PRESENTER (COUNTRY ONLY): Portugal
CONTROL ID: 3563341
FINAL ID: 0127
TITLE: The Impact of Hypertension on the Oral Health during Pregnancy.
ABSTRACT BODY:

Objectives: Pregnancy comprises a panoply of systemic changes that increase the susceptibility for some oral diseases. Several studies suggest a relationship between oral health status and cardiovascular risk factors, such as arterial hypertension. Moreover, the oral hygiene habits of the Portuguese pregnant population have been previously described as ineffective and high levels of dental biofilm have been reported. Therefore, we aimed to assess and correlate the oral health status of healthy and hypertensive pregnant women with their oral hygiene habits.

Methods: Participants were recruited at the Obstetrics Department of a tertiary centre, between 30 and 32 weeks of gestation. Decayed-missing-filled surfaces index (DMFS) and periodontal status (clinical attachment loss-CAL, bleeding on probing-BOP and plaque index-PI) were assessed in ten healthy(H) and seventeen arterial hypertensive pregnant participants(AHT). Oral health hygiene habits during pregnancy were recorded via questionnaires. U-Mann Whitney test, Spearman correlation and Point-Biserial correlation were used for statistical analysis.

Results: Hypertensive pregnant women scored significantly higher in all periodontal parameters when compared to healthy pregnant women (CAL≥4mm: H=2.77±3.96; AHT=21.05±18.57; p=0.007; BOP: H=13.79±15.49, AHT=27.01±11.94, p=0.003; PI: H=57.72±15.65, AHT=86.24±13.32, p<0.01), whereas there was no difference concerning DMFS (p=0.13). No differences were observed between the groups regarding the frequency of toothbrushing, use of complementary mean of oral hygiene or number of dental appointments in the previous year. Toothbrushing frequency was negatively associated with BOP (r=-0.41; p=0.033). Moreover, a negative correlation was observed between the use of a complementary means of oral hygiene, BOP (r=-0.43; p=0.026) and CAL≥4mm (r=-0.40; p=0.039).

Conclusions: The periodontal health of hypertensive women in the 3rd trimester of pregnancy was significantly worse than healthy women. Oral health habits highly impacted periodontal health. It is fundamental to raise awareness on the importance of maintaining good oral health during and after pregnancy in this population of hypertensive women.

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TITLE: Role of NLRP3 Inflammasome in Periodontitis : Friend or Foe?

ABSTRACT BODY:

Objectives: Periodontitis are highly prevalent chronic inflammatory diseases triggered by the dysbiosis of oral microbiota and characterized by gingival inflammation and the irreversible destruction of the periodontium (i.e. cementum, periodontal ligament and alveolar bone). Among periodontal microbiota, Porphyromonas gingivalis (P.g.) is a keystone pathogen responsible for oral dysbiosis and tissue homeostasis disruption leading to periodontal diseases. NLRP3 inflammasome, a major determinant of inflammation, is overexpressed in periodontitis. Recently, it has been suggested that NLRP3 inflammasome may actively participate in the pathogenesis of periodontitis. The objective of the present study is to characterize the role of NLRP3 inflammasome in periodontal destruction and alveolar bone resorption during P.gingivalis-induced periodontitis.

Methods: Periodontitis induction was performed on WT and NLRP3 KO mice by mean of ligature soaked with or without P.gingivalis and subgingivally placed into the palatal sulcus of the first upper right molar. The left molar remains intact and served as control. We replaced the ligature twice a week during four weeks. The alveolar bone resorption was monitored by micro-computed tomography (micro-CT) through out the experiment.
All mice were sacrified and processed for histological analysis.

Results: Micro-CT analysis revealed a protective role of NLRP3 against bone loss in periodontitis. This result correlates with the protection against periodontal soft tissue destruction. Moreover, we show that NLRP3 inflammasome is overexpressed in the connective tissue of WT mice after periodontitis induction. This upregulation increases strongly the local production of the pro-inflammatory cytokine such as IL-1beta in WT mice, as compared to NLRP3 KO mice. This expression actively participates in the recruitment of polymorphonuclear cells which subsequently form a PMNs-protective wall at the site of periodontitis induction.

Conclusions: During periodontitis, the upregulation of NLRP3 inflammasome has a protective effect against periodontitis through recruitment of neutrophils. However, this protective role is subverted by the presence of P.gingivalis.

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TITLE: Bone Loss Estimation in Periodontitis using Machine Learning on Periapicals
ABSTRACT BODY:

Objectives: To develop an automatic analysis of periapical radiographs from periodontitis patients and non-periodontitis subjects for the % alveolar bone loss (ABL) on the approximal surfaces of teeth using a machine learning technique based on artificial intelligence, i.e. convolutional neural networks (CNN).

Methods: A total of 1546 approximal sites from 54 subjects on mandibular periapical radiographs were manually annotated (MA) for a training set (n=1308), a validation set (n=98) and a test set (n=140). The training and validation sets were used for the development of a CNN algorithm. The algorithm recognized the cemento-enamel junction, the most apical extent of the alveolar crest, the apex and the surrounding alveolar bone.

