four groups ($n = 5$), and each group submitted to immersion in a solvent according to one of the following procedures: (i) Endosolv R for 1 minute, (ii) Endosolv R for 5 minutes, (iii) Endosolv E for 1 minute, (iv) Endosolv E for 5 minutes. The samples were dried for 24 h at 37°C. Before and after immersion and drying, the samples were weighed three times and the difference of the mean weights was calculated. The mean percentage loss of weight was calculated for each sample. The results were evaluated statistically using Kruskal-Wallis and Mann-Whitney tests. Level of significance was set at 0.05.

Results Solubility of AH 26 – normal was significantly higher than that of AH 26 – viscous ($P < 0.001$). Endosolv E caused significantly greater dissolution than Endosolv R ($P < 0.005$). There was no significant difference between 1 and 5 minutes immersion time.

Conclusions Although Endosolv E is believed to remove zinc oxide eugenol types of sealers and Endosolv R to remove phenolic resin type of sealers, under the conditions of this study, Endosolv E was a more effective solvent of AH 26. Increasing the powder-liquid ratio made AH 26 more resistant to dissolution.

Research Posters – Pathology

R63

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Presence of T cell subpopulations in apical granulomas and apical cysts

Aim To investigate immunohistochemically the presence of T helper/inducer (Thi) and T cytotoxic/supressor (Tcs) lymphocytes in apical granulomas and cysts.

Methodology Periapical lesions which were to be treated surgically were used in this study. Twelve histologically identified granulomas and nine apical cysts were immunohistochemically stained employing the immunoperoxidase technique. Positive cells were counted under the light microscopy and analysed statistically.

Results In both cysts and granulomas, the mean number of Thi cells was more numerous than Tcs cells, but with no statistically significant difference. The Thi/Tcs ratios for cysts and granulomas were 1.09 (±0.94) and 1.14 (±0.74) respectively, without significant difference between the lesions.

Conclusions There was no predominating presence of a certain type of T cell subpopulation associated with the periapical lesion type. The Thi/Tcs cell ratios found in both granulomas and cysts may indicate that both lesions were in chronic phase.

R64

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Pulpoperiapical osteosclerosis: prevalence in contemporary and archaeological populations

Aim To describe pulpoperiapical osteosclerosis in sample populations from the same geographical region (southern France) over a period of 4 millennia through an epidemiological study.

Methodology Sample populations were selected on the basis of jawbone condition in terms of clinical and/or anthropological data. The oldest sample included 50 individuals from the chalcolithic hypogea (–2000 BC) in Roaix (Vaucluse). The historic sample (IVth to XVIth century) included 250 individuals from the Notre-Dame-du-Bourg grave site (Alpes de Haute Provence). The contemporary sample included 223 individuals recruited from various dental practices in the Gard Department, France. Data for all individuals were obtained by digital radiographic imaging of the jawbones using an image processing software application (Sensor CCD Visualix, Gendex Dental Systems, Italy). Differences in lesion prevalence were analysed in terms of individual and dental features (Period, age, and gender; jawbone, dental group, and tooth type). Statistical analysis was performed using the Chi Square tests and Fisher exact test.

Results The prevalence of lesions showed an increase between archaeological samples and then decreased in the contemporary sample ($P < 0.001$). Regardless of the time period lesions were more likely to be found on the mandible ($P < 0.001$) and to involve the molar group ($P < 0.001$), especially the first molars ($P < 0.01$). Age and gender did not appear to be significant factors.

Conclusions Comparison with other archaeological samples is not possible since there are no previous reports on pulpoperiapical osteosclerosis. Most of the findings in the contemporary sample are in agreement with those reported elsewhere. The decrease in the prevalence of these lesions could be attributable to improvement in living conditions and to the development of dental care. This has not been the case for most other apical pathosis.

R65

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Influence of electronically controlled periapical instrumentation on periapical healing

Aim To study the influence of electronically controlled periapical instrumentation on the healing of chronic periapical lesions in the teeth of dogs.

