitrogen. Then the difference of pressure between P1 and P2 was set between 400 and 1000 hPa using a needle valve. The gas flow, φ, through the sample could be deduced from the volume variation, ΔV, and the pressure P1.

$$\varphi = P1dV/dt \qquad (1)$$

The difference of gas pressure between P1 and P2 was measured with a membrane pressure gauge (Testo). After setting the correct ΔP, the needle gauge was closed and pressure variation versus time was recorded.

RESULTS: SB-ZnO showed the best sealing with a mean of 826 seconds to leak 1 hPa, MC-GF had the worst seal with 104 seconds and MC-ZnO was intermediate with 216 seconds.

Table 1: Time in seconds for a pressure decrease of 1hPa (initial pressure: 450hPa).

SB-ZnO	516	506	43	2500	1050	340
MC-GF	500	7	20	25	74	0.6
MC-ZnO	196	490	264	37	150	156

DISCUSSION & CONCLUSIONS: This new quantitative method allows endodontic micro leakage to be measured. This liquid-free method is independent of the water wetting properties of the materials tested (fig. 2). Further studies are needed to compare it to traditional techniques (fluid filtration, dyes...) and more precisely to determine the surface energy dependence on water penetration at the dentin/material interface.

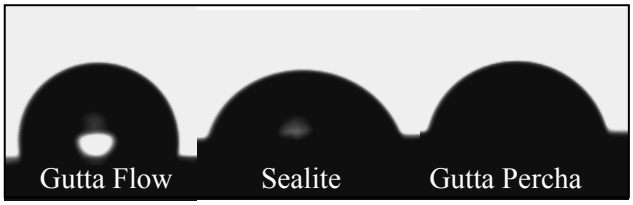

Figure 2: aspect of a drop of water on gutta flow (left), sealite (centre) and gutta percha (right).

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Rheology for a calcium phosphate ceramic in endodontics

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Introduction

The apex remains the key to endodontic success, and therapeutic action still focuses on this biological repair unit. Current filling materials are toxic to periapical tissues and calcium phosphate materials, due to their biocompatibility and bioactive properties, may be viewed as possible alternatives.
The aim of this study was first to determine whether an injectable bone substitute could be used to obtain further healing of apical tissue by the neoformation of a mineralized barrier. In the next step, the work focused on rheological measurements as a tool for physical characterisation and on improvement of the injection technique.

Materials and methods

Injectable bone substitute

The ceramic was a biphasic calcium phosphate (BCP, Biomatlante, Vigneux de Bretagne, France) with a 60/40 HA/ß TCP weight ratio. A cellulose-derived polymer (Methyl-Hydroxyl-Propyl-Cellulose HPMC) was used because of its biocompatibility and rheological properties, which confer injectability on the final composite. Briefly, a solution of 2 % or 3% of HPMC was prepared by dissolving dry raw HPMC powder (E4M Premium EP, Colorcon, Bougival, France) in bidistilled water with stirring for 48 hours. The composite biomaterial was obtained by mixing a 2% or 3% HPMC solution with the 40-to 80-µm BCP granules in different weight ratios. The resulting composite was sterilized at 121°C for 20 minutes.

Animal experiments:

Six adult female sheep, with a mean weight of 64Kg, were used for endodontic treatment (n=8). Single-root teeth were prepared using the Hero system, the haemorrhage was controlled, and the sterilized materials to be tested were injected with a "lentulo". The animals were sacrificed at 12 weeks.
All the teeth were then embedded in methyl methacrylate and cut into parallel sections (100µm) with a diamond saw (Leitz 1600).
Qualitative observations were performed by light microscopy on the stained sections (Goldner trichromatic system). Other Sections were observed by Scanning Electron Microscopy (SEM). Energy dispersive X-ray (EDX) microanalysis was performed with an EDAX.

Rheological study

Rheology concerns the flow and deformation of a suspension and, in particular, its behaviour in the transient area between solid and fluid.
Rheological measurements were performed using the RheoStress300 rheometer (ThermoHaake®, Germany), using a plane/plane geometry with a cone diameter of 60mm. The rheometer was equipped with a circulating water bath to control temperature.
Zero-shear viscosity was determined from the flow curve of viscosity against shear rate. The experiment was carried out in rate-controlled mode at 25°C.
Dynamic oscillatory measurements were carried out to determine the elastic (G') and viscous (G") moduli as a function of frequency (*f*) at a fixed stress (τ) and fixed temperature. The experiment was carried out in stress-controlled mode at 25°C.

Results and Discussion

The animal experiment results obtained were grouped as "success", "uncertain" or "failure" depending on the amount of calcium phosphate granules at apical level: complete filling, partial filling and absence. The *in vivo* experimentation showed radiological apical sealing in 5 samples (arrows). SEM revealed mineral formation at the apex level with mineral tissue conduction between the BCP granules for 6 teeth but, for 5 samples, the apical amount of BCP granules could be classified as "partial filling". Only one tooth showed good apical sealing with an amount of BCP classified as "good filling". The BSE image in SEM showed a homogeneous level of mineralization between and around the granules and in close contact with them. Its density was the same as that of the dentin walls.

To improve the injectable properties, rheological measurements were performed. An increase in the amount of BCP led to an increase in the elastic (G') and viscous (G") moduli. For suspensions in 48 and 50% by weight, G" was higher than G', which implied a liquid behaviour of the suspension at a frequency of 1 Hz and a stress of 1Pa. For a given frequency, G' became higher than G", which implied that the suspension presented a solid behaviour. For higher concentrations, G' was higher than G", which implied a solid behaviour.
The crossline occurred at a lower frequency with a larger amount of BCP granules. A first step of correlation between these results and injection experiments was performed to model the rheological properties of this suspension.

Conclusion

Calcium phosphate materials make it possible to create tight bonding with mineralized tissues, and the formation of a mineralized barrier could ensure apical hermeticity. There are several tests for characterizing materials rheologically and the selection of such tests will depend on both the application and the injectability in this type of work.

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Short- and long-term dentin bonding of experimental adhesives with a potential for chemical interaction at the dentin surface

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INTRODUCTION: There is growing evidence that the mechanism of certain dentin adhesives involves not only mechanical but also chemical bonding [1]. It was the aim of the present study to investigate the short- and long-term bonding to dentin of experimental adhesives. The adhesives contained bifunctional monomers with a potential capability to bond to the organic or the inorganic part of dentin surfaces.

METHODS: Extracted human teeth were used for the experiments. The teeth were given a flat dentin surface by grinding. The dentin surfaces were either demineralized with 25% phosphoric acid to give a surface rich in collagen, or deproteinized with 0.5% sodium hypochlorite to give a surface rich in hydroxy apatite [2]. Priming was carried out with a 35% aqueous mixture of HEMA. In the control group, the adhesive resin was a 40/60 mole/mole mixture of TEGDMA and UEDMA. In the experimental groups, three bifunctional monomers were investigated: MDP (10-methacryloyloxydecyl dihydrogenphosphate), 4-MET (4-methacryloyloxyethyl trimellitic acid mono ester) with a potential for bonding to hydroxy apatite, and 4-META (4-methacryloyl-oxyethyl trimellitate anhydride) with a potential for bonding to collagen. These monomers were mixed with TEGDMA and UEDMA in molar ratios of 15/34/51.

A cylindrical mold (h = 2.5 mm, d = 3.6 mm) was clamped to the treated dentin surface and resin composite (Filtek Supreme, 3M ESPE) was applied in the mold and light cured. The specimens were stored in water at 37ºC for 1 day or 1 year. The shear bond strength was then determined at a cross-head speed of 1 mm/min (n = 8).

RESULTS: The results are presented in Tables 1 and 2. Three-factorial ANOVA showed a significant influence of adhesive and storage time ($p <= 0.001$) but no interactions. There was no significant difference between the three bifunctional monomers with a potential for chemical interactions at the dentine surface ($p > 0.05$). The loss in bond strength due to long-term water storage was more pronounced in the demineralized group.

Table 1. Bonding (MPa) of experimental adhesives to H_3PO_4 - demineralized dentin. Means ± SD.

Adhesive	1 day	1 year
Control	17 ± 7	15 ± 9
MDP	28 ± 5	22 ± 7
4-MET	27 ± 5	18 ± 5
4-META	31 ± 6	20 ± 9

Table 2. Bonding (MPa) of experimental adhesives to NaOCl - deproteinized dentin. Means ± SD.

Adhesive	1 day	1 year
Control	18 ± 4	14 ± 3
MDP	24 ± 6	22 ± 8
4-MET	27 ± 5	27 ± 9
4-META	28 ± 5	25 ± 8

DISCUSSION & CONCLUSIONS: The potentially calcium-bonding adhesives (containing MDP and 4-MET) did not behave differently than the potentially collagen-bonding adhesive (containing 4-META). Thus, possible chemical interactions of the experimental adhesives were not reflected in the bond strengths. The bond strengths decreased as a consequence of long-term water storage. This is in agreement with earlier studies [3]. The presence of the hybrid layer in the demineralized group may have facilitated migration of water along the interface and thus increased the deterioration of the bond.

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Adhesive system self-etch functional monomer interactions with hydroxyapatite

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INTRODUCTION: Self-etch adhesives with intermediate pH (between 1 and 2) partly demineralise dentine and do not completely remove all the hydroxylapatite from the collagen fibers. In addition to micromechanical bonding, functional monomers could interact chemically with residual hydroxylapatite surfaces[1] and contribute to the overall adhesive performance of the self-etch systems. The functional monomer of AdheSE (Ivoclar Vivadent, Schaan, Liechtenstein) has two ionisable protons on the phosphonate group. Only the most acidic proton (pKa=1.82) participates in demineralization[2,3]. The neutralization of this phosphonate charge by divalent calcium cations produces crosslinks in the polymer structure. Our aim was to characterize by ^{31}P NMR the products of the reaction between dental hydroxylapatite and the phosphonate group of AdheSE.

METHODS: Dentin particles were prepared from sound molars extracted from patients with eruptive problems. Dentin disks were cut from crown segments parallel to the occlusal surface at the top of the pulp chamber with an Isomet saw. Occlusal enamel was removed by means of a second cut parallel to the sectioned dentin. Peripheral enamel was removed under water irrigation with a diamond burr. Dentin blocks were fragmented in a hydraulic press at 50 GPa pressure (Weber, Remshalden, Germany). The dentin particles collected were sieved to retain fragments smaller than 100 µm.
^{31}P NMR spectra were obtained in the solid state with a Bruker 400 MHz spectrometer (Bruker France, Wissembourg, France). The samples were studied pure and in three mixture ratios.

RESULTS : *Table 1. NMR table of results showing peak assignments.*

Products	NMR		
	Type	Spectra	Chemical shift, ppm
Dentin powder	hpec {^{1}H}	^{31}P	3.86 a
AdheSE primer + Dentin	hpec {^{1}H}	^{31}P	22.85 – 3.91 – 2.37
	CP	^{31}P-^{1}H	22.71 – 3.91 – 2.31
AdheSE + Dentin 1 / 1	hpec {^{1}H}	^{31}P	24.50 – 3.93 – 2.32
	CP	^{31}P	23.74 – 3.92 – 2.30 b
AdheSE + Dentin 2/ 1	hpec {^{1}H}	^{31}P	23.67 – 3.94 – 2.46
	CP	^{31}P-^{1}H	24.01 – 3.94 – 2.33 c

Fig. 1 ^{31}P NMR spectra AdheSE/dentin ratios : 0 in a, 1in b and 2 in c

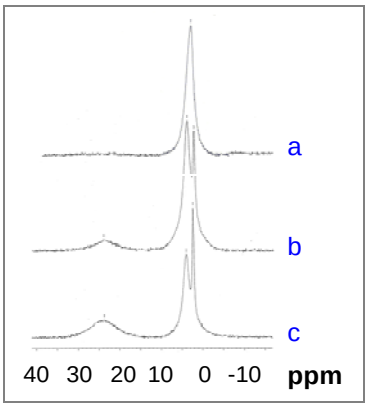

Figure 1 illustrates the NMR spectra. The analysis results is presented in Table1. The chemical shift for hydroxyapatite is at 3.9 ppm while that of the phosphonate is at 23 ppm. The broad absorption corresponds to the average signal of protonated and deprotonated phosphonates. When AdheSE reacts with the hydroxyapatite, the hydroxyapatite peak decreases in intensity as a function of the acid proportion and a new peak appears centered at 2.3 ppm. This absorption is identified as a new mineral phase: Brushite.

DISCUSSION & CONCLUSIONS: The results show that part of the hydroxyapatite is dissolved by the acidic monomer to yield brushite, a calcium phosphate with a lower Ca/P ratio than that of hydroxyapatite. The rest of the calcium ions dissolved neutralize the phosphonate groups according to the balanced reaction: $Ca_{10}(PO_4)_6(OH)_2 + 8\ RPO_3H_2 \rightarrow 6\ [CaHPO_4] + 4\ [Ca(RHPO_3)_2] + 2\ H_2O$.
Thus the phosphonate acid groups of AdheSE react with hydroxyapatite, are neutralized by the dissolved calcium ions and lead to the formation of a brushite precipitate. This new metastable phase present in the hybrid layer may evolve with time.

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Effect of dentin bonding systems on the expression of matrix metalloproteinases by odontoblasts

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INTRODUCTION: Recent studies have revealed the contribution of host-derived proteinases to the collagen matrix breakdown in the pathogenesis of dentin caries [1-2]. They could have potential implications in dentin bonding. Indeed, the results of these studies suggest that degradation of incompletely infiltrated zones within the hybridized dentin by host-derived dentin matrix metalloproteinases may proceed in the absence of bacterial enzymes [3]. *In situ* collagen degradation within incompletely infiltrated hybrid layers may also adversely affect the remineralization potential of denuded collagen fibrils *in vivo* [4] and *in vitro* [5]. Thus, the objective of this study is to determine the origin of matrix metalloproteinases: dentin bonding systems influence either the expression of metalloproteinases by odontoblasts or only active host-derived metalloproteinases within the dentin matrix.

MATERIALS & METHODS: Ten fresh, non-carious, human third molar teeth were extracted from patients 15-18 years old for orthodontic reasons (with their informed consent). Immediately after extraction, they were stored in the culture medium until used. Radiography was performed to visualize the extent of the pulp tissue. An occlusal cavity was prepared on each tooth with a diamond bur (1.6 mm diameter) under water-spray cooling combined with culture medium. The size of these cavities was standardized so that they did not extend over more than half the dentin thickness. Teeth were randomly assigned to two experimental groups (n=5). A dentin bonding system (Xeno III, Dentsply De Trey, Konstanz, Germany) was applied to each cavity of the first group. A flowable resin composite (Ceram X, Dentsply De Trey, Konstanz, Germany) was applied to all bonded specimens and light cured. Both dentin bonding system and composite were used according to manufacturers' instructions. The adhesive system and resin composite were light cured using a previously tested unit (Astralis 5, Ivoclar Vivadent, Saint Jorioz, France). The second group, without a dentin bonding system was used as control. The teeth were carefully sectioned and thick slices were cultured as described previously [6]. The slices were cultured for 7 days. The samples were fixed in 4% paraformaldehyde-PBS (48 hrs), washed in PBS, demineralized in acetic acid (1N) for 27 days, washed in distilled water, dehydrated in a graded ethanol series, cleared in toluene, and embedded in paraffin. Microtome serial sections (5 µm) were collected. The sections were cleaned of paraffin and treated for immunohistochemical analysis using Vectastain Elite ABC Kits according to the manufacturer's protocol (Vector Labs, Burlingame, California, USA). Two specific mouse monoclonal antibodies were tested: anti-MMP-2 and anti-MMP-9 (R&D Systems, Lille, France).

RESULTS: At T0, no differences in terms of staining intensity or distribution patterns were found for MMP-2 and MMP-9 with and without dentin bonding system. The staining was detected in the odontoblast layer and pulp tissue. After 7 days in culture, intense immunoreactivity was observed for MMP-2 and MMP-9 in the odontoblast layer and the pulp tissue respectively in the group with dentin bonding system.

DISCUSSION & CONCLUSIONS: The results of this study show that dentin bonding system directly influences the expression of metalloproteinases by odontoblasts. Further *in vitro* studies need to be performed to validate our hypothesis: metalloproteinases synthesized by odontoblasts may get into the hybrid layer through tubules and dentin fluid and then could contribute to damage to incompletely infiltrated collagen in the hybrid layer.

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Influence of two anti-oxidants on root canal dentin bonding

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INTRODUCTION: Endodontic irrigants like sodium hypochlorite and hydrogen peroxide reduce dentin bond strength. The mechanism of this compromised bonding remains unclear. Several hypotheses have been proposed to explain this problem: denaturation and of the collagen matrix or oxidizing action of the irrigants. Following the latter hypothesis, the oxidized dentin could alter the degree of conversion of both adhesive and composite resin monomers.
The aims of this study were: 1) To check the influence of endodontic dentin treatments on the degree of conversion of resin monomers and 2) To assess the ability of 2 anti-oxidants (Na ascorbate and GSH) to reverse the reduced adhesion to oxidized dentin.