Results: For 140 unseen images in the test set, the CNN scored a mean of 23.1 ± 11.8 %ABL, while the corresponding value for MA was 27.8 ± 13.8 %ABL. The intraclass correlation (ICC) was 0.0601 (p<0.001). Further sub-analyses for various tooth types and various bone loss patterns showed that ICC's remained significant although the algorithm performed better on incisors than molars.

Conclusions: A CNN trained algorithm on radiographic images, showed a diagnostic performance with moderate to good reliability to detect and quantify %ABL in unseen periapical radiographs.

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TITLE: Bone Regeneration in Apical Periodontitis

ABSTRACT BODY:

Objectives: It has long been discussed that the healing rate of asymptomatic apical periodontitis (AAP) is contingent on the treatment protocol in terms of number of visits, preparation techniques and medicaments. However, bone healing is an intricate multifaceted process of cellular proliferation. The aim of this study was to examine a potential link between patients' mitogen-induced inflammatory mediator release and the rate of bone healing following non-surgical endodontic treatment of AAP

Methods: Patients (n=20) diagnosed with AAP were enrolled in the study and underwent non-surgical endodontic treatment. Small field-of-view cone beam computed tomography was used to assess the lesion volume prior to and 10 months following treatment. Pre-treatment peripheral blood samples were collected from the patients and peripheral blood mononuclear cells were isolated and cultured in two different media: RPMI 1640 and RPMI 1640 + PHA. The supernatant was collected at the 24th and 48th hour and the levels of IL-1β and PGE2 were assessed by ELISA. Individual mitogen-induced reactivity (MIR) was calculated for each molecule. Logistic regression, extra tree classifier, ANOVA and heatmap methods were used to assess the association between the levels of MIR of each molecule and the rate of healing.

Results: The whole set of used algorithms outlined MIR PGE2/24h , MIR PGE2/48 and MIR IL-1β/48 as strong predictors of the rate of bone healing.

Conclusions: A link between the host's mitogen-induced reactivity and the rate of bone regeneration was observed. Further studies can shed light on the underlying pathways of the observed phenomenon and translate these finding into the clinical practice.

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TITLE: Time-induced Ageing of Titanium Dental Implants

ABSTRACT BODY:

Objectives: Titanium is used worldwide as the base material for dental implants due to its excellent biocompatible features. However, the chemical composition of implant surfaces, which plays a crucial role in the osseointegration, is unavoidably contaminated by organic compounds, causing the degradation known as "the ageing of titanium". Thus, this study investigates the surface chemistry of new and 5-year-old commercially available dental implants so as to compare the influence of the time-induced changes on the characteristics of their surfaces.

Methods: X-ray Photoelectron Spectroscopy (XPS) was employed to analyse the chemical composition of the titanium surfaces. First, wide scans were conducted to detect which elements were present on the surface, and then narrow scans were performed around the energy peaks of carbon (C), oxygen (O) and titanium (Ti), to quantify the relative concentration of each element. Both the new and 5-year-old surfaces were subjected to the same characterization process.

Results: XPS analysis showed higher C concentrations on the 5-year-old surfaces than on the new ones; the mean concentration was 31.23±0.56 C at. % (atomic concentration) (95% confidence interval (CI): 26.116-36.050) and 15.4±0.53 C at. % (95% confidence interval (CI): 14.085-16.714), respectively. Specifically, the hydrocarbon concentration was remarkably higher on both surfaces. Along with that, the concentrations of O and Ti were lower on the old surfaces (43.43±1.40 O at. %; 8.4±0.89 Ti at. %) comparing with the new surfaces (57.63±0.15 O at. %; 15.07±0.75 Ti at. %). These outcomes may be related to a reduction of the titanium bioactivity.

Conclusions: There are relevant differences in terms of the chemical composition of the 5-year-old implants with respect to the new ones, specifically in the concentration of carbon compounds, which are associated with the ageing of titanium.

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TITLE: Long-term antimicrobial effects of 4-META/MMA-TBB resin containing antibacterial agents

ABSTRACT BODY:

Objectives: We aimed to assess the long-term antimicrobial effects of a 4-META/MMA-TBB resin (Bondfill SB Plus, Sun Medical) containing antibacterial agents.

Methods: We employed two antibacterial agents, benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC), and prepared 4-META/MMA-TBB resins which contain either 5.0wt% BAC (BAC5.0wt%) or 5.0wt% CPC (CPC5.0wt%). Disk-shaped specimens (10-mm diameter; 2-mm thickness) were prepared and assessed the long-term antimicrobial effect using agar diffusion test (n=6/group) up to 24 weeks. Three oral microorganisms (Streptococcus mutans, Streptococcus sobrinus, and Actinomyces naeslundii) were inoculated in BHI agar medium, and the specimens were allowed to put on the agar. The growth inhibition zone was confirmed after aerobically cultured at 37 ° C for 24 hours. Twenty-four hours prior to each measurement of the inhibition zone, 4-META / MMA-TBB resin specimens were placed on new agar plates. The medium was changed every week until the third week, and then every two weeks thereafter. The obtained data were subjected to statistical analysis using two-factor repeated measures ANOVA followed by multiple comparison testing using Shaffer modified Bonferroni correction method. For two-factor repeated measures ANOVA, independent factors "type of resin", and "time", as well as the mutual interaction "type of resin" × "time" were included. The total evaluation period was 24 weeks.