Methodology After inducing periapical lesions in six mongrel dogs for 35 days, root canal treatment was performed using two different protocols. In group 1, 'intracanal instrumentation' (3 dogs – 17 canals), canals were instrumented to the apical delta with crown-down technique using ProFile (Maillefer Dentsply, Switzerland) instruments and then filled with Thermafil obturators (Maillefer Dentsply, Switzerland). In group 2, 'electronically controlled periapical instrumentation' (3 dogs – 18 canals), following the same procedure as in group 1, the apical delta was perforated and additional instrumentation was performed using a hand H–file to the length determined by a resistance type apex locator (EED–11, Struja, Croatia), on average 1.05 mm beyond the apex. Canals were filled as in the first group, 2 mm shorter then the apical foramen, where a new artificial apical constriction was created. Thirty-five days after filling, undemineralized sections 5–7 μm thick were stained with Toluidine blue and analyzed using light microscopy. Histomorphometric indices (lesion width, lesion length, osteoid surface and osteoclast index) were measured using a computer program (ISSA, VAMS, Croatia). Results were statistically analyzed using Mann-Whitney *U* test.

Results The difference between groups in terms of lesion width was not statistically significant (group 1: 2.76 mm; group 2: 2.67 mm;

$P > 0.05$). The differences in lesion length (group 1: 0.79 mm; group 2: 1.20 mm), osteoid surface (group 1: 7%; group 2: 30%) and osteoclast index (group 1: 75.96 mm^{-2}; group 2: 5.35 mm^{-2}) were statistically significant ($P < 0.05$).

Conclusions Electronically controlled periapical instrumentation resulted in enlarged periapical lesion (demonstrated by greater lesion length). After a 35-day healing period enhanced healing potential was demonstrated by lower bone resorption activity (e.g. greater osteoid surface and lower osteoclast index), in the electronically controlled periapical instrument group.

R66

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Periapical central giant cell granuloma: clinicopathologic study of four cases

Aim To ascertain the clinicopathologic features of periapically located Central Giant Cell Granulomas (CGCG) that were misdiagnosed as endodontic lesions.

Methodology Clinical and histopathologic data of biopsy specimens diagnosed as CGCGs over the past eight years were collected from the archives of the Oral and Maxillofacial Histopathology Laboratory, Geneva University, and were reviewed.

Results Four cases of periapical CGCG were found to be submitted with a clinical diagnosis of either radicular or residual cyst. In one case root canal treatment had been performed previously. The patients were two women and two men (three European, one North African). The age ranged from 31 to 85 years (mean age 59.2). Two cases were located in the mandibular premolar-molar region: one on the right side (from tooth 44 to tooth 46) and one on the left side (from tooth 33 to tooth 35). Two cases were situated in anterior-lateral region of the maxilla (one from tooth 11 to tooth 13, one from tooth 22 to tooth 23). Two lesions were submitted with a diagnosis of radicular cyst whereas the other two were submitted with a diagnosis of residual cyst.

Conclusions These data suggest that periapical CGCG may be misdiagnosed as an endodontic lesion due to its radiographic similarity to a routine inflammatory periradicular lesion. Post-treatment follow-up and routine submission of periapical surgical specimens are mandatory in order to avoid delay in the diagnosis and to perform appropriate treatment.

R67

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Investigation of the involvement of several non-collagenous proteins in the metabolism of human odontoblast-like cells

Aim To analyze proteins, known to be involved in the anabolic hard tissue metabolism of bone, in human odontoblast like (hol) cells over the course of six weeks.

Methodology Primary cell cultures were set up from caries free impacted wisdom teeth from donors no older than 18 years. They were pre-cultivated in an incubator up to transfer phase (T)2. In T3 they were seeded on a resin support material (Thermanox, Nunc, Germany) and transferred to a perfusion cell culture system (Minucells and Minutissue, Germany). Sampling of the hol cells was completed weekly over the course of six weeks. Analysis was made by quantitative RT-PCR (iCycler, BioRad, Germany). Different, non-collagenous proteins known to be involved in the anabolic hard tissue metabolism were quantitatively determined. These are osteonectin (SPARC), osteocalcin (BGLAP), osteopontin (SPP1), alkaline phosphatase (ALPL), fibronectin 1 (FN1) and dentin-sialophosphoprotein (DSPP).

Results An expression pattern for hol cells was identified showing an increased expression of the dentine specific DSPP in the course of cultivation. Human pulp derived cells differentiated into odontoblast-like cells in the perfusion cell culture system and expressed dentine specific, non-collagenous proteins.