MATERIALS & METHODS: First, the linear relation between the Knoop microhardness (KHN) of a selected composite, (Tetric Flow®, VIVADENT) and its degree of conversion (DC) was established. The DC of 12 samples of composite with different polymerization times were assessed by FTIR before their hardness was measured. Second, the influence of different endodontic solutions (5% NaOCl, Glyde® - Dentsply, ZOE-based sealer) on the KHN of composite was assessed. The composite was directly applied in contact with root dentin slabs. KHN was evaluated at different distances from the canal zone. The reverse action of 2 anti-oxidants (10% sodium ascorbate & 10% glutathione (GSH) was also tested. 7 batches of 5 teeth were treated endodontically with ProTaper files according to the manufacturer's instructions, and treated as described below.

Third, the same experimental plan was followed to check the influence of these different treatments on dentin bond strength (DBS). But according to the results of the previous approach, we limited the DBS test to samples resulting from batches 1, 2, 4 & 6. The selected test was a micro-tensile test.

Batch	Irrigant	filling	Treatment
1	0.9%NaCl		
2	NaOCl 5%		
3	NaOCl + Glyde		
4	NaOCl + Glyde	ZOE	
5	NaOCl + Glyde	ZOE	Na Asc
6	NaOCl + Glyde	ZOE	GSH
7	NaOCl + Glyde	ZOE	Na Asc + GSH

RESULTS: 1) A good linear correlation ($r^2 = 0.8$). between KHN and DC of Tetric Flow allowed KHN to be used to indirectly assess the DC. 2) The endodontic treatment significantly influenced the DC of the composite ($p < 0.0001$) but without a localisation effect ($p = 0.15$). 5% NaOCl had a deleterious effect on the polymerization of Tetric Flow®.($p< 0.0001$). 10 min. application of GSH or both GSH+NA ascorbate to root dentin allowed KHN values to be recovered that were close to those measured in contact with the sound dentin (batch 1,4 : n.s.). 3) Root dentin treated by 5% NaOCl (batch 2) or by overall endodontic solutions (batch 4) showed DBS values significantly lower than those obtained on sound dentin (respectively $p = 0.0016$ and $p< 0.0001$). 10 min. application of 10% GSH solution on the canal walls allowed the effect of oxidizing agents to be reversed. Batch 6 DBSs were not significantly different from those of the reference batch ($p= 0.2$).

DISCUSSION & CONCLUSIONS: This study first confirmed the linear correlation between KHN and DC for a given composite resin matrix as shown by Ferracane's study (1). The recorded linear function (KHN (f)DC) provided a reliable and simple method to assess the variation in polymerization of a composite directly applied to treated dentin. It has been shown that irrigant solutions commonly used in endodontics lead to decreased DBSs (2-3). It is likely that the dentin oxidation partially inhibits the polymerization of the resin matrix. This oxygen-inhibition also explains the reduction in DBS. But dentin treatment with anti-oxidant agents before bonding can reverse this drawback. GHS appears to be a more efficient reducing agent than Na ascorbate.

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Morphological study of interface between root dentin and fibre-reinforced composite post

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INTRODUCTION: The aim of the study was to analyze the interfaces, and thus the seals, between root dentin and fibre-reinforced composite posts. The interfaces were obtained by applying two amelo-dentin bonding systems, a one-bottle system used after application of phosphoric acid and a self-etch system, both associated with an adhesive cement.

METHOD: Twenty-six single-rooted front teeth extracted for periodontal reasons were prepared endodontically and then randomly assigned to one of two groups of 13 specimens: group 1: Excite DSC* one-bottle adhesive after etching with phosphoric acid; group 2: AdheSE DC* self-etch. On each specimen, after preparation of the canal and spreading of the adhesive, a translucid Postec FRC post* was bonded with Variolink II dual adhesive ciment*. Each specimen was embedded in epoxy resin and cut along the longitudinal axis of the tooth. The two halves obtained were prepared differently: one was demineralized then deproteinized at the cut surface so that the hybrid layer and cement layer could be observed; the other was prepared by completely dissolving the dentin substrate in order to observe the resin tags that had penetrated the dentin tubuli. The specimens were then dehydrated and sputtered with gold for SEM observation of the continuity of the hybrid layer and the interface with the adhesive cement, and to give a score to the resin tag morphology for each third of the root (at 1, 4.5 and 8 mm from the canal insertion position) [1]. A Mann and Whitney non-parametric statistical test was used to compare the hybrid layers obtained with each of the adhesives. The statistical test used to analyze resin tag scores was an ANOVA for repeated data.
*Ivoclar, Schaan, Leichtenstein

RESULTS:
Table 1. Ratio of length of hybrid layer : length of interface observed for each bonding system. There is no significant difference between the two adhesives ($p<0.05$ considered as significant) (*Ivoclar, Schaan, Leichtenstein)

Adhesive used with Variolink II* cement	**Length of interface observed in mm**	**Length of hybrid layer in mm**	**Ratio**
Excite DSC*	170.5	136.0	82.2%
AdheSE DC	181.4	160.1	99%

Table 2. Comparison of mean resin tag scores for different root observation positions and bonding systems (a, b, and c significantly different, *not significantly different)

Treatment	**Crown third 1 mm**	**Mid third 4,5 mm**	**Apical third 8 mm**	**Total**
Excite DSC	2.74	2.22	1.58	2.24*
AdheSE DC	2.91	2.00	1.01	2.08*
Total	2.84[a]	2.10[b]	1.72[c]	

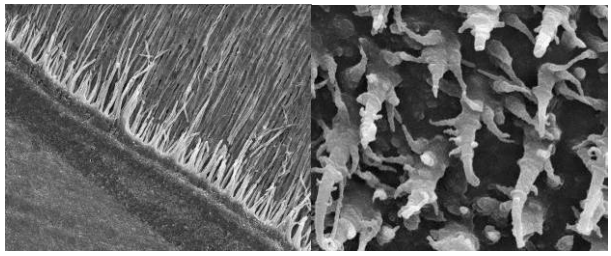

Fig. 1. Resin tags with side branches obtained in the mid third of the canal position with Excite DSC (right). Continuous hybrid layer with Excite DSC* (left)*

DISCUSSION & CONCLUSIONS: The statistical tests did not reveal any significant difference between the two bonding systems as far as the continuity of the hybrid layer and the morphology of the resin tags were concerned. The scores assigned to the resin tags, however, varied with position for a given adhesive ($p<0.001$), falling significantly from the crown third towards the apical third. The tags were denser, longer and had more side branches in the crown areas than in the apical areas. This difference can be explained by ultrastructural variations in the dentin substrate [2].

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Evaluation of adhesion between composite resins and an experimental mineral restorative material.

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INTRODUCTION: The purpose of this study was to evaluate the ability of new Ca_3SiO_5 based cement, used as a base in sandwich technique restorations, to bond to restorative composite resins. Adhesion was studied by evaluating marginal microleakage and shear bond strength of samples of composite resins bonded to the experimental cement with several different surface treatments.

METHODS: A three-step adhesive system (AllBond 2®, Bisco) and a silane coupling agent (porcelain primer, Bisco) were used to bond the composite resin (Enamel plus HFO GE3, Micerium) according to 9 different procedures (n=5). The marginal seal was evaluated by the silver nitrate penetration method after 3500 thermocycling cycles at 5 and 55°C. Shear bond strengths were evaluated on samples treated according to only five procedures (n= 10) two hours after bonding. Kruskall Wallis non parametric tests and Games-Howell *post hoc* tests were used to evaluate statistical differences between the experimental groups.

RESULTS: Figures 1 and 2 summarize the results. Groups with the same letter did not differ significantly.

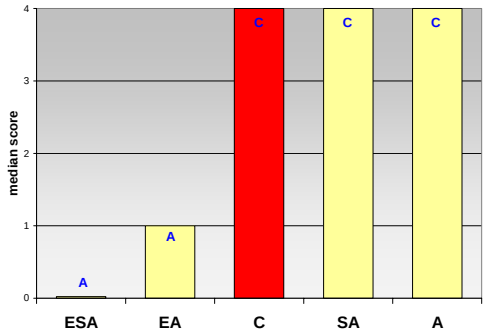

Fig1. Interfacial microleakage according to surface treatment.

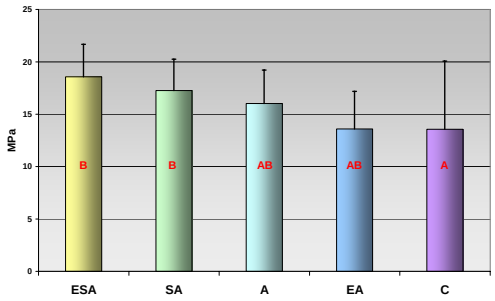

Fig. Mean shear bond strength according to surface treatment

The results presented here are those obtained with the five following procedures: control (no treatment) (C), adhesive resin (A), silane–adhesive resin (SA), etching-adhesive resin (EA) and etching-silane-adhesive resin (ESA).

DISCUSSION & CONCLUSIONS: Etching the surface of the experimental cement with a H_3PO_4 gel for 15s, then applying a silane coupling agent, before the adhesive resin, led to both the highest shear bond strength [18.57(3.04)MPa] and the lowest microleakage (median score = 0). This procedure seems to be the best when a composite resin has to be bonded to the experimental cement.

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A clinical study of a new Ca3SiO5-based material for direct posterior fillings

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INTRODUCTION: A new cement-based material for direct restorative posterior fillings (RD 94, Septodont, France) has been developed to circumvent the shortcomings of the traditional filling materials. This material is inorganic and non-metallic, and the main components are Ca3SiO5, CaCO3, ZrO2, and water. After evaluation of the genotoxicity, the cytotoxicity, the effects on the specific functions of target cells, and the marginal sealing of this new material, a multicentric clinical study was initiated to evaluate, in a three-year follow-up, the performance of this experimental calcium silicate cement (RD 94), versus a traditional resin composite (Z 100, 3M, US), in Class I and Class II restorations.

METHODS: From June 2005, every patient from 18 to 80 years old who, at the examination performed at the authors' University Clinic, needed a posterior restoration, was invited to join this randomized trial, under cover of Huriet's law. Each patient provided informed consent to participate in the study, which was approved by the ethics committee of the University of Marseilles (CCPPRB 1). All the patients invited participated in the study.

Two operators, both familiar with the new material, placed all restorations. All treated teeth were in occlusion, and the cavities were prepared with slightly convergent cavity walls, without bevels, and under rubber dam isolation. In Class II cavities, a thin metallic matrix band and wood wedges were used. All cavities were sprayed with water, and for the RD 94, no conditioning of the cavity or base material was recommended by the manufacturer. The restorations were finished after two weeks with polishing stones and strips. An examination book was created for each restoration, and a slight modification of the USPHS (United States Public Health System) criteria was used to evaluate the quality of the restorations by two calibrated observers [1-3]. Periapical radiographs and color slides were taken of all restorations, at dates D 0, D +15 days and D +6 months.

RESULTS: After ten months, 140 restorations had been performed in the Marseilles Dental School, 70 with RD 94 and 70 with Z 100. Forty-two of the restorations were Class I and ninety-eight Class II cavities. Ninety-four molars were treated, and forty-six premolars. Eighty teeth were treated in the upper maxillary and sixty in the lower.

In April 2006, thirty restorations had been evaluated at six months, including 11 Z 100 and 19 RD 94 restorations, and no non-acceptable clinical result was observed. Post-operative sensitivity was reported for two Z 100 restorations. A very good marginal adaptation and surface finish was observed on RD 94 restorations, although the color match of this new product was not yet perfect. None of the nineteen patients treated with RD 94 has lodged a complaint for pain, unpleasant physiologic or pathologic sensations, or objective or subjective reactions related to the material.

DISCUSSION AND CONCLUSIONS: Randomized clinical trials are considered the optimal way to validate the outcome of dental materials. However, randomized control groups require broad patient support, and are therefore time consuming and extremely demanding to conduct, thus contributing to the expensiveness of such studies. Nevertheless, the biological properties of this new material, combined with its interesting physicochemical characteristics, and with these hopeful preliminary results, justify the follow-up of this clinical study. After six months, the results indicated no significant differences, for direct restorations in medium sized cavities in posterior teeth, between a classical resin composite and the new Ca3SiO5-based material under test.

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Electron spin resonance, a useful analytical technique for studying the kinetic decay of free radicals trapped in dental resin

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INTRODUCTION Electron spin resonance (ESR) spectroscopy is used to detect and characterise free radicals present in solid or liquid phases such as organic radicalar species (allylic and propagating radicals) trapped in dental resins [1]. The aim of this presentation is to show how this analysis tool can be useful to follow the time evolution of the concentration of free radicals when dental resins are photopolymerised in various conditions and stored in various environments.

METHODS Experimental unfilled resin composites were prepared using a Bis-GMA/TEGDMA 70/30 mixture (wt%/wt%). A photoinitiator consisting of a camphorquinone/amine mixture (50/50 wt%) was added to the composites in the proportion of 1%.

Immediately after photopolymerisation in suitable moulds, we followed the kinetics of the EPR peak variation intensity for each radical for six hours, at ambient temperature. After the end of each kinetic follow-up, the resin bar was immersed in distilled water at 25 or 37°C. For some experiments, the storage environment was different (air, oxygen, nitrogen at controlled temperature). The peak intensities were followed over time (24 hours, 1, 2, 3 and 4 weeks) after the photopolymerisation. The samples were stored in the different environments between each spectroscopic examination.

RESULTS All the results showed decreasing free radical concentration kinetics that comprised two domains (*Fig 1*) :

-a first, between 0 and 24 hours, where the decay rate measured was rapid (200 u.a.day^{-1}), constant, and independent of the dental resin storage conditions. One interpretation of this observation involves the relaxation of the free volumes existing in the vitrified resin [2].

- a second, extending from 24 hours to one month and more, where the rate measured was weaker (3 to 100 u.a.day^{-1}), and decreased progressively or stayed constant depending on the storage conditions. A first analysis of this second domain suggested a free radical oxidation phenomenon.

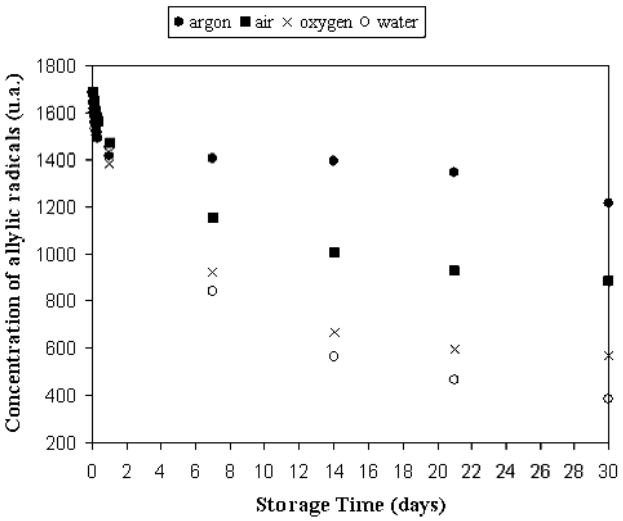

Fig. 1: Concentration decay of the allylic free radicals with time in various storage conditions of the resins.

PERSPECTIVES These results show how ESR spectroscopy can provide new information which may lead to a better understanding of the phenomena existing in dental resins. We plan to pursue and refine these experiments by using specific EPR methods (spin-trap for example) and to compare present and future results with those published in the literature concerned with the leaching in physiological media of compounds present in dental resins.

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Influence of Curing Mode upon Polymerization Contraction Kinetics of Resin Composites

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INTRODUCTION

Polymerization shrinkage is an inherent occurrence because monomer molecules get closer by exchanging van der Waals spaces for covalent bond spaces. The major drawback is the formation of a tooth-restoration interfacial gap. So, decreasing stress-strain behavior and volume changes of restoration polymers remains a current challenge. This study investigated the influence of the curing mode on the polymerization contraction of three resin composites.

METHODS

Three samples of three resin composites [Filtek P60 (P60), Filtek Flow (FLO) and Admira (ADM)] were light-cured using a LED unit (Elipar Freelight 2) for 20s and 40s in continuous mode and for 20s in exponential mode (5s of ramped activation and 15s with a maximum power). Curing contraction and kinetics were measured for a total duration of 360s using the deflected-disk technique according to the Watts and Cash method [1].