Results: Two-factor repeated measures ANOVA revealed that significant differences in inhibition zone of BAC5.0wt% and CPC5.0wt% for all microorganisms, as well as time and mutual interaction "type of resin" × "time". Inhibition zone of BAC5.0wt% was larger than that of CPC5.0wt% up to 4th week. However, the inhibition zone of BAC5.0wt% substantially decreased after 4th week. Finally, inhibition zone of BAC5.0wt% was comparable to that of CPC5.0wt%.

Conclusions: The growth of oral microorganisms was inhibited by 4-META/MMA-TBB resin containing antimicrobial agents (BAC or CPC).

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TITLE: Long-term Outcomes of Periodontal Regeneration with Enamel Matrix Derivative (EMD)
ABSTRACT BODY:

Objectives: To report the long-term outcomes in periodontal intrabony defects following regenerative surgery with and Enamel Matrix Derivative (EMD).

Methods: Periodontal patients treated with reconstructive surgery with EMD between 1999 and 2012 (follow-up of at least 8 years) were screened (n=548) and invited to participate in a clinical examination. The following clinical parameters were recorded and compared at baseline (at 6-months after non-surgical therapy) (T0), 6 months after surgery (T1) and after at least 8 years follow-up (T2): probing pocket depth (PPD), gingival recession (GR), clinical attachment level (CAL), plaque and bleeding scores. Tooth survival (0/1), smoking status and frequency of adherence to supportive periodontal therapy (SPT) were also recorded. The primary outcome variable was the CAL change.

Results: 41 patients with a total of 75 treated teeth were available for analysis. Out of these, 68 (tooth survival rate: 90.7%) reached the latest follow-up with a mean observation period of 10.3 years (range: 8.0–21.3). The most frequent reason for tooth loss was recurrence of periodontal disease. Tooth survival curves showed a statistically significant difference between smokers and non-smokers (p=0.028). Mean CAL changed from 8.43 ± 1.86 to 6.47 ± 1.70 (p<0.001) at T1 and 5.91 ± 1.83 (p<0.001) at T2. At T1, a CAL gain of >3 mm was measured in 35% of the defects (i.e. 24 of 68), while at T2 it was detected in 51% of cases (i.e. 35 of 68).

Conclusions: The present results have provided evidence that in the great majority of cases, the clinical improvements obtained with EMD, can be maintained on the long-term. However, smoking and tooth type (i.e. maxillary molars) were correlated with an increased risk for tooth loss.

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TITLE: Bonding of an experimental bioglass-based 2-step universal adhesive to dentin

ABSTRACT BODY:

Objectives: To determine the immediate dentin-bonding effectiveness of an experimental 2-step universal adhesive formulation to which experimental bioglass filler, being referred to as '0NaMBG', was incorporated or this bioglass filler was beforehand applied prior to adhesive treatment. The microstructure of the dentin surface, having received 0NaMBG, and of 0NaMBG itself was additionally characterized.

Methods: The immediate (1-week) micro-tensile bond strength (μTBS; n=10 teeth; two-way ANOVA: $p<0.05$) of an experimental BZF-29 (GC) adhesive, consisting of a 10-MDP primer and a hydrophobic adhesive resin, to which 5% 0NaMBG was self-added ('BZF-29$_{0NaMBG}$') and which was bonded in a 3-step etch&rinse (E&R) and 2-step self-etch (SE) mode, was measured to bur-cut dentin. The alternative bonding protocol involved the application of an aqueous 0NaMBG solution (0.015g/1.35mL distilled water) prior to BZF-29 bonding ('0NaMBG+BZF-29'). G2-Bond Universal ('G2B$_{uni}$', GC) and BZF-29 without filler ('BZF-29') served as controls. Additionally, 0NaMBG application on dentin was morphologically imaged by SEM and 0NaMBG's ultrastructure morphologically/chemically characterized using S(TEM)/EDXS.

Results: No significant difference in μTBS was recorded between BZF-29$_{0NaMBG}$ and 0NaMBG+BZF-29, with E&R bonding significantly outperforming SE bonding (graph). When applied in E&R mode, BZF-29$_{0NaMBG}$'s μTBS was significantly higher than G2B$_{uni}$'s and BZF-29's μTBS. When applied in SE mode, no significant differences in μTBS were recorded. SEM revealed dentinal tubule obstruction by 0NaMBG filler. S(TEM)/EDXS revealed the presence of 50 to 100-nm beam-sensitive glass particles consisting of an ordered atomic network of several elements (Si, O, Ca and P).