Conclusions The experimental model was established to compare expression patterns of odontoblast-like cells with those of anabolic hard tissue metabolism of bone to allow differences and similarities of the mineralization processes to be identified.

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R68

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Detection of Platelet Activating Factor-Receptor in periapical lesions

Aim To investigate the presence and localization of Platelet Activating Factor-Receptor (PAF-R) in periapical lesions associated with root filled teeth with post-treatment disease.

Methodology Sixty-three periapical lesions associated with root filled teeth with post-treatment disease were obtained during periradicular surgery and fixed in neutral formalin. The paraffin-embedded tissue samples were routinely processed and haematoxylin–eosin stained slides were examined under light microscopy. The avidin–biotin peroxidase complex method was used to localize PAF-R on the paraffin tissue sections. Rabbit polyclonal to PAF-R H963 (Abcam Ltd, UK) was used as the primary antibody and diluted to 1 : 100 in PBS before use.

Results The study of haematoxylin–eosin stained sections revealed that 25 (39.6%) of lesions were granulomas, 12 (19%) cysts, 4 (6.3%) scar tissue, 3 (5%) keratocysts while in the rest 19 cases (30.1%) an epithelial lining together with an inflamed connective tissue was found (inflamed periapical cysts). The immunoperoxidase staining reaction product appeared as brownish deposits on the tissue sections. PAF-R staining was detected mainly on mononuclear inflammatory cells such as lymphocytes, macrophages, lymphoplasmacytes as well as in endothelial cells. A strong correlation between PAF-R expression in these cells and presence of ongoing inflammation was seen especially in granulomas and inflamed cysts. Interestingly, in epithelium erosion sites a strong infiltration by PAF-R positive cells was noted. In periapical cysts and scar tissue specimens only a weak labelling for PAF-R was observed. Fibroblasts and epithelial cells were not stained for PAF-R.

Conclusions PAF-R positive cells are present in periapical granulomas and inflamed periapical cysts and their presence appears to be positively related to inflammatory activity.

R69

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Development of a protocol for *in vitro* culture of pulpal cells

Aim To apply previously established protocols for cell culture to human pulpal cells and to optimise them; and to use Relative

Quantification-Real time PCR to detect and quantify the expression of dentine sialophosphoprotein (DSPP) by the cultured cells, over time.
Methodology Pulp tissue from 12 human extracted third molars was removed and cultured *in vitro*. The protocol for culture was modified until cell growth was obtained and confirmed by microscopy. After sub-culturing to 100% confluence at 2, 4, 6 and 8 days, the RNA was isolated from a sample of the cultured cells from two pulp explants. RT-PCR was used to make complementary DNA and Relative Quantification-Real time PCR was used to quantify DSPP expression at the different time points relative to the expression of the 18S gene.
Results The initial protocols resulted in fungal and bacterial contamination or lack of adherence of the initial explant. Three of the twelve pulp tissue explants were eventually successfully and predictably cultured. A protocol to culture human pulpal cells has been proposed. Histologically, the cultured cells resembled pulpal fibroblasts. Relative Quantification-Real time PCR confirmed the expression of DSPP by the pulpal cells. There was no significant difference in the levels of DSPP expression after 2, 4, 6 and 8 days of culture.
Conclusions It was possible to culture human pulpal (fibroblast-like) cells using a modified protocol. Expression of DSPP by the cultured pulpal cells was confirmed but no change in expression levels was detected over 6 days in culture. A base-line model has been proposed for testing inductive changes in the cultured cells to better understand their function.

Research Posters – Cytotoxicity and Biocompatability

R70

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In vitro cytotoxicity of root canal sealers