RESULTS

Resulting data are displayed in Tables 1 and 2. According to the Kruskal-Wallis tests, and whatever the curing mode, P60 showed significantly the lowest contraction, and FLO the highest. Remarkably, among all the materials tested there was no significant difference between the 20s and 40s continuous curing mode. In the exponential mode, all the materials displayed the highest final curing shrinkage. For the first three-second period, the polymerization contraction was slower in the exponential mode than in the continuous mode and the polymerization rate was higher for all the materials during the 3-10 second period in the exponential mode. From the 20th second, the curing rate was identical whatever the curing mode.

Table 1. Polymerization shrinkage means (%vol.)

Material	Curing mode	Shrinkage means % (SD)
ADM	Led 20s	2.62 (0.04) ^{a}
	Led 40s	2.56 (0.05) ^{a}
	Led exp	2.77 (0.03) ^{b}
P60	Led 20s	1,73 (0,03) ^{c}
	Led 40s	1.77 (0.03) ^{c}
	Led exp	2.00 (0.01) ^{d}
FLO	Led 20s	3.89 (0.03) ^{e}
	Led 40s	3.90 (0.02) ^{e}
	Led exp	3.98 (0.05) ^{f}
Results with the same superscript letter are not different		

Table 2. Polymerization shrinkage kinetics rate (vol%.s^{-1}).

Material	Curing mode	Polymerization kinetics (vol%.s^{-1}) between: 0-3s	3-10s	10-20s	20-40s	40-60s
ADM	Led 20s	0.281	0.131	0.041	0.011	0.004
	Led 40s	0.294	0.118	0.036	0.012	0.005
	Led exp	0.167	0.182	0.048	0.013	0.004
P60	Led 20s	0.210	0.077	0.025	0.007	0.003
	Led 40s	0.202	0.081	0.025	0.008	0.004
	Led exp	0.083	0.145	0.035	0.009	0.003
FLO	Led 20s	0.228	0.251	0.084	0.017	0.005
	Led 40s	0.249	0.241	0.075	0.020	0.007
	Led exp	0.067	0.294	0.103	0.021	0.006

DISCUSSION & CONCLUSIONS

Curing contraction and polymerization rate depend on the composite composition: monomer type, size and viscosity; filler type and amount; photoinitiator amount [2]. Because of a lower filler quantity, FLO has a lower viscosity, and its shrinkage is higher than that of P60 or ADM. ADM contains much more TEGDMA than P60 and its shrinkage is higher than P60. Besides, the curing mode is an important parameter: Use of a high intensity LED cure unit in continuous mode leads to fast formation of a rigid network, which rapidly decreases the capacity of the monomers to flow. The ramped activation decreases the polymerization rate and allows a molecular rearrangement taking away the gel point. Such a phenomenon entails a decrease in the strain-stress behavior, which potentially diminishes interfacial failures. [3]. It seems possible to reach a clinical compromise between the curing shrinkage and the reduction of internal stresses in the resin composites by choosing a curing mode in accordance with the polymers used. Moreover, for a material thickness of 1.64mm (which is the thickness of the experimental disks) a continuous light activation for 20s is quite sufficient to obtain the terminal conversion rate of the resin composites tested.

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MECHANICAL PROPERTIES AND POLYMERIZATION SHRINKAGE OF DENTAL NANOHYBRID COMPOSITES

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INTRODUCTION

A good restorative material has to stand up to the strain and wear induced by high stress during chewing. The mechanical properties of light-activated composites have been greatly improved, but their Young modulus – revealing their strain-resistance, and their hardness – revealing their wear-resistance, are still much weaker than those of native dental enamel and dentin. In this study we measured the influence on these properties of two different light curing systems: a halogen and a LED lamp. Polymerization shrinkage was also measured on SEM observations since it is one of the most important parameters to insure a long lasting restoration without recurrence of decay.

METHODS

Two nanohybrid restorative composites - Tetric®EvoCeram (Ivoclar-Vivadent) and CeramX™ (Dentsply) - were compared with a microhybrid material - Tetric®Ceram (Ivoclar-Vivadent). The halogen lamp was Astralis®10 (Ivoclar-Vivadent) and the LED one was Bluephase® (Ivoclar-Vivadent).

Three point bending test: 20 bars were made of each material and polymerized with one or other of the lamps for the time recommended by the manufacturer, i.e 10" for Tetric EvoCeram, and 20" for the other materials. After 24 hours in distilled water at 37°C, flexural strength and modulus were determined in accordance with the NF EN ISO 4049 standard.

Vickers micro-hardness: 10 bars (10x4x2 mm) of each material were polymerized with each LCU and stored for 24 hours in distilled water at 37°C. 10 other bars were stored for 10 days in the same conditions. Vickers micro hardness was then determined.

Polymerization shrinkage measurement: cavities were made in 8 wisdom teeth per material and per LCU and then filled (without adhesive) with composite. After 24 hours in distilled water at 37°C, the teeth were longitudinally cut and polished and then were observed by SEM. Polymerization shrinkage was measured near the tooth surface, at 2 and 4 mm deep and at the back of the cavity.

An ANOVA test and an unpaired t-test were performed to analyze the results.

RESULTS

Young moduli were higher when the materials were cured with the halogen LCU. Tetric®EvoCeram gave the weakest Young modulus value (4 GPa with Bluephase®). Vickers micro-hardness results were equivalent with both lamps except for Tetric®EvoCeram which was harder when cured with the halogen LCU; CeramX™ always showed the best modulus values. In some cases, samples had a higher hardness after 10 days of storage in distilled water: Tetric®Ceram cured with Astralis®10 and Tetric®EvoCeram with Bluephase.

It was observed by SEM that the shrinkage increased with the depth, whatever the material used. Results obtained with Tetric®EvoCeram and CeramX™ were not affected by the type of LCU, while those obtained with Tetric®Ceram were better with the LED unit. Tetric®EvoCeram gave the lowest shrinkage with the two LCU, Tetric®Ceram the highest.

DISCUSSION & CONCLUSIONS

Hardness was high for CeramX and Tetric®Ceram, and average for Tetric®EvoCeram while shrinkage was very high for Tetric®Ceram, average for CeramX™ and very low for Tetric®EvoCeram. These results suggest that it is not only the degree of conversion that acts upon the polymerization shrinkage, but also the composition and structure of the material. CeramX™ and Tetric®EvoCeram are nano-divided composites containing high proportions of fillers which reduce polymerization shrinkage. Tetric®EvoCeram can thus be considered as a good restorative material since its use leads to very little shrinkage, despite a low supposed polymerization rate considering its micro hardness. However, more studies (like ^{1}H-RMN or FTIR) need to be carried out to obtain more information about the degree of conversion of the monomers, and then to optimize the time of curing.

ACKNOWLEDGEMENTS

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Effect of Chemical Structure of Monomers on Shrinkage Stress in Experimental Light-cured Dimethacrylate-based Resins

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INTRODUCTION: The interfacial integrity of composite resins depends on many factors, especially the polymerization shrinkage stress related to the dimethylacrylate based matrix of these products. The aim of this study is to analyse the influence of the monomer (structure and concentration) on the shrinkage stresses of experimental matrices.

METHODS: Seven experimental light-cured dimethacrylate-based resins were tested in this study, three pure monomer (bis-GMA, TEGDMA and UEDMA), two co-monomer bis-GMA/TEGDMA (70/30 and 50/50 % by weight) and two co-monomer UEDMA/TEGDMA (88.5/11.5 and 66.5/33.5 % by weight). In order to make the matrices light-curable, 0.2 wt*%* of CQ (camphorquinone) and 0.2 wt*%* of CEMA (*N, N*-cyanoethylmethylaniline) were dissolved in each of the monomer mixtures. *Preparation (mixing):* A centrifugal mixer, SpeedMixer DAC 150* (FlackTech), was used. *Viscosity (η):* A rheometer, RFS II* (Rheometric Scientific), determined the viscosity of the matrices at ambient temperature (20 ± 0.1°C). *Shrinkage stress:* A mechanical testing machine, INSTRON 5569* (Instron Ltd), equipped with a 2 kN load cell recorded the stresses in the samples (150 mg ; C = 2,4 ; n = 5) polymerized over a period of 80s using the conventional high power density curing mode (1.200 mW/cm^{-2}) of the Optilux 501* (Kerr-Hawe) light-curing unit. *Glass transition temperature (T_g):* The T_g values of monomers were determined using a differential scanning calorimeter (DSC Pyris 1*, Perkin-Elmer) at a scanning rate of 10 C°.min^{-1}.

RESULTS: *1. Viscosity (η):* The viscosities of the UEDMA bases were exactly the same as those of the the bis-GMA ones. Thus the results showed 2 pairs of different viscosities, each pair allowing the behaviour of the bis-GMA and UEDMA bases to be matched with each other. *2. Shrinkage stress*: The mean values of the maximum shrinkage stress (MSS) after 600 s are listed in Table 1.

Table 1. T_g,viscosity and shrinkage stress of the matrices tested.

Matrix (wt%)	T_g monomer (T°)	Viscosity (Pa.s)	Shrinkage Stress (MPa)
Bis-GMA	3.3	1188	0.3
UEDMA	- 28	22	6.8
TEGDMA	-82	0.01	14.2
bisGMA/TEGDMA (70/30)	-39	3.5	4.4
UEDMA/TEGDMA (88.5/11.5)	-37	3.5	8
bisGMA/TEGDMA (50/50)	-58	0.28	8.2
UEDMA/TEGDMA (66.5/33.5)	-53	0.28	10.6

Statistically, there was a negative correlation between viscosity, T_g and shrinkage stress.

DISCUSSION & CONCLUSIONS: It would seem that the viscosity of the matrices studied and the concentration of the diluting monomer (TEGDMA) of the co-monomer matrices, are the determining elements influencing the development of shrinkage stresses [1]. The large differences in stress values for the pairs with the same viscosity, shows us that it is not the viscosity in itself which has a dominating influence on stresses (via the DC) but the molecular mobility, which is linked to the monomer structure and characterized by the glass transition temperature [2]. A better understanding of the phenomenology of the internal stresses necessitates an evaluation of the volume contraction and the degree of conversion of these matrices.

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USE OF HYPERBRANCHED POLYMER IN DENTAL RESINS TO REDUCE POLYMERIZATION SHRINKAGE

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INTRODUCTION: Linear polymers (BisGMA, TEGDMA, etc.) have been commonly used in the matrix of composites. Recently, new types of polymers, hyperbranched polymers (HBP), have been synthesized. HBP are very large molecules with many branches and a large number of functional groups on each molecule in comparison with traditional linear polymers. In a previous study [1], a linear relationship was found between the volume contraction and the actual number of double bonds converted. A reduction of the polymerization shrinkage may obviously be expected from the addition of molecules allowing the double bond conversion per unit of volume of resin matrix to be decreased. HBP are thus attractive because their functionalities can be modified to obtain molecules with a high volume and few double bonds. The objective of this study was to investigate the potential of HBP to decrease polymerization shrinkage of the composite matrix while maintaining double bond conversion.

METHODS: The resins investigated consisted of a conventional mixture, BisGMA/TEGDMA (50/50 wt/wt), used as a reference, in which three HBPs, with different shell chemistry, were added at several weight ratios (5, 10, 20, 30, and 40%). A photoinitiation system was added to each mixture in a proportion of 1%. Degree of conversion (DC) of the polymers was determined by Raman spectroscopy. The volume contraction was measured by pycnometry and by a density column.

RESULTS:

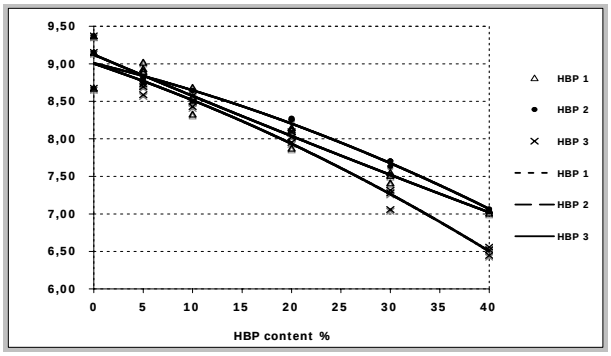

Fig. 1: Polymerization contraction of the resins as a function of the HBP content

Fig. 1 shows that polymerization shrinkage decreases when an increasing weight of HBP is

added to the BisGMA/TEGDMA 50/50 mixture. The lowest volume shrinkage achieved was about 6.5%, which is approximately 30% less than the reference (9%). The most pronounced effect on shrinkage reduction was achieved when HBP3 was added.

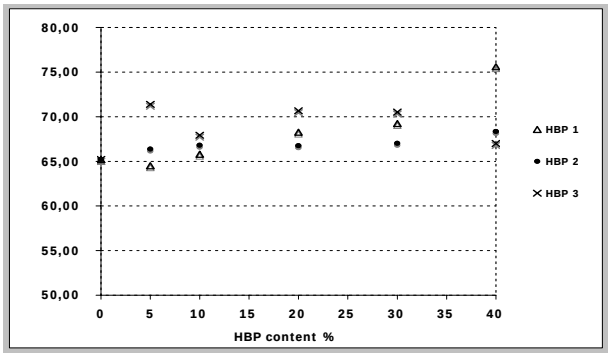

Fig. 2: Degree of conversion of the resins versus HBP content.

The DC for the BisGMA/TEGDMA (50/50), used as the reference, was not significantly different from that of any of the modified systems, as shown in Fig. 2.

DISCUSSION & CONCLUSIONS: By decreasing the volume shrinkage and maintaining the DC, the modified resins are likely to give an improved organic matrix in comparison with the commonly used BisGMA/TEGDMA matrix. However, mechanical properties and viscosity should not be affected by the presence of HBPs. Increased viscosity would have several negative effects on the manufacture or application of dental composites. In a recent study [2], the addition of HBP to a mixture of BisGMA/TEGDMA (1:1) resulted in improved mechanical properties without increasing the viscosity. Work along this line is in progress.

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Study of the thixotropic behaviour of flowable resin composites: analysis of the destructuration-restructuration process

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INTRODUCTION: The purpose of this study was to investigate the mechanism of the destructuration-restructuration process of thixotropic experimental flowable composites and to determine whether it had an influence on the final mechanical properties of the polymerized materials.

METHODS: Experimental resin composites were prepared using a Bis-GMA/TEGDMA 50/50 mixture (wt%/wt%) filled at 60% with silanized dental glass as a macrofiller and partially hydrophobic silica as a microfiller, in the proportions 60/0, 57/3, 55/5, 52/8 and 50/10 wt%. A photoinitiation system, a camphorquinone/amine mixture (50/50 wt%), was added to the composites in the proportion of 1%.

Samples were prepared in moulds of 25mm x 2mm x 2mm as specified in ISO specification 4049 [1]. Composites were heavily destructured (by forcing them through the application tip of a syringe) before being placed in the moulds, and were polymerized after a resting time of 0min, 3min or 90min. A last group of sample was polymerized without prior destructuration as a control. Four samples were prepared in each group.

Samples were then placed in a 3-point setup bending at a rate of 0.75mm/min until fracture occurred in order to measure their elastic moduli and their flexural strengths. Statistical analysis was performed using a two-way ANOVA and post hoc Scheffe's tests at $p<0.05$.

To analyse the modifications in the microscopic structure of the materials after deformation and during restructuration, samples were sliced using a microtome and Transmission-Electron Microscopy (TEM) micrographs were taken for each sample.

RESULTS: Statistical analysis performed on the results obtained for the elastic moduli and those obtained for the flexural strengths of the samples showed an influence of the delay before polymerization as well as an influence of the ratio between macrofillers and microfillers. Elastic moduli of the materials tested are shown in Table 1.

Table 1. Elastic moduli of the materials tested versus the time elapsed between destructuration and photopolymerization.

%micro-fillers	0min	3min	90min	∞
0	6.25(1.39)	7.30(0.69)	4.83(1.42)	8.13(0.70)
3	6.38(0.44)	7.65(0.39)	8.00(1.48)	8.20(1.83)
5	6.28(0.81)	7.63(0.56)	7.63(0.30)	7.78(0.30)
8	6.70(0.46)	4.48(0.51)	6.93(0.46)	7.14(0.45)
10	6.18(0.17)	6.93(0.38)	6.28(0.79)	8.37(0.29)

TEM micrographs showed no difference in the microscopic structures between samples that were destructured and samples that were not. Figure 2 shows the sample with 5% of microfillers, both in the rest state and immediately after deformation.

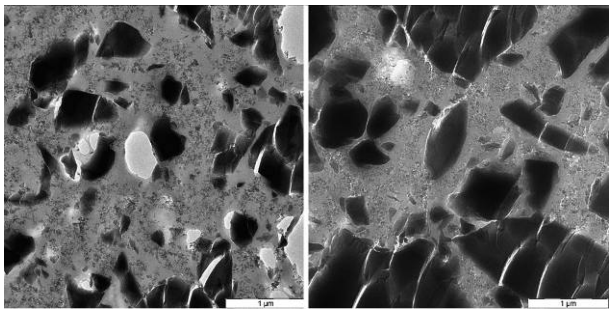

Fig. 2: Microscopic structure of the composite with 5% of microfillers in rest state (left) and immediately after destructuration, (right).