Conclusions: The 0NaMBG-containing 2-step UA bonded equally well to dentin as when the bioglass filler was beforehand applied, hereby representing two options for a potential bioactive co-functionality. Research is ongoing to determine the aged μTBS and to characterize interfacial interaction by TEM.

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TITLE: Dentin Regeneration by DMP-1/BMP-2-Plasmid-Modified Nanoparticle-Treated DPSCs on Dentin Scaffolds
ABSTRACT BODY:

Objectives: Investigation of the potential of Calcium Phosphate Nanoparticles (CaP NPs), carrying plasmid DNA encoding Dentin Matrix Protein 1 or Bone Morphogenetic Protein 2 (DMP-1/BMP-2-CaP NPs), combined with human Freeze-Dried Dentin Scaffolds (hFDDSs) to promote odontogenic differentiation by Dental Pulp Stem Cells (DPSCs).

Methods: DPSCs were isolated, characterized and seeded on EDTA-treated hFDDSs. CaP NPs were synthesized and characterized [colloidally, by Dynamic Light Scattering; microscopically (SEM) for particle size; and spectroscopically (UV-vis) for functionalization]. Control CaP NPs conjugated with Cy-5 fluorescent dye or carrying the plasmid encoding green fluorescent protein were tested at a range of concentrations (0.5-8µg Ca/ml) for 24h-uptake and 48h-transfection efficiency, respectively (Confocal Microscopy, Flow Cytometry). The effect of CaP NPs on cell viability was assessed by MTT assay. Two most favorable for viability/transfectability concentrations of each DMP-1 or BMP-2-CaP NPs were selected to evaluate cell morphology on hFDDSs (SEM), viability (Live/Dead Staining), and gene expression of odontogenic markers, including DMP-1, BMP-2, DSPP, RunX2, Osterix, MSX-1 and MSX-2 (real-time PCR).

Results: Increasing concentrations of CaP NPs reduced cell viability, while increased uptake and transfection efficiency by DPSCs in a dose- and time-dependent manner. The uptake efficiency reached 51% of the total cell population at the concentration of 8µg/ml, while the transfection efficiency was 37%. Considering the transfectability/viability balance, the concentrations of 1 and 4µg/ml causing reduction of cell viability that did not exceed 45% for the DMP-1 NPs and 38% for the BMP-2 NPs, 72h post-treatment, were further evaluated in DPSC-seeded hFDDSs. Real-time PCR results showed a NP-specific, time-dependent upregulation of odontogenic markers (BMP-2, DMP-1 and DSPP), with downregulation of the transcription factors (Osterix, RunX2, MSX-1, MSX-2). Studies are in progress to evaluate in vitro biomineralization leading to dentin-like tissue formation.

Conclusions: The combination of DMP-1/BMP-2-CaP NPs and DPSCs on hFDDSs presents a promising approach in dentin tissue engineering.

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TITLE: BACH1 binding links the genetic risk for severe periodontitis with ST8SIA1

ABSTRACT BODY:

Objectives: Genome-wide association studies identified various loci associated with periodontal diseases, but assigning causal alleles remains difficult. Likewise, the generation of biological meaning underlying a statistical association has been challenging. Here, we characterized the genetic association at the gene ST8SIA1 that increases the risk for severe periodontitis in smokers.

Methods: We used CRISPR/dCas9 activation and RNA-Sequencing to identify genetic interaction partners of ST8SIA1 and to determine its function in the cell. We used reporter gene assays to identify regulatory elements at the associated SNPs and to determine effect directions and allele specific changes of enhancer activity. Antibody electrophoretic mobility shift assays proved allele specific transcription factor binding at the putative causal SNPs.

Results: We found the reported periodontitis risk gene ABCA1 as top up-regulated gene following ST8SIA1 activation. Gene set enrichment analysis showed highest effects on integrin cell surface interactions (AUC = 0.85 (q = 4.9×10^{-6}) and cell cycle regulation (AUC) = 0.89 (q = 1.6×10^{-5}). We identified two associated repressor elements in the introns of ST8SIA1 that bind the transcriptional repressor BACH1. The putative causative variant rs2012722 decreased BACH1 binding by 40%. We also pinpointed ST8SIA1 as the target gene of the association.

Conclusions: ST8SIA1 inhibits cell adhesion with extracellular matrix proteins and integrins, cell cycle, and enhances apoptosis. Likewise, tobacco smoke reportedly results in inhibition of cell adhesion, decrease in integrin-positive cells and cell growth. We conclude that impaired ST8SIA1 repression, independently caused by reduced BACH1 binding at the effect T-allele as well as by tobacco smoke, contribute to higher ST8SIA1 levels and in smokers who carry the effect T-allele, both factors would be additive with damaging effects on the gingival barrier integrity. The activity of ST8SIA1 is also linked with the periodontitis risk gene ABCA1.