Aim To study cytotoxicity of the root canal sealers Diaket (3 M ESPE, Germany), IRM (Dentsply, USA), SuperEBA (Bosworth Company, USA), Hermetic (Pharma Gmbh + Co.KG, Germany) and GuttaFlow (Roeko, Coltene/Whaledent GmbH + Co.KG, Germany) on human laryngeal carcinoma cells (HEP) *in vitro*.
Methodology The sealers were prepared according to the manufacturer's instructions under aseptic conditions and a 0.02 mL increment of each sealer was placed at the bottom of a 24-well plate. The sealers were covered with a 1×104 HEP cell suspension for 1 h, 24 h, 48 h, 7 days and 1 month after mixing. Four samples and respective controls without sealer were prepared. After 5 days of incubation the number of cells was determined using an electronic counter; the number of viable cells was determined under light microscopy following the addition of methylene blue. The number of cells in the experimental dishes was calculated as a percentage of the controls.
Results GuttaFlow showed increased cytotoxicity over time after mixing. After one month, cytotoxicity was significantly higher (22%) than after 1 h (80%), 24 h (68%), 48 h (66%) and 7 days (50%) ($P < 0.01$). Other tested materials showed strong cytotoxic effects. There were no viable cells for all tested periods.
Conclusions All tested materials except GuttaFlow showed strong cytotoxic effect at all periods but cytotoxicity for GuttaFlow was dependent on time.

R71

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In vitro cytotoxicity of an antibacterial self-etch adhesive system containing MDPB

Aim To evaluate the cytotoxic potential of a 12-methacryloyloxydodecylpyridium bromide (MDPB) – containing antibacterial self-etching primer (Clearfil Protect Bonds, Kuraray, Japan) on L-929 fibroblasts.
Methodology The primer and adhesive components of the test material was diluted serially with the culture medium at a ratio of 1 : 1000 and 1 : 4000 (v/v). Cytotoxicity was identified by plating the L929 cell lines in 24-well culture plates in Dulbecco's modified Eagle's Medium supplemented with 10% foetal calf serum, penicillin (100 U/ml) and streptomycin at 37°C. After 24 h, the medium was changed with fresh medium containing different dilutions of the primer or adhesive components of the test material. Cells were further maintained for five days. A two-step self-etch primer/adhesive system (FL Bond, Shofu, Japan) was utilized for comparisons. Changes in cell number within each test group was assessed statistically using Friedman Test. Inter-material comparisons were made with Kruskal-Wallis Test ($P = 0.05$).
Results Following exposure to both dilutions of primer, the number of cells did not differ significantly within time, although the total cell number was markedly lower than that of the control group ($P < 0.05$). Compared to cells exposed to 1 : 4000 (v/v) of primer, some of which showed rounding or slight divergence from normal cell morphology, a marked cellular degeneration and rounded cells were observed at lower dilution factor, indicating strong toxicity. Similar to the primer, the total cell number in the adhesive was lower than that of the control group. At 1 : 1000 (v/v) dilution, cells displayed more cellular alterations than those exposed to higher dilution, including nuclear condensation and cell degeneration.
Conclusions The primer of the tested antibacterial bonding system displayed less cytotoxicity than its adhesive component, while a higher dilution factor resulted in reduced cytotoxic effects.

R72

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Effect of root canal dressing on the regeneration of inflamed periapical tissue

Aim To evaluate the effects of an intra-canal application of chlorhexidine and calcium hydroxide on apical periodontitis in rats.
Methodology An experimentally induced apical periodontitis was established on the mesial roots of maxillary molars of Wistar rats by exposing the root canals to the oral cavity for 14 days. In the positive control group ($n = 10$ teeth) the root canals received no further treatment other than the coronal access cavities were filled with composite. In the negative control group ($n = 10$ teeth) partial pulpotomies were performed aseptically and the access cavities immedi-

ately sealed. In a third control group ($n = 10$ teeth) the canals were instrumented, left empty and the access cavities were sealed. In the experimental groups the root canals were instrumented and either filled with 2% chlorhexidine gel (Speiko, Münster, Germany) or calcium hydroxide paste freshly prepared from pure calcium hydroxide (Merck, Darmstadt, Germany) ($n = 10$ teeth per group). After 7 days (= 21 days after initial canal exposure) all rats were killed and the histological sections were stained for microscopic analysis of periapical regeneration. The data of the subjective evaluation were analyzed with the Kruskal-Wallis test. Lesion sizes were measured and statistically analyzed using the ANOVA and post-hoc Scheffé test.

Results The two treatment groups showed significantly lower average inflammatory scores and smaller lesion sizes than the positive control group and the third control group ($P < 0.05$). No statistically significant differences were observed between the two treatment groups ($P > 0.05$).

Conclusions Chlorhexidine used as an intracanal medicament showed good periapical regeneration, suggesting that it may be an alternative to calcium hydroxide.