DISCUSSION & CONCLUSIONS: From the results above, it appears that the thixotropic behaviour of resin composites does not seem to be linked to filler particle movements but to more complex factors (including hydrogen bonds involving filler particles). The time elapsed between destructuration of the materials and their photopolymerization has an influence on their final mechanical properties.

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ACKNOWLEDGEMENTS: The authors would like to thank Voco GmbH (Drs I. Braun & R. Maletz) for the preparation of the experimental flowable resin composites, and Thérèse Glorieux and Pascale Lipnik for their help.

Anatomy and shaping of the mesial intercanal groove of the lower molars

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INTRODUCTION: Root canal anatomy, especially in molars, has always been a subject of study in endodontics. In the mandibular molars, a groove situated on the pulp-chamber floor between the mesial-canal orifices has been paid specific attention. According to Yesilsoy et al., after the canal shaping, potentially infected pulpal tissue could persist in the groove and might compromise the treatment prognosis [1]. The aim of this work is to study, on a set of first and second mandibular molars, the impact of using shaping instruments and ultrasonic tips on the depth and surface of this anatomical feature.

METHODS: This study was carried out on 18 healthy mandibular molars having two roots, one mesial and one distal, which were separate and fully formed. The crowns were cut horizontally at the level of the cemento-enamel junction [2]. After radiographs (mesial view), the roots were embedded in transparent resin cubes. An isomet was used to make a mesio-distal cut between the mesiobuccal and mesiolingual orifices. Then, an experienced operator mechanically prepared all the teeth in the same way. First, the canal orifices were enlarged and the mesial root canals prepared with HEROShaper® files (Micro-Mega Besançon, France). Subsequently, the intercanal groove was specifically prepared with ultrasonic tips under constant irrigation. The teeth were randomly split into two groups and prepared using the ETD 20 or the ET 25 tip (Satelec, Acteon Group, Mérignac, France). For each operating stage (before the treatment, after the shaping and after the ultrasonic preparation), three views (occlusal, internal, external) were taken with an operating microscope. The pictures were analyzed with the Photoshop® software. The peripheral limits of the canal orifices and the groove were drawn in order to calculate the depth and the surface area of the groove. These parameters were calculated after the mesial root canal shaping and the groove ultrasonic preparation. The depth and surface area data were converted into percentages to allow comparisons from one face to the other and between the different teeth. These data were then analyzed by a paired series Student test, before and after the ultrasonic preparation. The analysis of the variance of the effect of the two types of ultrasonic tips was also performed with a risk α=5%.

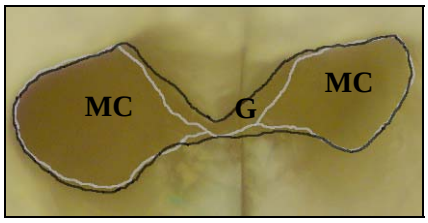

Fig 1: Canal orifices and groove limits, on occlusal view

— Limit after the root canal shaping

— Limit after the groove ultrasonic preparation

G: groove; MC: mesial root canal

RESULTS: The ultrasonic preparation allowed a significant gain in surface area of the groove on the occlusal, internal and external views. This preparation also provided a significant deepening on the sagittal views.

There was no significant difference between the groove preparation using the ETD 20 and the ET 25 tips (p=0.3722 to 0.9904 depending on the views).

Table 1: Depth and surface area average gain

View	Parameter under study	Average (%)
Internal	Depth	182.51
	Surface area	244.79
External	Depth	197.09
	Surface area	246.05
Occlusal	Surface area	35.98

DISCUSSION & CONCLUSIONS: Ultrasonic preparation enables the groove to be widened and deepened without distorting the root canal orifices. It allows the cleansing of this area by favouring the elimination of the pulpal tissue and by forming a reservoir for the irrigation. Moreover, thanks to a significant gain in surface area, it creates a "beam" between the mesial root canals which, prepared in this way, could be used to bond and fix coronoradicular restorations.

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Mechanical behaviour of a nickel-titanium root canal instrument: a non-linear finite element approach

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INTRODUCTION: The study of the mechanical behaviour of Ni-Ti endodontic instruments is a difficult challenge for many reasons: the instrument geometries are complex, the mechanical behaviour of Ni-Ti SMA is strongly non-linear and very much affected by the multiaxiality of the mechanical loading [1-2]. Some interesting experimental investigations have been performed in recent years to determine the effect of different parameters (rotational speed, curvature of root canals, microstructure, etc. [3]) on the failure of endodontic instruments. These indispensable studies are time-consuming and can be performed only on existing instruments. Our investigations concern an alternative and complementary approach based on non-linear finite element simulations to evaluate the effect of the cross-sectional shape on the mechanical behaviour of Ni-Ti endodontic instruments.

METHODS: Since the geometry of endodontic instruments is complex, three-dimensional computation based on the finite element method is required to determine the stresses, the strains and the behaviour of root canal instruments. Some results have already been published concerning the study of the mechanical behaviour of endodontic instruments by numerical methods [4]. In all cases, the mechanical behaviour of Ni-Ti has been considered as elastic. This hypothesis allows the duration of simulations to be reduced but it does not take into account the real mechanical behaviour of Ni-Ti (*i.e.*, superelastic non-linear behaviour). In this study, an *ad hoc* model recently proposed by Bouvet *et al.* [1] was implemented in the finite element code CAST3M® developed by the CEA (Commissariat à l'Energie Atomique), and used in the simulations. This model takes into account the martensite transformation, the effects of cyclic loading and the effects of the multiaxiality of the loading.

RESULTS: Figures 1 and 2 show the cross section and the finite element mesh of the endodontic instrument respectively. The finite element mesh consists of 6210 elements and 8281 nodes. It takes into account both the cross sectional size and the thread variations. The left face was blocked and bending was applied to the right face. Figure 3 shows the stress and the martensite volume fraction distributions in the instruments.

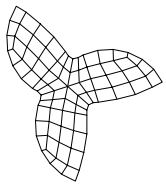

Fig. 1: Cross section of the instrument.

Fig. 2: Finite element mesh of the instrument.

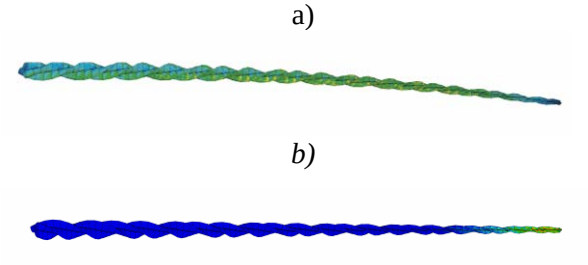

Fig. 3: von Mises stress and martensite volume fraction distribution in the instruments.

DISCUSSION & CONCLUSIONS: The results obtained show that it is now possible to estimate the stress field and the phase transformation rate in the complex geometry of a Ni-Ti endodontic instrument. The next step of this work will concern failure prediction under representative mechanical loading.

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ACKNOWLEDGEMENTS: The authors would like to thank Micro-Mega for its assistance.

Cleaning qualities of Rinsendo®: an *in vitro* study

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INTRODUCTION: The purpose of this study was to investigate root canal rinsing devices *in vitro* and to evaluate their cleaning qualities, especially in the apical third.

METHODS: Ninety human single-rooted permanent teeth, stored in 70° alcohol, were randomly divided into 3 groups, their crowns cut off, the root pulps removed and their empty chambers rinsed with 5ml of a 2.5% NaOCl solution. The root canals were treated mechanically using ENDOFLARE®, HERO SHAPER® 6%, or HERO SHAPER® 4% (MICRO MEGA®). Each step was followed by 5ml NaOCl rinsing, and a final rinse of 5ml of EDTA followed by 5ml of 2.5% NaOCl was carried out. Rinsing was performed by means of either a laterally opened syringe (group 1), or an ultrasonic device of 15/100th files SATELEC® (group 2), or RINSENDO® DUERR DENTAL® (group 3). Roots were split into 3 fragments (cervical, middle and apical) which then were broken axially and prepared for observation under a VP 1450 ZEISS Scanning Electron Microscope (Jena, Germany) under 7mA acceleration. A program was used to analyse the cleaning effectiveness of each rinsing device on the cervical, middle and apical root canal surfaces.

RESULTS: All systems tested seemed indicated for root canal surface cleaning. However, the cleaning quality was variable and depended on the surface location and the technique employed. The cervical third was best cleaned by the syringe rinse; the ultrasonic device ranked second and Rinsendo® was the least effective. The middle third was best cleaned by the ultrasonic device, followed by the syringe rinse and Rinsendo®; and the apical third was best cleaned by Rinsendo®, followed by the syringe rinse and ultrasonic device. Furthermore, the cleaning effect was not uniform. In all samples, tiny and occasionally large strands of waste were observed. Moreover, when the foramina vacuities were checked by means of a K15 file, in about 50% of all apical samples, a jet of liquid signifying an excess of NaOCl was noted.

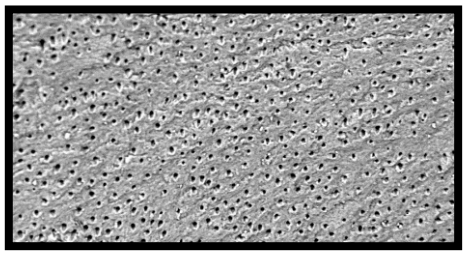
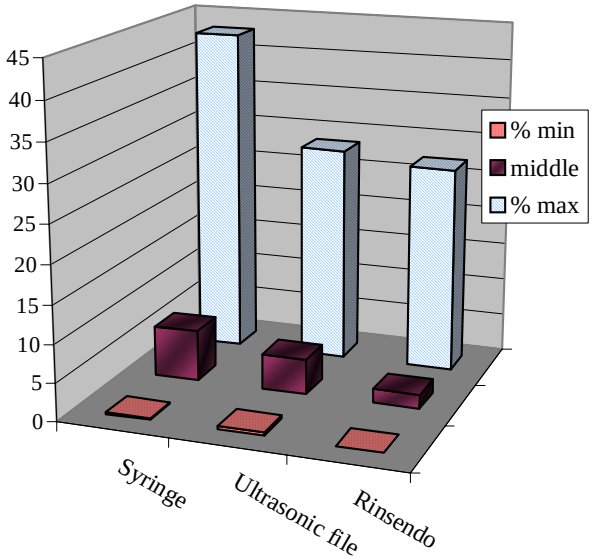


Fig. 1: Surface quality of the apical third after irrigation with Rinsendo® (X500)

Table 1. Comparison of the three systems in the irrigation of the apical third.



DISCUSSION & CONCLUSIONS: All the systems tested seemed indicated for apical root canal cleaning. In this *in vitro* study, the best results were obtained with RINSENDO®. Some problems were observed with the RINSENDO® device: the syringe piston blocked after the first or second rinse. In immature permanent teeth and in root resorption, special care should be taken with regard to NaOCl excesses, which were observed with all rinsing systems.

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Comparative evaluation of interface between two bonding systems and crown and root dentine using optical microscopy

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INTRODUCTION: Adhesion to dental tissues is achieved by using bonding systems which lead to the formation of a hybrid layer, a mixture of adhesive polymers and the demineralised tissues of the tooth. The dentin morphology is one of the factors that influence the effectiveness of the seals obtained with bonding systems. The aim of this study was to compare, by optical microscopy, the morphology of the hybrid layer produced in crown and root dentin after application of two bonding systems, a phosphoric acid etch-and-rinse system and a self etching system.

METHODS: Twelve healthy molars extracted because of developmental pathologies were used. A 2-mm-thick dentin disk was cut from each tooth and randomly assigned to one of two groups: Group I to receive Excite* one-bottle system preceded by prior etching with phosphoric acid, which is a photopolymerizable product having an ethanol-based solvent; or Group II to receive AdheSE* self-etch, which is photopolymerizable and has a water-based primer applied in two steps.

Twelve single-rooted human teeth extracted for periodontal reasons were prepared endodontically with Hero system 6-4-2, and randomly assigned to one of two groups: Group I to receive Excite **DSC*** simplified dual system preceded by prior etching with phosphoric acid, or Group II to receive AdheSE DC*, a dual self-etch system applied in two steps with an endodontic microbrush.

[1]The specimens were prepared for optical microscopy and stained. To standardize the observations, microphotographs at 320x magnification were taken systematically.

*Ivoclar Vivadent, Schaan, Leichtenstein

RESULTS: The stain used coloured the dentin yellow and the resin bright pink.

The interface observed in groups I and III showed a regular hybrid layer with a thickness sometimes reaching 10µm, and short, v-shaped tags (14 to 28µm). These tags were conical and sealed the upper parts of the tubuli. The interface observed in groups II and IV showed a regular hybrid layer varying in thickness (1.5 to 3 µm). Tags were numerous, long and fine. Tag lengths were at least 21µm and sometimes reached 98µm, their length depending on the location in the canal. They ensured intimate contact with the dentin walls.

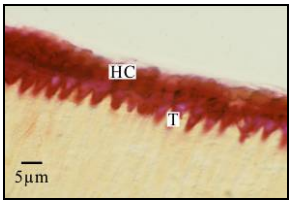

Fig.1. Hybrid layer obtained with Excite applied to crown dentin (x320)

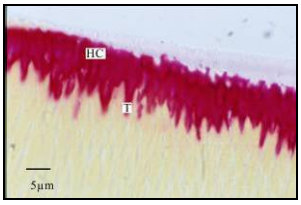

Fig.2. Hybrid layer obtained with Excite DSC applied to root dentin (x320)

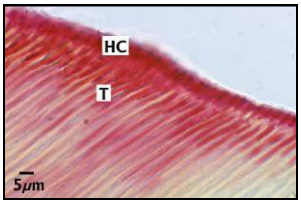

Fig. 3. Hybrid layer obtained with AdheSE applied to crown dentin (x320)

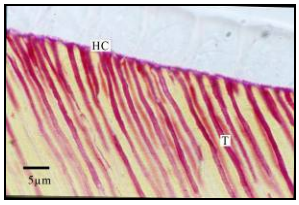

Fig.4. Hybrid layer obtained with AdheSE DC applied to root dentin (x320)

DISCUSSION AND CONCLUSION: The hybrid layer obtained with the self-etch system was thinner than that obtained with a phosphoric etch preceding adhesive application, and the results of our study are in agreement with the literature.[2] The difference may be explained by the fact that, with self-etch systems, the presence of the dentin smear layer will result in a smaller depth of dentine infiltration. The regular morphology of the hybrid layer obtained with the self-etch systems is due to the fact that the demineralization and infiltration take place simultaneously.

The results show a good contact between the tags and the walls of the tubuli with both adhesive systems, which is favourable for good sealing

Our results show that there are no differences in the morphology of the hybrid layer produced in the crown and the root after application of two bonding systems. But the tag density decreases from the crown third to the root third.[3]

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Root canal sealers: scanning electron microscope study of tubular penetration.

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INTRODUCTION: Root canal obturation is obtained by associating a semi-solid hard nucleus, the gutta-percha, with a root canal sealer ensuring only the sealing of obturation thanks to its cohesion, its adaptation to the canal walls and its tubular penetration.
The purpose of this study was to evaluate the penetration of 5 root canal sealers into dentinal tubules: a zinc-oxide-eugenol-based sealer (Endobtur®, Septodont, Saint-Maur, France), a glass-ionomer sealer (Ketac Endo®, ESPE, Seefeld, Germany), a calcium-hydroxide-based sealer (Acroseal®, Septodont, Saint-Maur, France), an epoxy-resin-based sealer (AH Plus®, Dentsply/De Trey, Konstanz, Germany), and a silicon-based sealer (Roeko Seal Automix RSA®, Roeko, Langenau, Germany).

MATERIALS AND METHODS: Forty-two permanent single-rooted freshly extracted teeth were used in this study. After coronal section and determination of the working length, the canals were shaped, respecting the difficult sequence of the HeroShaper® system (MicroMéga, Besançon, France). Instrumentation was associated with repeated irrigation by NaOCl 3% (12ml). The smear layer was eliminated by the use of EDTA 15% (3ml). A final rinse was performed with NaOCl 3% (5ml). The teeth were divided into 5 groups according to the chosen sealer then obturated with Herofill® obturator. Two unobturated teeth formed the control group. The samples were cut transversely at the level of the apical, middle and coronal thirds, to be studied with the Scanning Electron Microscope.
For each cut, the minimal and maximal value of the depth of penetration of the sealer were noted so that the average penetration value could be calculated. The results were statistically analysed with Fisher's PLSD test and the repeated measurements ANOVA.