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TITLE: Release of Bisphenol A from Dental Polycarbonate Materials
ABSTRACT BODY:

Objectives: Polycarbonates are polymers of bisphenol A (BPA), an endocrine disruptor associated with various pathological conditions. The use of polycarbonates in prosthodontics includes denture base resins, prefabricated temporary crowns and CAD/CAM-fabricated occlusal splints. This study evaluated the release of BPA from dental polycarbonates to methanol and artificial saliva using liquid chromatography/mass spectroscopy (LC-MS).

Methods: Prefabricated polycarbonate crowns (mandibular first premolars, 3M, USA) were scanned using an optical strip-light scanner to prepare their polycarbonate replicas using 3D-printing (Freeformer, Arburg, Germany) or milling from CAD/CAM blocks, Temp Premium Flexible (TPF, Zirkonzahn, Italy) and Tizian Blank Polycarbonat (TBP, Schütz Dental, Germany). Control replicas were milled from polymethyl methacrylate (PMMA) blocks, Temp Basic (Zirkonzahn, Italy). The crowns/replicas were stored at 37°C in methanol or artificial saliva (AS) (n=5), and their eluates were collected after 1, 7, 28 and 84 days. BPA concentrations were measured using a LC-MS/MS method with dansyl chloride derivatization. The amounts of released BPA were expressed in micrograms per gram of material (µg/g).

Results: Significantly more BPA was released to methanol. The highest amounts of BPA were detected after the first day in the eluates of the milled polycarbonates TBP (methanol: 32.2±3.8µg/g, AS: 7.1±0.9µg/g) and TPF (methanol 22.8±7.7µg/g, AS: 0.3±0.03µg/g), followed by 3D-printed replicas (methanol: 11.1±2.3µg/g, AS: 0.1±0.1µg/g) and the 3M prefabricated crowns (methanol: 8.0±1.6µg/g, AS: 0.07±0.02µg/g). Between day 7 and 84, the daily release of BPA to methanol and AS decreased below 2µg/g and 0.6µg/g, respectively. No BPA was released from PMMA to AS, and the cumulative amount released to methanol was 0.2±0.06µg/g.

Conclusions: BPA release from polycarbonates was considerable, especially to methanol, which represents the worst-case scenario of BPA release. The current tolerable daily intake of BPA (4µg/kg bw/day) was not exceeded, but clinicians should remain vigilant, because the biological effects of BPA have not been fully understood yet.

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TITLE: Osteogenic Potential of Human Periosteal-Derived Cells for Bone Regeneration

ABSTRACT BODY:

Objectives: Critical bone defect regeneration is a challenging issue as it requires a scaffold to provide mechanical resistance and Mesenchymal Stem Cell (MSCs) recruitment to regenerate new bone. Bone marrow is known as the major source of osteogenic stem cells. However, despite the scant data available, periosteum stem cells represent a real interest for bone regeneration. This study was designed to characterize Periosteum-Derived cells and their osteogenic potential by studying their osteogenic differentiation and matrix mineralization abilities.

Methods: human Periosteum-Derived Cells (hPDCs) were obtained by the outgrowth method and characterized by immunofluorescence with antibodies to CD9, CD34, CD45, CD90, CD105. Their proliferation was compared to pulp fibroblasts at day 3, 5 and 7 with an MTT assay. To investigate their differentiation toward the osteogenic lineage, cells were cultured in osteogenic induction medium for 1 month. Alkaline Phosphatase (ALP) activity was quantified every week. Similarly, Alizarine Red Staining (ARS) of calcium depositions was performed and quantified every week to determine matrix mineralization.

Results: hPDCs displayed a cuboidal shape, characteristic of periosteum cells. They expressed CD9, CD90, CD105 MSC-specific markers, the osteoprogenitor CD34 marker and displayed a significantly higher proliferation rate as compared to pulp fibroblasts. Use of osteogenic induction medium did not modify ALP activity but significantly increased the formation of the mineralized matrix as measured by Alizarin Red at the 4th week.

Conclusions: This work demonstrates that human Periosteum-Derived Cells display a MSC phenotype as demonstrated by a high proliferation rate, expression of stem cell markers and an osteogenic differentiation potential. They also have an osteoprogenitor cell profile as demonstrated by their osteogenic differentiation and mineralized matrix synthesis which highlights a bone regeneration capacity. In conclusion, hPDCs hold promise for bone regeneration and may be of interest in the management of critical bone defects.

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TITLE: The microbiome of endodontic infections and its association with clinical features

ABSTRACT BODY:

Objectives: To describe the endodontic infection of teeth with primary and secondary apical periodontitis and explore differences related to symptomatology.

Methods: Twenty-nine teeth with primary or secondary apical periodontitis were extracted and cryo-pulverized. Amplicons based on the V4 hypervariable region of the 16S rRNA gene were sequenced using Illumina MiSeq. The data was processed in line with the UPARSE pipeline and subsampled at equal depth (6400 reads/sample). The microbiome profiles were ordinated using Principal Component Analysis (PCA), and tested for differences between groups with permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis distance. If significantly different, the microbial profiles were further analyzed using the LDA effect size biomarker (LEfSe) discovery tool.