R73

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In vitro evaluation of the biocompatible properties of mineral trioxide aggregate (MTA) as a root-end filling material

Aim To evaluate and compare the biocompatible property of root-end filling materials, MTA and IRM, using the osteoblast-like cell culture.

Methodology Cell suspension (murine MC3T3-E1 osteoblast) was added carefully over the material disks, which had been set for 2 days at 37°C. They were incubated for 3 weeks with α-MEM containing 10% FBS, ascorbic acid, and ß-glycerophosphate. After 1 day and 2 weeks, the adhering cell number on the materials was examined using Hoechst 33258 staining under a fluorescence microscope. Furthermore, areas in contact with the material surface were observed under a transmission electron microscope.

Results The number of cells adhering on MTA was significantly higher than that on IRM. In addition, ultrastructural analysis revealed numerous collagen fibrils with mineralization directly attached to the surface layer of MTA.

Conclusions MTA possesses better cytocompatible property than IRM.

R74

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Comparison of biocompatibility and cytotoxicity of two new root canal sealers

Aim To investigate, remote organ toxicity and connective tissue reaction of two new root canal sealers (GuttaFill and EndoRez) and compare with Kerr sealer using some biochemical and histopathological parameters.

Methodology Sixty white Wistar-Albino rats (weighing 200–250 g) were used. A total of 0.1 mL of GuttaFill (Roeko, Germany), EndoRez (Ultradent Products, USA) or Kerr sealer (Kerr Romulus, USA) were administered subcutaneously into the dorsal thoracic middle of rats (15 of each). Control rats were given saline only. Rats were decapitated at 24 h, on day 7 and on day 30 of the experiment and the tissue samples from lung, liver, kidney and skin were removed for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. Tissues were also examined histologically. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, and creatinine, urea concentrations (BUN) were determined to assess liver and kidney functions, respectively. Tumor necrosis factor (TNF) and lactate dehydrogenase (LDH) were also assayed in serum samples.

Results No statistically difference was found among the control and EndoRez, GuttaFill and Kerr root canals sealers regarding tissue MDA, GSH levels or serum parameters ($P > 0.05$) at all times.

Conclusions Both new root canal sealers showed good compatibility and acceptable tissue toxicity.

R75

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The additive effect of rhBMP-2 on the cytotoxicity of MTA cement

Aim To compare the cytotoxicity of MTA (Mineral Trioxide Aggregate) cement with combination treatment of MTA and BMP-2 (Bone Morphogenetic Protein-2) in MTT assays of MG-63 human osteosarcoma cells.

Methodology MG-63 human osteosarcoma cell line (ATCC CRL-1427, Rockville, MD, USA) was cultured in MEM media supplemented with foetal bovine serum, antibiotic-antimycotics, sodium pyruvate, nonessential amino acids, sodium bicarbonate in culture dish. MTA cement (0.2 g, Dentsply Tulsa, USA) was mixed according to manufacturer's instructions with or without one microgram of rhBMP-2 (R&D Systems, Minneapolis, MN, USA). After 1, 24, 48, and 72 h of setting time, each group of 15 samples was incubated with 2 mL culture media at 37°C, in 5% CO_2 and 95% humidity for 24 h. Confluent cells were counted with a haematocytometer (2.5×105 cells/ml), seeded 96-well microplate and incubated with the supernatant of each sample for 24 h. Mitochondrial dehydrogenase activity of the MG-63 cells was determined using MTT (Sigma, St Louis, MO, USA), which was converted to blue water-insoluble product formazan that accumulated in the cytoplasm of viable cells. Each group of cells was treated with MTT solution for 4 h. The absorbance of formazan was measured at 540 nm with a spectrophotometer (Specgene, Techne, France). The MTT assay was performed in triplicate and repeated in six cultures. Data were statistically analyzed with Mann-Whitney U test ($P < 0.05$).

Results The scores of MTT assay increased, and the cytotoxicity of all samples decreased time-dependently. In the early period, the MTA and BMP-2 group was less cytotoxic than the MTA group until 24 h ($P < 0.05$). After 48 h, the difference was not statistically significant ($P > 0.05$).

Conclusions The cytotoxicity of MTA and combination treatment of MTA and BMP-2 decreased with time. The addition of BMP-2 had a beneficial effect that reduced intitial cytotoxicity of freshly mixed MTA cement.