RESULTS: Ketac Endo was the only sealer which did not penetrate into the dentinal tubules. In the apical third, only AH Plus penetrated the dentinal tubules. In the middle and coronal third, Acroseal and AH Plus showed the best results ($p<0.03381$), ahead of Endobtur and RSA.

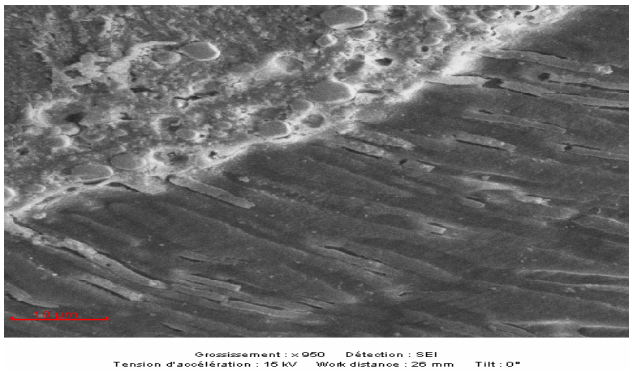
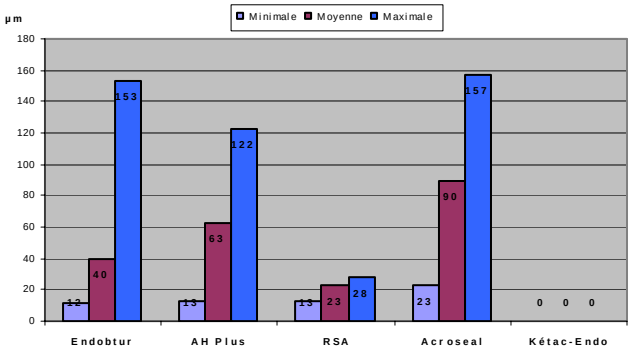


Figure 1 AH Plus is perfectly adapted to the peritubular dentin, its texture is homogeneous and builds little circular sticks.



Graph 3: Values of the minimal, average and maximal intratubular penetration of each sealer in the coronal third.

DISCUSSION AND CONCLUSIONS: In this study, AH plus and Acroseal behaved best. Beyond the obturation technique, the factors directly influencing the sealer penetration were the diameter of the dentinal tubule and the type of sealer used: its surface tension, plasticity, dimensional stability to setting, and particle diameter.

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Spectrofluorometry study of interface between root dentin and one-bottle adhesive. Preliminary results

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INTRODUCTION: The aim of this work was to study the molecular interaction between the radicular dentin and the bonding system in the hybrid layer when a fibre-reinforced composite post was attached. The technique chosen for determining the characteristics of this reaction was fluorescence resonance energy transfer (FRET) between the autofluorescent dentin (donor) and a bonding system labelled with a fluorescent compound (dextran FITC) (acceptor). The degree of resolution of the technique (10 to 100 nm) enabled molecular interactions to be observed.

METHOD: Spectrofluorometry was performed using an Aminco SPF 500C spectrofluorometer in order to study the absorption and excitation spectra of the radicular dentin and the bonding system, Excite DSC (Vivadent, Schaan, Liechtenstein). Dextran fluorescein labelling of the adhesive (methacrylate polymer) was used to help show up the FRET as the fluorescence spectra of the adhesive and dentin are very similar, which is not the case for FITC. The adhesive samples, alone or mixed with 0.1 or 0.5% dextran fluorescein (MW=10000, Molecular Probes) were polymerized on a slide. Roots of single-rooted teeth conserved in a 0.1% chloramine T solution were prepared to receive a post by removal of the pulp, and preparation of the canal with reamers appropriate to the splint size (Moser n°1 pre-reamers and reamers for the mandibular and lateral maxillary incisors, and n° 3 for the canines and central maxillary incisors). The roots were then cut longitudinally with an Isomet saw (Isomet, Buehler, Lake Bluff, NY, USA) and examined by fluorescence spectroscopy, either without preparation or after etching of the endocanular part and polymerization of a layer of adhesive on the surface.

RESULTS: *Table 1: Excitation and emission characteristics of the compounds involved*

	λmax Excitation	λmax Emission
Dentin	343 nm	400 nm
Excite DSC	330 nm	360 nm
Dextran FITC	490 nm	526 nm

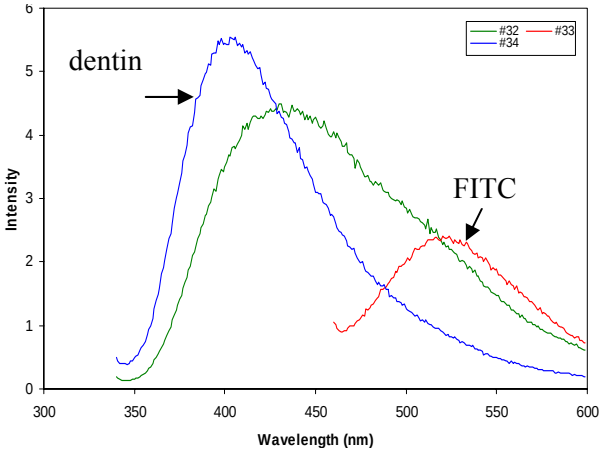

Fig. 1. Emission spectra of dentin (blue), and Excite DSC with fluorescein (red) after polymerization, showing FRET between dentin and labelled adhesive (green curve)

DISCUSSION & CONCLUSIONS: The preliminary spectrofluorimetry studies showed:

- The validity of an Excite DSC-FITC mixture, without FRET between the two compounds, for showing up FRET between the dentin and the adhesive.
- The potential of energy transfer (FRET) between autofluorescent dentin and Excite DSC adhesive-FITC. The FRET enables molecular interactions between the adhesive and the dentin in the hybrid layer to be observed with a nanometric level of resolution (10 to 100nm) and a "molecular map" of the interface to be obtained.

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Fatigue resistance of different glass fiber posts

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INTRODUCTION: Fatigue is considered as one of the main causes of structural failure in operative dentistry. Fatigue tests can reveal the resistance level of each type of post under cyclic loading that simulates the normal occlusal and masticatory functions [1]. The aim of this study is to evaluate the fatigue resistance of various fiber posts and to check the existence of a correlation between their resistance and their composition.

METHODS: The posts of the various brands are not all cylindrical and do not all have the same diameter. In order to compare the fiber posts amongst themselves, the manufacturers have designed perfectly cylindrical experimental posts 25 mm in length and 1.8 mm in diameter. The nature and the proportions of their various components are identical to those that are proposed to dentists. Five types of fiber posts were selected for this study. They were Group 1: Aestheti-Plus exp (RTD,Fr), Group 2: Light-Post exp (RTD,Fr), Group 3: Snowpost exp (Carbotech,Fr), Group 4: Snowlight exp (Carbotech,Fr) and Group 5: FRC Postec exp (Ivoclar-Vivadent, Liech).
Ten posts from each group were tested in a fatigue machine (Procyon Systèmes, Fr).
The three-point bending method of loading was applied with a loading angle of 90°. A cyclic force ranging from 25N to 100N was applied at a frequency of 3 Hz. The two supports and the central loading anvil had a diameter of 3 mm and the distance between the two supports was 9 mm. All the tests were carried out at room temperature. The device has a counter that measures the number of cycles and stops when the specimen breaks. The tests were stopped voluntarily after 2 million cycles, which would simulate about four years of physiologic occlusal and masticatory activity. After studying ANOVA validity conditions, we chose the Fisher's protected least difference (PLSD) parametric test to identify differences between pairs of groups.

RESULTS: Group 1 = 2.000.000a ± 0; Group 2 = 2.000.000a ± 0; Group 3 = 1.740.555a ± 436.140; Group 4 = 612.631b ± 503.538; Group 5 = 2.000.000a ± 0. Means with the same letter indicate non-significant differences ($p>0.05$). Snowlight exp showed fatigue resistance that was significantly lower than that of any other post tested ($p<0.0001$). 100% of Snowlight exp and 30% of Snowpost exp were fractured during the test.

DISCUSSION & CONCLUSIONS: As compared to the resin matrix, fibers form the stiffer component in a post and a higher fiber density should lead to greater fatigue resistance [2]. Nevertheless Snowligtht exp is composed of the highest percentage of fiber (64%) embedded in a polyester methacrylate matrix, which differentiates these posts from the others that contain less fiber and are composed of epoxy resin or UDMA. The percentage of fibers does not seem to be a factor determining the fatigue resistance. The methods of fabrication, the modulus of elasticity of the resin matrix, how the fibers are silanized prior to embedding in the matrix, and/or voids present within the resin or in the discontinuities along the interfaces between fibers and matrix can provide an explanation for the differences in resistance to fracture of the posts under cyclic load [3].

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On the chemical nature of the organic matrix and its influence on fiber post adherence

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INTRODUCTION: Composite posts are manufactured by embedding fibers in an organic resin matrix. Our aim was to evaluate the influence of the chemical nature of this organic phase on the experimentally measured adherence strength at the interface with a dentin bonding agent. Experimental posts were specifically designed and made according to the same criteria as standard posts to determine the sole effect of the resins by comparison tests.

METHODS: 10 Light-Posts made with 60% quartz fibers by volume and 40% Novolac-type epoxy resin made up the reference group (G0). 30 experimental posts were divided into three groups according to the nature of the matrix : G1 with bis-GMA + hexanedioldimethacrylate, G2 with epoxy bisphenol A, and G3 with polyurethane. Push out tests were performed to evaluate shear bond strength at the interface with the dentin bonding agent [1]. A special Teflon mold was used to construct the test and maintain the applied force parallel to the post axis. A Synergie 2000 MTS system was used at a crosshead speed of 1mm/min. One-step (Bisco) dentin bonding agent was placed on the post then dried and light-cured 20 sec. A microhybrid Lumiglass (RTD) composite was compacted around the post in the teflon mold and light-cured for 40 sec at 800mW/cm2 with a Spectrum 800 (Dentsply). The specimens obtained were stored away from light and humidity for 7 days then tested. Shear strength, in MPa was calculated as:

$$\sigma = F/\pi dh$$

(F=force in N, d=diameter in mm and h=height of the sample in mm). After studying ANOVA validity conditions, we choose the Fisher's protected least difference (PLSD) parametric test to identify differences between pairs of groups.

RESULTS: the shear strengths and standard deviation found were (in MPa): G0 = 27.7^{a} (2.8); G1 = 34.3^{b} (1.5); G2 = 34.4^{b} (1.6); G3 = 29.3^{a} (3.1). Means with the same letter indicate non-significant differences ($p>0.05$). The experimental groups made of bisGMA and epoxy bisphenol A exhibited retention values that were significantly higher than the control group (G0 vs G1 and G2 ; $p < 0.0001$).

DISCUSSION & CONCLUSIONS: The chemical nature of the organic matrix seems to play a significant role in shear bond strength. All the matrixes tested were cured at high temperature to reach near 100% conversion rate. This guarantees dimensional stability and good intrinsic mechanical properties for the posts. However the surfaces become more inert and less prompt to react covalently with functional groups on the adhesive. The exact nature of the link between resin and adhesive is, as yet, unknown. Resin choice should be guided not only by the bonding capacity but also by the mechanical results combined with further studies to assess the behavior of the posts upon aging. Moreover hydrolytic stability is required to maintain post integrity [2].

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ACKNOWLEDGEMENTS: we are pleased to thank RTD for material support.

The use of guided tissue regeneration principles in endodontic surgery

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INTRODUCTION: Periradicular surgery has become an established treatment option in endodontic surgery. The major objective is to obtain periradicular healing. The application of guided tissue regeneration (GTR) in periapical surgery has seen an increase in recent years based on favourable outcomes reported in periodontal applications. Through a clinical case (Fig 1) and a review of the literature, this work presents the use of GTR as an adjunct for healing in endodontic surgery.

METHODS: Review and case series with at least 12 months' follow-up were researched. Data sources included electronic databases and hand-searched journals from 1996 to 2006. The evaluation was made on clinical, radiographic and histological parameters.

DISCUSSION & CONCLUSIONS: Clinical and radiographic conditions improved during the study period. Using GTR in endodontic surgery leads to an increase in the amount of bone, periodontal ligament and cementum. The main applications are periodontic-endodontic lesions. A classification of these lesions is required.

This conclusion must be tempered by the fact that there are only a small number of studies. Controlled clinical trials investigating this topic are difficult to design in humans

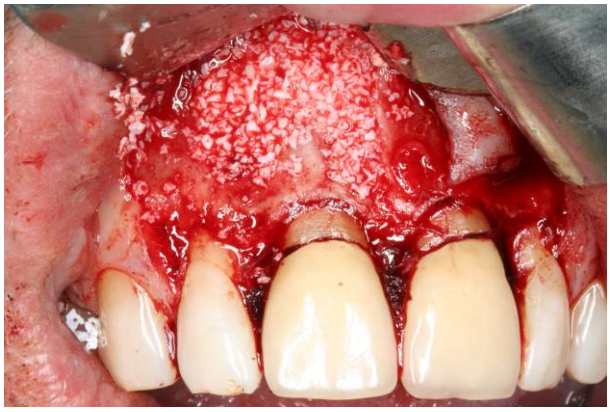

Fig 1: Use of a bone replacement graft for a periodontic-endodontic lesion.

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Accidental ingestion and aspiration of root canal instruments and other dental items in a French population

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INTRODUCTION: There is no doubt among endodontists that using a rubber dam for root canal treatment is mandatory. Postgraduate endodontic students are taught the importance of a rubber dam and how to place it under even the most difficult clinical conditions, even on crownless or cone-shaped teeth. However, in spite of the serious consequences of ingestion and aspiration, most surveys showed that most of the general practitioners do not use rubber dams when performing routine endodontic treatment, regardless of the country. Our own experience showed that patients swallowed or inhaled not only endodontic instruments but also other items such as crowns, burs, and copper band.
There are published case reports describing swallowing and aspiration of dental items but no study gives the prevalence of this type of accident.

METHODS: The data were provided by two insurance companies representing 24,651 French general dentists over 11 years.
For aspiration and ingestion, the following data were recorded:

1) The endodontic instrument or dental item involved and the percentage of occurrence of either aspiration or ingestion. The prevalence of accidental aspiration or ingestion was calculated.
2) The need for hospitalization. A chi2 test was performed to compare aspiration and ingestion. Two other chi2 tests, for ingestion and aspiration, were performed to compare endodontic versus non-endodontic items in order to know whether endodontic instruments are more dangerous and required hospitalization more frequently. The significance level was set at 5%.

RESULTS :

Table 1. *Number of endodontic instruments or dental items involved and the percentage of occurrence of either aspiration or ingestion.*

	Aspiration	Ingestion
Endodontic file	1	57
Barbed broach		27
Bur		125
Temporary crown	5	15
Prosthesis	27	136
Matrix band		14
Piece of amalgam	2	17
Screw post	3	9
Extracted tooth		7
Orthodontic bracket		8
Inlay core	7	49
TOTAL	44	464

Table 2. *Number of cases requiring hospitalisation.*

	Aspirati of non endo item	Aspiration endo tool	Ingestion non-endo item	Ingestion endo tool
Without hospital	0	0	237	61
With hospital without intervention	0	0	94	21
With hospital and intervention	43	1	37	14
TOTAL	44		464	

CONCLUSIONS:
The percentage of endodontic instruments aspirated or ingested were 2.2% and 18% respectively.
For the endodontic instruments, the prevalence for aspiration was 0.0009 per 100,000 root canal treatments and the prevalence for ingestion was 0.08 per 100,000 root canal treatments.
The Chi2 test showed a statistically significant difference between aspiration and ingestion ($p<0.0001$). All aspiration cases (100%) required hospitalisation compared to 36% for ingestion. No statistically significant difference was found between ingestion and aspiration of endodontic instruments compared to non-endodontic items.

Inquiry on practices in endodontically-treated-tooth restoration

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INTRODUCTION: The restorations performed on endodontically-treated teeth (RCR) are many and varied. Three objectives are described in the report of the ANAES (National Agency for Accreditation of Health Establishments): retention of the coronary restoration, reinforcement of corono-radicular cohesion and ensuring the durability of the tooth on the dental arch at the biological and structural level. [1]. Few studies on the clinical practices in France have been published on this set of themes. One of the rare studies available, Sabek's study, 1997, [2], is no longer in relation with current practice, given the development of materials and techniques. To try to highlight the state of the practice of RCR in France, a questionnaire was developed for use in an investigation carried out at the end of a postgraduate training session on this topic. The analysis of the data made it possible to check the assumption of adequacy between the initial, post-university training, professional experience, and the data of the literature.