Results: The 334 thousand sequences that passed quality filtering were clustered into 276 Operational Taxonomic Units (OTUs) and classified into 126 genera or higher taxa. The predominant genus in the entire sample set was Fusobacterium. The microbiomes of the endodontic infections were significantly associated with endodontic status (primary/secondary infection) ($F=2.2$, $P=0.015$) as well as with the presence or absence of pain ($F=2.3$, $P=0.013$). There were no associations between gender and microbiome or gender and the presence or absence of pain. The relative abundance of several OTUs differed based on pain existence. For example, Streptococcus, Prevotella 7 and Bifidobacterium were more abundant in asymptomatic, while Fretibacterium, and Peptostreptococcus in symptomatic apical periodontitis. The Streptococcus OTU was further identified as S. mutans and even was the second most abundant OTU in the asymptomatic group. Secondary apical periodontitis showed a higher relative abundance of e.g. a Tannerella and Mogibacterium OTU.

Conclusions: The microbial profile of primary endodontic infection differed from that of secondary. The presence or absence of pain in apical periodontitis was related to the microbial ecology of the root canal. These results should be taken into consideration in future treatment strategies.

PRESENTER: Athina Christina Georgiou

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TITLE: >3Years Follow-up of a Variable-Thread Tapered Implant.

ABSTRACT BODY:

Objectives: The aim of this prospective, single-center study was to document, for the NobelActive implant, survival rate, success rate and potential predictive variables for early bone loss (e.g. smoking, periodontal bone loss according to age, quality and quantity of bone, crestal gingival thickness, guided bone regeneration (GBR) procedures, ISQ and torque values).

Methods: From March 2011 to September 2016, 286 NobelActive implants were installed in 157 subjects. Separate analyses were performed for implants placed via a 1- or 2-stage procedure, and relative to the timing of placement/loading. At implant placement, abutment connection, functional loading, at 6, 12, 24 and up to 58 months post-loading, clinical and radiographic data were recorded.

Results: The cumulative survival rate after 12 months and 58 months of loading was respectively 99.7% and 98.6%. The mean marginal bone loss (MBL) of 276 patients between loading and 12 months was 0.05 mm (±0.77). 58 months post-loading, a mean bone loss of 1.05 mm (±1.43) was observed in 26 patients. Periodontal bone loss over age, smoking, crestal gingival thickness, torque values, bone quality, bone quantity and GBR influenced significantly the bone loss over time.

Conclusions: The presented 58 months (4.9 years) follow-up of functional loading data shows a divergent bone level around the NobelActive implants.

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TITLE: Biological Responses of Calcium-Silicate-Based Cements on a 3D Pulp Analogue

ABSTRACT BODY:

Objectives: Aim of this study was to evaluate biological responses of two bioactive calcium silicate-based cements (MTA and Biodentine™) on a three-dimensional, tissue-engineered organotypic pulp analogue.

Methods: For the establishment of the pulp analogue, Human Umbilical Vein Endothelial Cells (HUVEC) and Stem Cells from Human Exfoliated Deciduous teeth (SHED) were first co-cultured in 2D conventional culture flasks at a ratio of HUVEC:SHED=3:1. After 24h, the co-cultures were trypsinized and the pellet incorporated into a collagen I/fibrin hydrogel (total protein content 3.5mg/ml), at a concentration of $(2.5-6.5)\times10^5$ cells/hydrogel, depending on the experimental procedure. Biodentine™ and MTA cylindrical specimens (6.5×2mm) were prepared and placed in direct contact with the cell-seeded hydrogels 48h later, whereas hydrogels without specimens served as control. Live/dead staining, MTT assay and Scanning Electron Microscopy (SEM) were used to evaluate cell viability/proliferation and morphology. Real-time PCR to evaluate expression of angiogenic markers (PECAM-1, VEGFa, VEGFR1, VEGFR2, ANGPT-1, Tie-2).

Results: Live/dead staining at day 3 after treatment showed that viable- dominated over non-viable cells (not exceeding 10% for both materials). Cells were evenly distributed inside the hydrogels exposed to the bioactive cements without any differences compared to the control (untreated) cultures. MTT analysis at day 1 and 3 showed that viability/proliferation inside the cell-seeded hydrogels was not influenced by the presence of the bioactive cements. Real-time PCR at day 3 showed upregulation of VEGF and ANGPT-1 in both treated and untreated hydrogels, whereas the respective receptors VEGFR2, VEGFR1 and Tie-2 were downregulated. These alterations were more pronounced in the silica cement-treated groups.

Conclusions: Both calcium silicate-based materials are biocompatible and exert a positive angiogenic effect on the organotypic pulp analogue, validating further application in vital pulp therapy of deciduous teeth.

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TITLE: Dynamic Changes In Orthodontic Tooth Movement In Rats: 3D Analysis

ABSTRACT BODY:

Objectives: To evaluate the changing pattern of orthodontic tooth movement (OTM) throughout time with a constant orthodontic force (OF) by an improved 3D analysis in a rat model, overcoming the limitations of previous 2D analysis.

Methods: Six adult male Wistar rats were randomly assigned to two groups by implanting a mini-screw in one hemimaxilla (split-mouth design). In Group 1 (n=6) a constant OF (25 g) was applied between the upper first molar (M1) and the mini-screw. In Group 2 (n=6) no force was applied on the contralateral hemimaxilla (control). All rats were longitudinally followed up with in vivo micro-CT before OTM (baseline, T0), after 10 (T1), 17 (T2), 24 (T3) and 31 days (T4). The OTM and bone mineral density (BMD) were measured with in 3D by a rigid voxel-based superimposition method. Values were compared between different timepoints and experimental sides by repeated measures MANOVA.

Results: M1 displaced significantly more in Group 1, while the other teeth didn't. Increased tipping of M1 was observed at T0-T1 and T3-T4, while at T1-T3 bodily movement was predominant. OTM had a significant effect on BMD (P=0.03*) but time did not (P=0.10). BMD was significantly lower in Group 1 at all timepoints except for baseline. Interestingly, BMD in Group 1 decreased at T1 but increased gradually from T1 to T4, parallel to the changing pattern of M1 displacement.

Conclusions: The changing pattern of OTM is not constant throughout time. Initially, M1 displacement was mainly due to tipping, while after BMD decreasing at T1, bodily movement dominated. The later increase of BMD from T2 to T4 led to a mix of bodily movement and tipping in M1 displacement.

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TITLE: Regenerative Endodontic Treatment with Blood Clot and PRF: A Clinical Report

ABSTRACT BODY:

Objectives: Blood clot (BC) and platelet-rich fibrin (PRF) have been used as scaffolds in regenerative endodontic treatment (RET). The aim was to compare the performance of PRF with BC in inducing root development after RET of two traumatized immature necrotic permanent teeth of a patient.

Methods: A 6 year-old boy with trauma to maxillary central incisors applied to pediatric dentistry clinic. Clinical and radiographic examinations revealed both incisors having necrotic pulp without apical periodontitis. In the first appointment both teeth were treated by preparing access cavities and pulp removal. The canal was irrigated with 1.5 % NaOCl. Calcium hydroxide was inserted into the canal for 3 weeks.

At the second appointment, both canals were irrigated with EDTA. Blood was drawn from the patient's forearm for preparation of PRF. After provoking periapical bleeding, the PRF was placed into the canal space of maxillary right central incisor while blood clot alone to the maxillary left central incisor. Mineral trioxide aggregate was placed directly over the PRF and blood clot. Teeth were temporarily restored with glass ionomer cement. Two weeks later, both teeth were permanently restored with composite resin. The patient was followed-up for 28-months, clinically and radiographically.

Results: After 28 months clinical examination revealed no sensitivity to percussion or palpation tests in both teeth. Root elongation, dentinal wall thickening and continued apical closure in both teeth were observed in radiographs. Cone-beam computed tomography images revealed that tooth treated with BC showed narrower root canal in the apical region and tendency for root obliteration compared to tooth treated with PRF.

Conclusions: Both PRF and BC exhibited successful clinical outcomes and continued root canal development in the regenerative endodontic treatment. Nevertheless, PRF may be potentially an ideal scaffold compared to BC in terms of long-term pulp obliteration.

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TITLE: Prognostic value of Ultra-High Frequency Ultrasound in primary Sjögren Syndrome

ABSTRACT BODY:

Objectives: Primary Sjögren's Syndrome (pSS) is a chronic autoimmune disease causing progressive impairment of lacrimal and salivary glands due to the development of inflammatory infiltrate in the glandular tissues. The characterization of the glandular infiltrate is extremely relevant as a prognostic factor for the development of lymphoma. In particular, a focus score (FS) ≥3 is considered as a threshold significantly contributing to lymphoma development. The aim of this study is to evaluate the potential role of Ultra-High frequency Ultrasonography (UHFUS) in the identification of patients with a FS ≥3 at higher risk of lymphoma.

Methods: Consecutive patients with suspected pSS were enrolled. All the patients underwent a complete rheumatological diagnostic work-up, UHFUS of minor salivary glands, and surgical biopsy. UHFUS images were evaluated according to the OMERACT scoring system (0= normal glandular parenchyma to 3= diffuse presence of hypoechoic areas in the absence of normal glandular parenchyma, glandular fibrosis).

Results: In total, 168 patients were included. Out of them a diagnosis of pSS was confirmed in 81 patients (48.2%, mean age 55.17±14.34). Seven patients presented a FS ≥3. None of the patients with UHFUS score 0 presented a FS ≥3; only the 3.7% of patients with UHFUS score 1-2 displayed a FS ≥3, whereas the 16.7% of the patients with a UHFUS score 3 had a FS ≥3 ($p < 0.05$).