METHODS: The anonymous questionnaire intended for the experts was drawn up with the aim of being exhaustive while remaining limited in length. The items selected depended on the two direct and indirect types of corono-radicular reconstitution. The questionnaire was tested on a group of 15 private expert practitioners and modified for better comprehension of the terminology according to remarks made by this reference group. The investigation concerned a population of experts interested in the subject at a meeting of the French Dental Association (ADF) on the topic "Reconstruction of the non-vital tooth".

RESULTS: One hundred seventy six questionnaires were collected. By comparing the data with those collected in 1997 [2], some noteworthy points were brought out:
Glass ionomer cements, and in particular resin modified glass ionomer cement (RMGIC), have acquired an important place in recent years. Composite resins are slowly replacing amalgam as the material inserted in the plastic phase. The use of fiber posts is becoming increasingly widespread. The materials necessary for the reconstruction of non-vital teeth are heterogeneous. No coherent unit used by a majority of experts was highlighted by this investigation. The use of adhesive systems showed certain inadequacies. It appears that the systems with 2 clinical steps are preferred. The adhesive system used by more than a third of the participants is photopolymerizable. The reconstruction resin is also photopolymerizable for half the participants. A current trend is the increasingly frequent use of translucent fiber posts. The number of posts inserted per reconstitution is one for premolars but, for molars, half the experts questioned place only one post while the others use two. The reconstruction techniques used for non-vital teeth are direct and indirect whatever the position of the tooth on the dental arch. Inlay-cores are part of everyday practice, preferably made out of non-precious alloys, in spite of the risks of corrosion. The choice of sealing cements is particularly between CVI and cement with zinc phosphate.

DISCUSSION & CONCLUSIONS: The comparison of the data between the age classes of the experts showed that the oldest experts had kept up to date with new techniques and materials. Doubt persists, however, on the correct training of the experts on the most recent materials, in particular adhesive systems and the reconstitution resins used with the fiber post. The results of this investigation gave useful information but it should be noted that the experts questioned were particularly aware of this subject since all these people had chosen to attend a meeting on the topic of the reconstruction of the non-vital tooth.

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COMPARATIVE STUDY OF THE BUCCO-DENTAL STATE OF STUDENTS FROM 3 SCHOOLS OS, SPB AND SPJA.

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INTRODUCTION: In the Ivory Coast, few studies have been carried out on the oral and dental heath of students in general and of students in dentistry in particular, who are in charge of patients from the 4th year of study. This study was performed in order to evaluate the impact of lectures on dental health received by students in dentistry by comparing their dental health with that of other students.

METHODS: 210 students from three schools were chosen. The schools were the school of dentistry (OS), the school of pharmacy and biology (SPB), and the school of law and administration (SPJA) of the University of Cocody. They were subjected to a survey and an oral and dental examination.

RESULTS:

Table 1: Prevalence of caries distribution

	UFR OS	UFR SPB	UFR SPJA	TOTAL
Prevalence of caries	57.6%	40.38%	61.11%	53.8%

The prevalence of caries increased progressively from UFRSPB (40.38%), to UFROS (57.6%), and UFRSPJA (61.11%**).**

DISCUSSION & CONCLUSION

The results show that students in SPJA had most caries (with a prevalence of 61.11%), followed by those in OS (57%) and those in SPB (40.3%). In preventive care, the students of OS (86%) had good dental hygiene compared to students of SPJA (51.8%) and SPB (48%).

In spite of their greater awareness, through lectures received, the students in OS do not behave in a different way from their colleagues regarding the regularity of visits to a dental office.

The teaching in the curriculum appears to have an impact on the student's behaviour regarding dental care.

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BUCCO DENTAL STATE AND NEED IN OPERATIVE DENTISTRY AND ENDODONTICS OF STUDENTS OF DENTAL SCHOOL OF ABIDJAN.

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INTRODUCTION: In the Ivory Coast, many studies have been carried out since 1984 in the aim of establishing the situation concerning caries according to different ages of OMS dental students. There is no study on the oral health of the students who are active participants in the sanitary system. This descriptive study was carried out to assess the oral and dental health of students of dentistry and evaluate the impact of lecturing received according to their cycle of training.

METHODS: 104 students (from the 2nd to 6th years of study) regularly registered in the school of dentistry of Abidjan were chosen and divided into 3 groups: group 1 (pre-clinical 2nd and 3rd year), group 2 (clinical 4th and 5th year), group 3 (post-clinical 6th year). They answered a survey and were given an oral and dental examination in order to reach the goal of this paper. All data were analyzed using the EPI INFO software.

RESULTS: From this study, the result was a prevalence of caries of 27.8% in group 1, of 16.3% in group 2, and of 13.5% in group 3. Awareness concerning conservative care was more evident among clinicians (47%) than post-clinicians (34%) and pre-clinicians (28%).

DISCUSSION & CONCLUSION: The prevalence of restorations was high. It rose among clinicians, while the prevalence of irreversible pathologies was high among the pre-clinicians.
Clinicians had better dental health than pre-clinicians and post-clinicians.

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U Scheinin (1974) *Incidence of dental caries among a group of University students in Turkey*. Acta. Odontol. Scand.n°32, P. 335-344, 1974. F Maatouk (2001) *profile of student of dental school in Tunisia* Eastern Mediterranean Health Journal, volume 7, n° ½ January-March 2001, P. 52-59, Jr. Meister al. (1980) *Comparison of the oral hygiene and periodontal health status of a class of dental students as freshmen and es seniors*. Journal of preventive dentistry, 6: 245-52.

French versions of two indices of dental anxiety and patient cooperation

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INTRODUCTION: This study aims to produce French language versions of two psychometric indices that could be used for epidemiological or clinical dental studies in French speaking countries. The Dental Anxiety Scale[1] (DAS) is designed to evaluate the level of anxiety experienced by adults on suggestion of four dental situations. It may be used both in epidemiological studies and in longitudinal clinical studies. The modified Venham scale[2] offers a qualitative and quantitative description of behaviour and anxiety in one score and may be repeated several times over a treatment session. It is used to evaluate patient cooperation during dental care.

METHODS: The Dental Anxiety Scale was translated by a turn/return process by three French and three English dental experts. Correspondence between the initial and final version was validated by one English and one French expert. The modified Venham scale[2] was translated and modified by adding new descriptions in order to improve its use with adult subjects. Reliability for the French Venham scale version was tested by a class of 31 final year dental students who scored thirteen different video sequences of dental care viewed at weekly intervals over three weeks.

RESULTS:

Table 1: French modified version of the Dental Anxiety Scale. The DAS gives a global score for dental anxiety that ranges from 4 to 19 (DAS≥13: mild anxiety; DAS≥17: severe anxiety).

(Une seule réponse par question; le score global correspond à la somme des scores obtenu pour chaque question))

Question N°1: Si vous deviez aller chez le dentiste demain, comment vous sentiriez vous ?

Question N°2 : Chez le dentiste, lorsque vous attendez votre tour pour passer sur le fauteuil, comment vous sentez-vous ?

Question N°3: Lorsque vous êtes installé(e) dans le fauteuil, en attendant que le (la) dentiste prépare la fraise avec laquelle il (elle) s'apprête à soigner vos dents, comment vous sentez-vous ?

Question N°4: Lorsque vous êtes assis(e) sur le fauteuil pour un nettoyage de vos dents, pendant que le (la) dentiste prépare les instruments qu'il (elle) va utiliser pour racler vos dents à côté des gencives, comment vous sentez-vous ?

Propositions de réponse pour la question 1 :

Je penserai que cela va être une expérience assez agréable (score 1) / Cela me serait indifférent (score 2) / J'aurai peur que cela soit désagréable et douloureux (score 3) / J'aurai terriblement peur de ce que le dentiste pourra faire (score 4).

Propositions de réponse pour les questions 2-4 :

Détendu(e) (score 1) / Pas tout à fait à l'aise (score 2)/ Tendu(e) (score 3)/ Angoissé(e) (score 4) / Je serais tellement anxieux(se) que certaines fois je pourrais transpirer ou avoir presque envie de vomir (score 5).

Table 2: French modified version of the Venham scale. Intra-and inter examiner reliability was verified respectively with the evaluation of the intraclass correlation coefficient α=0.92 ($CI_{95\%}$: [0.91; 0.94]) and with Anova (p=0.973).

0 Détendu, souriant, ouvert, capable de converser, meilleures conditions de travail possibles. Adopte le comportement voulu par le dentiste spontanément, ou dès qu'on le lui demande.

1 Mal à l'aise, préoccupé. Regard direct. mais expression faciale tendue. Observe furtivement l'environnement. S'appuie spontanément sur le dossier du fauteuil. Les mains restent baissées ou sont partiellement levées pour signaler l'inconfort. Pendant une manœuvre stressante, peut protester brièvement et rapidement pour montrer son inconfort. Le patient est disposé à – et capable de – dire ce qu'il ressent quand on le lui demande Respiration parfois retenue. Capable de bien coopérer avec le dentiste.

2 Tendu. Le ton de la voix, les questions et les réponses traduisent l'anxiété. Multiplie les demandes d'informations. Mains crispées aux accoudoirs, peuvent se tendre et se lever, mais sans gêner le dentiste. S'appuie au dossier spontanément, mais la tête et le cou restent tendus. Accepte le main-dans-la-main. Regard direct. Pendant une manœuvre stressante, protestations verbales, pleurs discrets possibles. Le patient interprète la situation avec une exactitude raisonnable et continue d'essayer de maîtriser son anxiété. Les protestations sont plus gênantes. Le patient obéit encore lorsqu'on lui demande de coopérer. La continuité thérapeutique est préservée.

3 Réticent à accepter la situation thérapeutique, a du mal à évaluer le danger. Soupire souvent. Protestations énergiques, pleurs possibles. S'appuie au dossier après plusieurs sollicitation, la tête et le cou restent tendus. Légers mouvements d'évitement. Mains crispées, regard parfois fuyant. Accepte le main-dans-la-main. Hésite à utiliser les mains pour essayer de bloquer les gestes du dentiste. Gigote un peu. Proteste verbalement, larmoyant. Protestations sans commune mesure avec le danger ou exprimée bien avant le danger. Parvient à faire face à la situation, avec beaucoup de réticence. La séance se déroule avec difficultés.

4 Très perturbé par l'anxiété et incapable d'évaluer la situation. Crispation importante, Sourcils froncés, regard fuyant, les yeux peuvent être volontairement fermés. Pleurs véhéments sans rapport avec le traitement. Mouvements d'évitement brusques. Pose ses mains sur sa bouche ou sur le bras du dentiste mais finit par laisser faire. Serre les lèvres mais finit par garder la bouche ouverte. Soulève fréquemment sa tête du dossier. Rejette le contact corporel, mais peut encore accepter le main-dans-la-main. Importantes contorsions, nécessitant parfois une contention. Le patient peut être accessible à la communication verbale et finir, après beaucoup d'efforts et non sans réticence, à essayer de se maîtriser. La dissociation est partielle. La séance est régulièrement interrompue par les protestations.

5 Totalement déconnecté de la réalité du danger. Inaccessible à la communication. Rejette le contact corporel. Serre les lèvres et les dents. Referme la bouche et serre les dents dès que possible. Agite violemment la tête. Pleure fort à grands cris, hurle, dit des injures, se débat, est agressif ; inaccessible à la communication verbale, et visuelle. Quel que soit l'âge, présente des réactions primitives de fuite. Tente activement de s'échapper. Contention indispensable.

DISCUSSION & CONCLUSIONS: The French versions of the Dental Anxiety Scale and Modified Venham Scale could be used for studies with French speaking patients and investigators. However, the validation process needs to be completed.

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ACKNOWLEDGEMENTS: The author thanks Pr Paul Allison, Pr Paul Riordan and Dr Derek Debuse for their participation in the translation process of the DAS.

Assessment of the management of pain related to conservative care in the Dental Center of Nantes Hospital.

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INTRODUCTION:

Dental pain has an inflammatory and/or infectious origin often due to carious injury, but can also be induced by dental treatment. It is therefore surprising that iatrogenic pain (per and post-operative symptoms) during restorative and endodontic therapeutics is always considered as a normal event by practitioners and patients [1,3].

To respond to the objectives of the second four-year plan for pain management of the French Ministry of Health [2], the Dental Center of Nantes Hospital carried out an epidemiological study to assess the management of pain related to conservative care.

The aim of this study was first to characterize the symptoms induced by the treatment, and secondly to assess pain management. This research work was the first step of a quality assurance programme and was undertaken to establish reference procedures for providing effective pain management during conservative care.

MATERIAL AND METHODS:

This prospective study was carried out on a random sample of 258 patients. The patients were questioned following treatment and at the beginning of the next consultation,

using a standardized and validated clinical questionnaire. Pain intensity was estimated with a numerical rating scale [3].

Various data were collected: the first concerning the patients and their behaviour towards dental care; the second examining the incidence, the type, the intensity, the duration of the iatrogenic pain; and the third assessing pain management through the quality of practitioners' information and advice, anaesthesia, prescriptions, patient's compliance, and self medication.

The statistical analysis was performed using the SPSS software (Statistical Package for Social Sciences). The descriptive statistics were supplemented by comparative tests (Chi^2, Student, Mann Whitney, Wilcoxon tests). Correlations were investigated to measure the intensity of linear links for scores and scales. Statistical significance was determined as $p<0.05$.

RESULTS:

This study showed that iatrogenic pain was moderate. Per-operative symptoms were reported for 33% of the patients, whereas 24% had painful post-operative effects. Post-operative pain appeared immediately after the consultation for 51% and lasted about 0.7± 0.7 day. 8% of the patients were still in pain at the next consultation.

Anaesthesia was not systematically proposed to patients for restorative care on vital teeth. 44 % of the patients were informed of the possibility of pain during treatment and 33% of possible pain after treatment.

The frequency of prescriptions was only 8.1%. When an analgesic prescription was given, it was generally after the care. The patients increased the amount of prescribed medication in 17.5% of cases.

DISCUSSION AND CONCLUSIONS:

The main findings suggest a lack of communication between health professionals and their patients and reveal that pain management during conservative dental care is insufficient, particularly for the operative act and its immediate effects, which are not sufficiently taken into account. Moreover, post-operative pain is underestimated.

The results of this study will be followed by a quality assurance programme intended to improve pain management during conservative care.

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ACKNOWLEDGEMENTS: Sincere thanks to Doctor L.Moret of the unit of medical practices evaluation (PIMESP) for her contribution to this work.

A quality assurance programme for improvement of pain management during conservative care in Dental Center of Nantes Hospital: Methodology, preliminary results.

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INTRODUCTION:

In France, the quality of care has been a public health priority for several years. National programmes have been implemented to improve pain management [1]. In 2001, a first evaluation carried out by the French Society of Public Health [2] showed that the health professionals were becoming aware of this problem. In 2002, a new national plan was defined to prevent the pain caused by care and surgery [3].

To respond to its objectives, the Dental Center of Nantes carried out a quality assurance programme to promote the management of pain related to conservative care.

MATERIAL AND METHODS:

The quality assurance programme was undertaken in several parts:

1) A descriptive prospective study:

To characterize the symptoms induced by treatment and to assess pain management, a random sample of 258 patients were questioned using a numerical rating scale and a standardized clinical questionnaire.

The conclusions of this preliminary work brought out a lack of iatrogenic pain management in conservative care.

2) Proposals for Reference Operative Procedures for each type of conservative treatment. Drafting of advice for the patients.

3) Training of undergraduate students and practitioners for optimal control of iatrogenic pain.

4) Re-assessment:

Several dates were randomly fixed for the re-evaluation of pain management during conservative therapeutics.

RESULTS - DISCUSSION:

The first step of this quality assurance programme showed that iatrogenic pain was not a marginal event during conservative care (per-operative symptoms: 33%; post-operative symptoms: 24%). Because it was generally moderate, it was ignored by both the practitioners and the patients, who considered this pain as a normal event. The conclusions of this study reveal a lack of information of the patients, as well as a lack of analgesic prescriptions and therapeutic means to prevent a possible per- and post-operative pain.

The Reference Operative Procedures were defined after analysis of these preliminary results and were proposed for each type of conservative care according to the pain risk. Emphasis was laid on prevention and control of pain during and after the treatment with simple and effective measures: informing patients of possible pain, proposing loco-regional infiltration before the operative act on teeth with vital pulp, systematic prescription of analgesics according to the intensity of the symptoms, etc.

The preliminary results of the re-evaluation stage show that some changes in iatrogenic pain management during conservative care have been reported. Nevertheless, other re-assessments should be performed before concluding that there is a long-term improvement in pain management.

CONCLUSIONS:

The quality assurance programme carried out by the Dental Center of Nantes Hospital should continue to succeed in changing the behaviour of both health professionals and patients towards dental pain during conservative care.

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Role of *Fusobacterium nucleatum* in volatile sulphur compound production on the dorsum of the tongue

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INTRODUCTION: Oral malodor is mainly associated with volatile sulphur compounds (VSC), particularly hydrogen sulphide (H_2S) [1].These compounds are highly toxic. The dorsum of the tongue is the major site of oral malodor production [2]. One possible pathway of forming VSC involves the microbial degradation of sulphur-containing amino acids. Methionine and cysteine are the substrates for this reaction, but the best characterized enzyme implicated in H_2S formation (L-cysteine desulfhydrase) shows the best affinity for cysteine [3]. The aim of this study is to investigate the VSC metabolism and its regulation by tongue microbiota. We also evaluate a medium (CSH agar) able to count bacteria releasing H_2S from cysteine.

METHODS: Ten healthy adult males aged 18 to 55 years and non-smokers for three months were recruited by the Clinical Investigation Center of the University Hospital of Lille. All subjects gave their informed consent. Samples were taken by dorsum tongue scraping using a sterile plastic loop (1 µl), immediately immerged in prereduced transport medium (8 ml cysteinated 1/4 strength Ringer solution- CR). All samples were transported to the laboratory and processed in less than 4 hours. 10 fold dilutions in CR were plated on Columbia blood agar to establish the total anaerobic counts and on Columbia cysteine agar (CSH) to establish counts of –SH liberating bacteria. –SH liberating bacteria produce identifiable black colonies on this medium. All plates were incubated for seven days under anaerobic conditions. From plates with 15 to 150 CFU, the total number of colony forming units was determined. From CSH plates, black colonies were presumptively identified. Pure strains with –SH producing ability were analyzed by *in vitro* assays. H_2S release was quantified using a portable sulphide monitor (Halimeter®- RH 17 Interscan).

RESULTS: The mean count under anaerobic conditions was 8.53 log CFU/ml. -SH producing bacteria counts were 2 log lower than total anaerobes (Fig. 1).

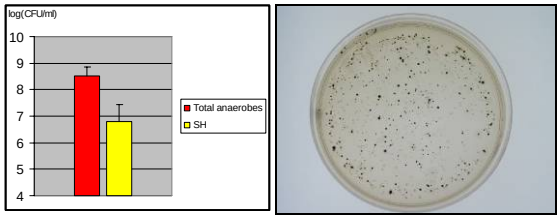

Fig. 1 Counts of total anaerobes and –SH producing bacteria (left); –SH-releasing black colonies on Columbia cysteine agar plate (right).

From CSH plates (Fig.1), *Fusobacterium nucleatum* was detected in 9 subjects and *Prevotella sp*. in 1 subject.

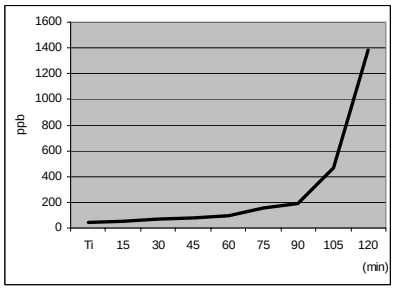

Fig. 2: Halimeter® measurements: in vitro H_2S production by a F. nucleatum strain (starting after 23 hours of culture).

H_2S was rapidly released by *F. nucleatum* strains after 24 hours of incubation (Fig.2). Measurable amounts of H_2S were formed when viable counts exceed 7 log CFU/ml.

DISCUSSION & CONCLUSIONS: *Fusobacterium nucleatum* is the most frequent –SH producer (by proteolysis) on the dorsum of the tongue. *In vitro* assays show a release in end logarithmic growth phase. Tongue cleaning should be recommended to avoid thick biofilms.

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Caries risk assessment by different approaches before fixed orthodontic treatment

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INTRODUCTION: Previous data have suggested a high prevalence of caries in patients undergoing orthodontic treatment but little is known concerning the carious risk of these patients before the beginning of treatment. The objective of the study was to assess the caries risk of young patients before fixed appliances were placed.

Fig.1: Dental plaque of a 13 year old patient with fixed appliance

METHODS: 31 healthy young patients scheduled for treatment were enrolled in this pilot study and received a clinical and X-Ray examination. The exclusion criteria were low salivary flow rate and active caries lesions. In order to assess the caries risk, three prediction tests were performed immediately before the beginning of treatment: the ClinproCarioLPop (CCLP, 3M ESPE, Germany) assessing the metabolic activity of cariogenic bacteria by their production of lactic acid, the Cario-Analyse (CA, Pierre Fabre, France) assessing the buffer capacity and, by RT-PCR, the numbers of Streptococcus Mutans and Lactobacillus in a sample of saliva, and, thirdly, by plate culturing cariogenic bacteria (PCCB) from a sample of plaque.

RESULTS: The patients (mean age 15 ± 4.6 years) had a DMFT of 2.9 ± 3.4 and DMFS of 4.5 ± 5.9 (mean ± SD). Judging from each of the tests, no patient had a low caries risk and a high risk was predicted for 68% with the CCLP and for 42% with the CA *(Table1)*. Furthermore, Streptococcus Mutans scores were higher than 10^4 CFU /ml saliva for 55% of the patients and higher than 10 CFU per sample of plaque for 32% of them.

Statistical analysis only showed poor concordances between the three indicators of caries risk (K<0.2) (*Table 2*).

Table 1. The distribution of the carious risk according to the three different tests

	low	moderate	high
CCLP	0 %	32 %	68 %
CA	0 %	58 %	42 %
PCCB	0 %	94 %	6 %

Table 2. The concordance (coefficient kappa) between the three different tests

	Same determination of caries risk	Coefficient K
CCLP/CA	54 %	0,14
CCLP/PCCB	32 %	-0,03
CA/PCCB	64 %	0,17

DISCUSSION & CONCLUSIONS: According to the present data, most of the patients selected for orthodontic treatment already present a high caries risk, underlining the need for better instruction of the patients before the beginning of orthodontic treatment.

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PRE-CLINICAL STUDY OF GALVANIC CORROSION BETWEEN ORTHODONTIC BRACKETS AND WIRES IN FLUORIDATED DENTAL RINSES

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INTRODUCTION:

A precedent study has shown galvanic corrosion of certain bracket-wire combinations after two months´ immersion in fluoridated dental rinses, [1]. The aim of this new study was to approach clinical conditions, by testing successive two-minute immersions, similar to the use of dental rinses by patients. This was done with a new robotic system. We analysed the solutions tested and the specimen surfaces to evaluate corrosion resistance.

METHODS:

Specimens:

We tested a FeCrNi bracket composed of (wt%) Fe: 65.5 , Cr: 18.5 , Ni : 12.3 , Mo: 2.4; and a CoCr bracket, composed of Co: 60.3 , Cr: 31.6 , Mo: 8 , Fe: 1.2. These specimens were joined with NiTi wires, composed of (wt%) Ni: 55 , Ti: 45. Each bracket-wire pair was fixed on a PTFE cylinder.

Test solutions:

The reference electrolyte used was Fusayama Meyer saliva. The composition was KCl (0.4 g/l), NaCl (0.4 g/l), $CaCl_2.2H_2O$ (0.906 g/l), $NaH_2PO_4.2H_2O$ (0.690 g/l), $Na_2S.9H_2O$ (0.005 g/l), Urea (1 g/l). The first dental rinse was Meridol®, containing 125 ppm Olafluor amine fluoride and 125 ppm stannous fluoride. The second dental rinse was Eludril®, containing chlorhexidine.

Set up:

The robot (EPSON- FRANCE) was able to test three pairs at the same time: it was connected to a computer with software allowing the choice of different immersion times in the different test solutions. Each pair was subjected to a 6-minute cycle: 2 minutes immersion in test solution, 2 minutes in the air and then 2 minutes rinsing in artificial saliva without Urea. 100 cycles were repeated for each pair, corresponding to the cumulated rinsing with mouthwashes for a patient, between the changes of the wires by the orthodontist.

Corrosion resistance analysis:

At the end of the 100 cycles, the different test solutions and rinsing solutions recovered were analyzed with Inductively Coupled Plasma-Atomic Emission Spectrometry. The surfaces of the different pairs of brackets and wires were examined using a scanning electron microscope.

RESULTS:

Released ions (µg/l)	NiTi/ CoCr Saliva	NiTi/ CoCr Méridol	NiTi/ CoCr Eludril
Ions analysed in test solutions	Cr: <5 Ni: <5	Cr: <5 Ni: <5	Cr: <5 Ni: <5
Ions analysed in rinsing solutions	Cr: <5 Ni: 20	Cr: <5 Ni: 18	Cr: <5 Ni: 5

Table 1: Concentration of released ions for NiTi/CoCr pair

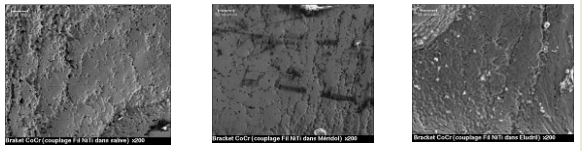

Fig 1: Photomicrographs (x200) of CoCr bracket joined to NiTi wire in the different test solutions.

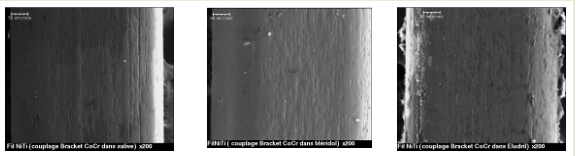

Fig 2: Photomicrographs (x200) of NiTi wire joined to CoCr bracket in the different test solutions.

DISCUSSION & CONCLUSIONS:

For the FeCrNi bracket with NiTi wire, no corrosion was detected in the different solutions: ICP-AES techniques did not detect released ions and the surfaces analysed by MEB did not show any deterioration of the materials. For the CoCr bracket, small concentrations of Ni were detected in the rinsing solutions and slight deterioration of the materials was observed by surface analysis. From the clinical point of view, the final result of orthodontic treatment could have been compromised by corrosion. Moreover corrosion products (Ni^{2+}) can lead to toxicity symptoms and allergic reactions [2]. This study indicates that the NiTi/CoCr combination might be more susceptible to corrosion in Méridol® (fluoridated mouthwash) than in Eludril® (chlorhexidine mouthwash).

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Influence of temperature on fluoride release by glass ionomer cements

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INTRODUCTION: Fluoride release by glass ionomer cement (GIC) has been widely studied according to different parameters such as the powder/liquid ratio, the composition, and the media.[1,2] But there is little information about the incidence of temperature[3,4]. The aim of this study was to determine the influence of temperature on fluoride release using a conventional GIC (C-GIC) and a resin-modified GIC (RM-GIC).

METHODS: Five disc-shaped samples (12 mm in diameter and 1.2 mm thick) of a C-GIC (Fuji IX, GC, Tokyo, Japan) and five samples of a resin-modified glass ionomer cement (Fuji II LC) were made. The RM-GIC samples were light-cured for 60 s on each side (mode HIP, Astralis 7, Ivoclar-Vivadent, Saint Jorioz, France). Specimens were immersed in flasks containing 6 ml of distilled water. The temperature was maintained constant using a climatic chamber (Secasi Technologies, SH 340, Pessac, France). The experiments were conducted at 5°C, 37°C and 55°C. The solution was changed at 1, 2, 3, 4, 7 and 14 days. Then, the samples were immersed for 1 hour in a solution of potassium fluoride at 0.1 M (1900 ppm) and the same method as above was conducted for 7 days instead of 14 days. Fluoride release was measured using an Ion Selective Electrode (ISEC301F-9, Radiometer Analytical, Villeurbanne, France).

RESULTS: Statistical analysis was performed with the Kruskal-Wallis test and the Bonferroni-Dunn test.

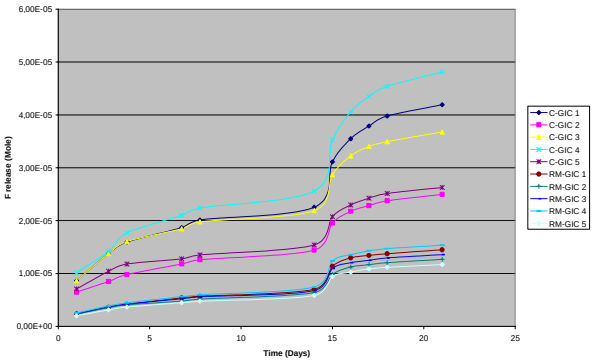

Fig. 1: Initial fluoride release and release after re-uptake at 5°C

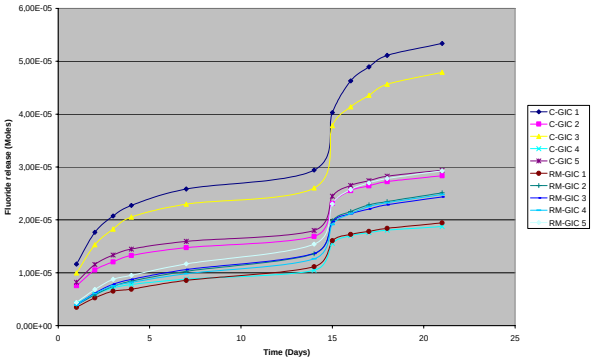

Fig. 2: Initial fluoride release and release after re-uptake at 37°C

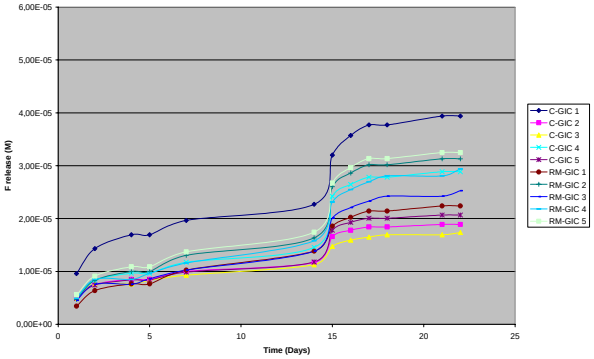

Fig. 3: Initial fluoride release and release after re-uptake at 55°C

Statistical analysis showed that temperature had no significant influence on the initial fluoride release and release after re-uptake for the C-GIC whereas it had an influence on the RM-GIC except between 37°C and 55°C for the fluoride release after re-uptake (Figure 1-3).

DISCUSSION & CONCLUSIONS: Fluoride release is high during the maturation phase for the 2 materials. When the materials are quite mature, the release is linear. It seems that the fluoride release is influenced by the temperature increase for the material containing a polymer matrix.

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Neutron diffraction study of hydroxyapatite crystallites on implant

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INTRODUCTION: A hydroxyapatite (HA) coating on titanium alloy substrate allows reconstituted bone to preserve the texture of the natural bone [1]. Recently, coating by nano-HA has received much attention as it may achieve better osteo-integration and improve the mechanical properties of HA-coated implants [2]. In this work, nanosized hydroxyapatite (HAP $Ca_{10}(PO_4)_6(OH)_2$) powders were characterized before and after deposit on the (Ti-6Al-4V) substrate using neutron diffraction and scanning electron microscopy (SEM). The aim was to evaluate the diffracting domain size, by neutron diffraction, of hydroxyapatite HAP and nano-HAP powders befote via PTS. Moreover, the local morphology of n-HAP was observed by scanning electron microscopy SEM.

METHODS: Two commercial hydroxy-apatite powders ($Ca_{10}(PO4)_6(OH)_2$) were analyzed by neutron diffraction. i.e. HAP and n-HAP. The nano-HAP powder was used as feedstock to generate partially nanostructured coatings on the surface of a titanium alloy (Ti-6Al-4V) via the Plasma Thermal Spray (PTS) process followed by 2h of heat treatment to reduce the amorphous part (n-HAP-Ti). This coating powder was analysed after scraping using a file tool. The structural study was carried out by neutron diffraction (ND). ND patterns were collected on the PSD diffractometer D20 (ILL Grenoble, λ = 2.4 Å, 2θ domain 5 – 122.78°, step 0.1°). The Rietveld refinements were carried out using the program FullProf.

RESULTS: It appears that the use of agglomerated particles as feedstock preserves the nanostructure of deposited HAP coating in the morphology of the layers. The substructure of nano-crystals is about 100-135nm in size. Nano-coating may provide nucleation sites for the reconstituted bone, allowing faster stabilization of the implant in the bone.

Table 1: Diffracting domain sizes of the different HA studied

Samples	HAP	n-HAP	n-HAP-Ti
Ds (nm ave.)	188	90	113

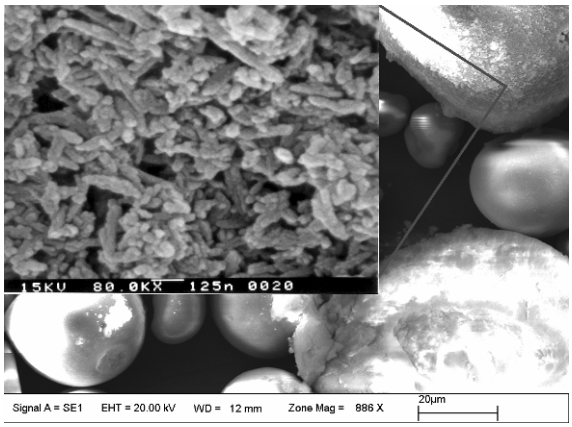

Figure 1: SEM micrograph of the deposited coating, showing a large spherical geometry of agglomerated particles (35µm ave.) consisting of nanocrystals 100-135nm in size

DISCUSSION AND CONCLUSION: The crystallite sizes of nano-HA (smaller than obtained with the conventional coating of HA (10-100µm)) are close to those of biological apatite carbonated in the bone (<50nm) [3]. Consequently, the bioactivity should be higher with nano-HA, which allows an acceleration of osseointegration.

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EFFECT OF A PRIMING AGENT ON BOND STRENGTH OF INDIRECT COMPOSITE TO DENTIN USING VARIOUS CEMENT SYSTEMS

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INTRODUCTION: The use of indirect resin composites, associated with resin-modified glass-ionomer cement (RMGIC) or resin-based adhesive cement (RBAC) has increased. Materials and technique have been improved and patient demand for aesthetic restorations has risen[1,2]. In this study, shear bond strength (SBS) between dentin and composite inlays sealed with dental cements (2 RMGIC and 3 RBAC) was measured. In a second part, the effect of a priming agent on bond strength was evaluated. Three more cement systems are being tested and will soon be added.

METHODS: Gradia indirect resin composite cylinders were fabricated according to laboratory standards. Extracted third molars were sliced so that flat coronal dentin surfaces were exposed. The cylinders were "bonded" to the dentin surface *(Fig. 1[left])* using 5 different cement systems (*Fuji Plus Capsules, GC; Fuji Plus Powder/Liquid, GC; Super-Bond C&B, Sun Medical; RelyX Unicem, 3M-ESPE; Maxcem, Kerr)* according to the manufacturers' instructions. A priming agent *(Composite Primer, GC, Fig. 1 [right])* was applied or not, prior to bonding.
Each group was composed of ten specimens. SBS was measured with a universal testing machine (Ametek Lloyd LRX Plus) one hour after bonding. Dentin surfaces of all tested specimens were examined by binocular microscopy to determine failure modes. A direct resin composite (Filtek Z250, 3M-ESPE) was bonded (OSP: Optibond Solo Plus, Kerr) to dentin and tested similarly as a control.

RESULTS: Mean values of SBS with and without priming agent are presented in *Tables I and II* respectively. A statistical analysis (one-way ANOVA, $p < 0.05$) was performed. The highest values with priming agent were obtained with Super-Bond C&B *(Table I)* while the highest results without priming agent were found in the Super-Bond C&B and RelyX Unicem groups *(Table II).*
All groups showed a majority of adhesive failures except Fuji Plus (P/L) which had primarily adhesive-cohesive failures.

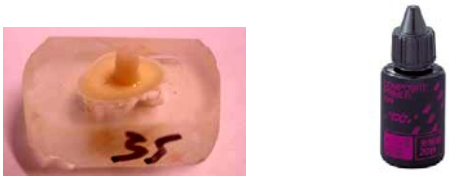

Fig. 1: Bonded specimen [left] – Priming Agent (GC Composite Primer) [right].

Table I. SBS with priming agent. Means with same letter are not significantly different

Group		Mean and Std deviation (MPa)
Super-Bond C&B	A	23.01 (6.95)
OSP+Z250	B	15.79 (5.79)
Fuji Plus (Caps.)	B	13.46 (5.59)
RelyX Unicem	C	9.97 (2.19)
Fuji Plus (P/L)	C	7.92 (3.73)
Maxcem	C	5.03 (3.37)

Table II. Without priming agent.

Group		Mean and Std deviation (MPa)
Super-Bond C&B	M	21.29 (6.15)
RelyX Unicem	M N	19.6 (6.89)
OSP+Z250	N O	15.79 (5.79)
Fuji Plus (Caps.)	O	15.11 (4.06)
Fuji Plus (P/L)	P	8.13 (4.83)
Maxcem	P	5.21 (2.6)

DISCUSSION & CONCLUSIONS: Super-Bond C&B showed high SBS with or without priming agent. Fuji Plus (Caps.) had significantly higher values than Fuji Plus (P/L). After statistical analysis to compare the SBS of each cement system with and without priming agent, adhesion values without priming agent always appeared higher than or not significantly different from values with priming agent.

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ACKNOWLEDGEMENTS: Thanks to the manufacturers for providing materials and information.

Influence of power density on microhardness of resin composite

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INTRODUCTION: It is generally accepted that sufficient light energy has to be transmitted to dental composites if their mechanical properties are to be good. In a recent study, Peutzfeld and Asmussen[1] showed that these properties were determined by not only energy density but also power density. The aim of this study was to assess the effect of power density on the microhardness of composites when the energy density was fixed.

METHODS: Two light sources, one LED (Bluephase®/Ivoclar-Vivadent) and one QTH (Astralis®10/Ivoclar-Vivadent), were used to irradiate two composite resins (CeramX™/ Dentsply, and Tetric Evoceram®/Ivoclar-Vivadent). For each light source, power densities were fixed at 400 and 1000 mW/cm² by varying the distance between the lamp tip and the composites, and energy was fixed at 8 J/cm² by varying the exposure duration (energy = power density x duration). Five samples (3mm in depth) were cured for each resin / curing-unit / power-density combination. The specimens were stored at 37°C for 24 h. For each specimen, the Vickers hardness number (VHN) was measured at the top and the bottom surfaces using a microhardness meter (Buehler model 5104).

RESULTS: Measurements of VHN showed (fig.1):

- for the LED unit: no significant differences between 400 and 1000 mW/cm² either at the top surface or at the bottom one for both composite resins,

- for the QTH unit: no significant differences between 400 and 1000 mW/cm² at the top surface for both composite resins, but at the bottom surface, resins (both materials) cured at 400 mW/cm² had the greatest microhardness values.

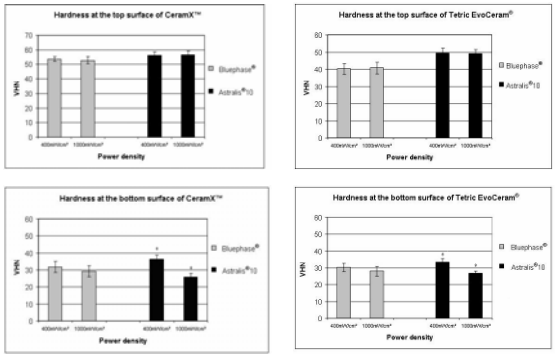

Fig.1: Microhardness of composites cured with the LED unit and the QTH unit at 8 J. Asterisks represent significant differences ($p<0.001$) (ANOVA, Tukey, $\alpha=0.05$) between the two power densities.

DISCUSSION & CONCLUSIONS:

The present study underlines that, for the QTH unit, at the bottom surface, for both resins, the lower power density induced the highest VHN. The same results have been found by Peutzfeld and Asmussen[1] concerning the degree of curing of resin composites. This may be explained by the kinetics of photopolymerisation: lower power densities delay the formation of a rigid network[2], which allows polymerisation of the composite at depth. Thus, an increased exposure duration may be more advisable for curing composite resins at 3 mm depth than a high power density.

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DENTIN ALTERATION OF DECIDUOUS TEETH IN HUMAN HYPOPHOSPHATEMIC RICKETS

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INTRODUCTION: The aim of the present study was to analyze the structure and mineralization of human dentin, and the composition and distribution of ECM components, associated with familial hypophosphatemic rickets.

METHODS: Hypophosphatemic and age-matched control deciduous teeth were collected. They were processed for SEM or immunohistochemistry examination. In addition, dentin blocks were prepared and immersed for 8 days in EDTA (4.13%). The supernatant was analyzed by SDS-PAGE and by Western blotting in order to visualize and to identify the proteins extracted by this procedure. Antibodies directed against SIBLINGs, osteocalcin (OC) and chondroitin /dermatan sulfate glycosaminoglycans were used.

RESULTS: Compared to the age-matched controls, the dentin of deciduous teeth from hypophosphatemic patients exhibited several differences: - presence of large interglobular spaces resulting from the lack of fusion of calcospherites in the dentin; defective mineralization in the interglobular spaces contrasting with normal Ca-P levels in the calcospherites on X-ray microanalysis; abnormal presence of low molecular weight protein complexes recognized on Western blots by antibodies against matrix extracellular phosphoglycoprotein (MEPE), dentin sialoprotein (DSP), osteopontin (OPN), and osteocalcin; accumulation in the interglobular spaces of immunolabelling with antibodies against SIBLINGs and OC, whilst chondroitin /dermatan sulfate glycosaminoglycans were associated with normal mineralization.

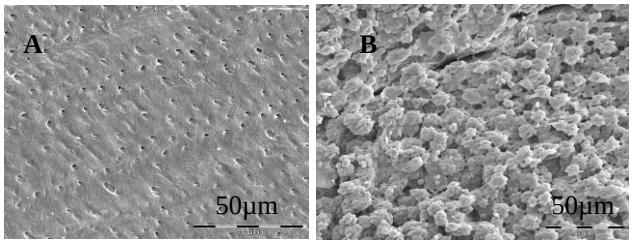

Fig.1: Large interglobular spaces between unmerged calcospherites were seen in circumpulpal dentin in XLH patients (B) whereas control teeth showed homogenous dentin (A).

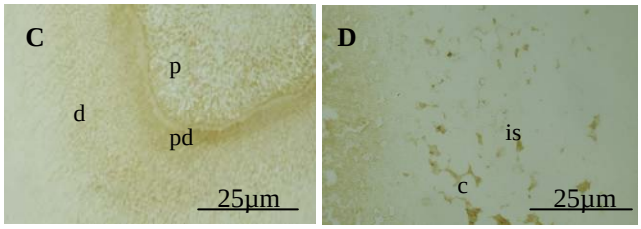

Fig.2: Indirect immunodetection of DMP-1: In the control teeth (C), inner part of dentin tubules is labelled. Interglobular spaces are exclusively labelled in XLH dentin (D). p: pulp, c: calcospherites, d:dentin, pd: predentin.

DISCUSSION AND CONCLUSIONS: These data suggest that hypophosphatemia, and/or defective PHEX function in the odontoblasts, strongly alter the dentin ECM composition and distribution. Alterations of some of the ECM molecules are key-factors in the formation of the defective hypophosphatemic dentin.

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Tissue engineering of dental pulp: controlled release of bioactive molecules from biodegradable polymer microparticles

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INTRODUCTION: The Transforming Growth Factor ß1 (TGF-ß1) is a multifunctional protein which promotes dental tissue repair by inducing odontoblast differentiation.

Microparticles made of biodegradable polymers allow a controlled release of bioactive molecules and seem to make possible modulation of cellular function and tissue formation at the injured site.

The objective of the study was to use a biodegradable copolymer (poly(DL-lactic-co-glycolic acid) (PLGA)) as a delivery vehicle to encapsulate the growth factor.

METHODS: Microparticles of PLGA copolymer were fabricated using a double-emulsion [(water-in-oil)-in-water] solvent-extraction technique. TGF-ß1 with a molecular weight of 25 kDa, was incorporated into PLGA microparticles along with bovine serum albumin (BSA) with a molecular weight of 68 kDa which served as a porogen to enhance the release of TGF-ß1.

Microparticle characterization: The particle size distribution was measured with a Coulter Counter Multisizer and confirmed by scanning electron microscopy (SEM).
In vitro release study: The concentration and the release kinetics of the proteins were studied using High Performance Liquid Chromatography (HPLC), with a Zorbax GF-250 column, for BSA. The TGF-ß1 results were analyzed by enzyme-linked immunosorbent assay (ELISA) using a quantikine kit.

RESULTS: The fabrication variables had significant effects on the surface of the microparticles and their internal morphology, on the drug entrapment efficiency and its distribution, and also on the release profile of the proteins.

The mathematical modelling of in vitro BSA release allowed the fabrication process of microparticles to be optimized.

DISCUSSION & CONCLUSIONS: The encaspulation of proteins in biodegradable polymer microparticles allows a controlled release of TGF-ß1 and BSA to be obtained.
The next stage will be to study the controlled release of TGF-ß1 in the in vitro model culture of human dental pulp and to observe its influence on the tissue repair process.
Further investigations will determine if TGF-ß1 released from PLGA microparticles is able to maintain its biological activity and if it is able to enhance the proliferation and differentiation of odontoblasts.

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ACKNOWLEDGEMENTS: Special thanks to J. Frelichowska.

POLYSACCHARIDE-BASED HYDROGEL FOR SMOOTH MUSCLE CELL CULTURE

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INTRODUCTION: Cardiovascular diseases related to vessel wall thickening may require surgery techniques such as multiple bypasses. Unfortunately, healthy vascular tissue from the patient is not always available to carry out this type of graft. Recent investigations in the tissue engineering field have aimed at finding biocompatible materials and scaffolds that could be seeded with cells and used as vascular substitutes[1]. To this end, we developed biodegradable polysaccharide-based hydrogels[2] that could serve as scaffolds for vascular engineering.

METHODS: Polysaccharide-based hydrogels were prepared using pullulan (Mw 200,000, Hayashibara Inc.). Chemical crosslinking was carried out using the reticulating agent STMP (trisodium trimetaphosphate, Sigma). The mixture was poured into Petri dishes and incubated at 50°C for 20 min. The resulting gels were washed in PBS pH 7.4 and a circular punch was used to cut 6 mm diameter-scaffolds. The internal structure of the gels was analyzed using ESEM (Environmental Scanning Electron Microscope). Rabbit Smooth Muscle Cells (SMC Rb1 line) were seeded on the top of the gels (10^5 cell/cm^2) then maintained in culture for up to 7 days in DMEM with 10% Fetal Bovine Serum. In some experiments, cells were labeled prior to the seeding step with a fluorescent dye (PKH26, Sigma). Cellular adhesion to the gels was evaluated by direct observation by light microscopy, fluorescence microscopy and confocal microscopy. Cell viability at day 4 was assessed using a Live/Dead assay (Calbiochem). A metabolic assay (MTT) was performed at days 1, 4 and 7 to determine the total number of cells per gel. Gel samples were fixed in formaldehyde, OCT-embedded then cryosectioned. Histological stainings were carried out to localize the cells within the gels.

RESULTS: Pullullan solution was cross-linked in order to obtain thin discs designed for cell culture support. This polysaccharide gave homogeneous, transparent and easy to handle gels (Fig.1A). Water content was found to be higher than 90%. Environmental SEM analysis revealed a smooth surface, on which SMCs were successfully seeded. Fall-out cells were observed but, overall, more than 50% of the initial cells remained associated to the gels. Cells attached in less than 2 hours. Cells were observed spreading out on the gel surface, forming numerous links between the initial cell aggregates (Fig.1B). The absence of toxicity of the gels was evidenced by the high viability of the cells. Histological observations confirmed the presence of cells in the scaffolds.

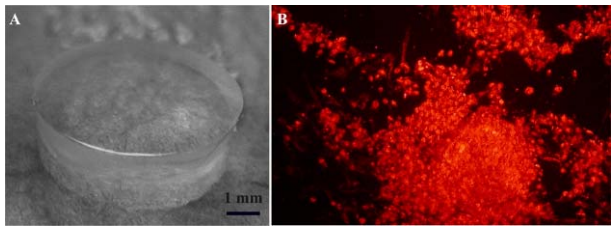

Fig. 1:(A) Transparent and easy to handle gel (disc, 6 mm diameter). (B) Fluorescent labeling of the SMCs prior to their seeding enabled their morphology and distribution to be observed.

A metabolic assay (MTT) demonstrated the cell proliferation over time with a progressive increase of the cell density from day 1 to day 7 (Fig.2).

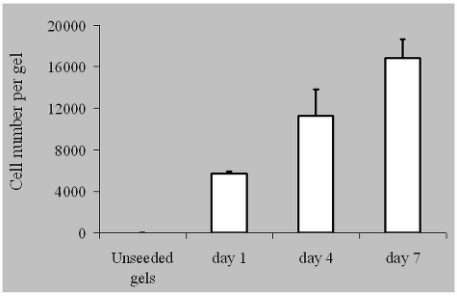

Fig. 2: MTT assay results expressed as the mean value of the total cell number per gel ± SEM (n=4)

DISCUSSION & CONCLUSIONS: We prepared biodegradable polysaccharide-based hydrogels that could serve as scaffolds for vascular engineering. Hydrogels allowed SMC adhesion, spreading and proliferation. Future studies will focus on the culture of vascular cells on tubular-shaped hydrogels and *in vivo* implantation.

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