Conclusions: UHFUS score 3 tends to identify patients with a FS ≥3, thus highlighting the high correspondence between the UHFUS scoring and the histology. UHFUS could potentially become an integrating tool in risk assessment and stratification for lymphoma development in pSS patients.

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TITLE: Mechanical Properties of a Graphene-based Composite for Dental Restorations

ABSTRACT BODY:

Objectives: Recently, graphene has attracted both academic and industrial interest because it showed to enhance mechanical, physical and chemical properties of biomaterials.
This work aimed at comparing the mechanical properties of a graphene-based composite and a conventional and commercially available nanohybrid resin-based dental composite, used as control.

Methods: The composites were subjected to the following mechanical tests: 3-point flexural strength (FS) test, compressive strength (CS) test and Vickers hardness (VH) test. For each different mechanical test, ten samples of each composite were prepared (n=10), according to following specimen design: bar-shaped (2 x 2 x 25 mm) for FS, cylindrical (4 mm diameter, 8 mm height) for CS, disc-shaped (4 mm diameter, 2 mm height) for VH. All tests were performed using a universal testing machine (Lloyd Instruments- LR30KPlus). Means (and standard deviations) were calculated and compared using the Student-t tests (P< 0.05).

Results: In all mechanical tests performed, the ultimate strength/hardness observed for the graphene-based composite appeared comparable or slightly increased compared to the nanohybrid resin-based dental composite. Moreover, both the flexural modulus of elasticity (recorded on the FS test) and the compressive modulus of elasticity (recorded on the CS test) were significantly reduced for the graphene-based composite, compared to the control.

Conclusions: The experimental graphene-based composite tested in this study showed ultimate strength and hardness at least as good as those observed for conventional dental composites. At the same time, the significantly reduced flexural and compressive moduli describe a considerably more elastic material. Further studies are needed, to definitely understand the clinical meaning of the mechanical differences herein observed.

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TITLE: Effect of Biomimetic Mineralization on Demineralized Dentin: A SEM/EDS Analysis

ABSTRACT BODY:

Objectives: To investigate the effect of Riboflavin as biomimetic mineralization on the chemical composition and crystallinity of demineralized dentin samples in vitro.

Methods: Dentin samples from human teeth were randomly divided into 4 groups: Surfaces treated with Universal bonding agent (Kerr Optibond (Orange, CA, ABD) (Group B), surfaces treated with tri-calcium phosphate (fTCP) (3M ™ Clinpro ™ 5000) followed by 2% Riboflavin (Group CR),) surfaces treated with Universal bonding agent and 2% Riboflavin (Group BR) and control surfaces with no pretreatment (Group C). To assess the morphological changes of the surface was analysed by SEM and quantitative analysis of tissue penetration was performed using energy-dispersive X-ray spectroscopy (EDS). Data was statistically analyzed using Mann Whitney U test for comparison between means at a significance level of 0.05.

Results: The results of microscopic observation of dentin morphology showed that Group CR and Group BR showed a large degree of remineralization on the surface of the dentin treated and the dentinal tubules were generally occluded. The remineralization in fTCP with 2% Riboflavin group was more even than that in bonding with 2% Riboflavin group. The crystals formed during remineralization in dentin have been observed in tri-calcium phosphate and bond with Riboflavin groups. Element mapping revealed a high Ca content within the dentinal tubules. EDS analysis demonstrated Calcium (Ca) content within dentin for Universal Bond group and fTCP with 2% Riboflavin group were 36,8±2,28 and 21,93±3,3 respectively (p=0.083). In addition, also Carbon (C) (p=0.121) and Oxygen (O) (p=0.041) were detected within the precipitates.

Conclusions: Within the limitations of this study, the surface treatment with Riboflavin combined with tri-calcium phosphate resulted in remineralization of demineralized dentin.

Further investigations will be necessary on potential innovative bioactive materials of dentine remineralization as minimal intervention is an important concept in clinical restorative dentistry.

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TITLE: Surface decontamination for surgical treatment of peri-implantitis: A systematic review

ABSTRACT BODY:

Objectives: To assess the impact of different decontamination protocols and peri-operative systemic antimicrobials on the outcomes of surgical treatment of peri-implantitis.

Methods: Randomized clinical trials (RCTs) on surgical treatment of peri-implantitis were selected through an electronic search on Medline, Embase, Scopus, and Central databases. Only studies directly comparing two or more anti-infective strategies were included. Following data extraction, two different sets of meta-analyses were performed. Firstly, overall impact of different implant surface decontamination methods was assessed by comparing baseline values with outcomes at 6-12 months. Secondly, pairwise comparisons evaluated the potential benefit of adjunctive systemic antimicrobials over placebo. Results were expressed as weighted mean effect (WME), weighed mean difference (WMD) or risk ratio (RR).

Results: Sixteen RCTs were included. No pairwise comparisons were available for different surface decontamination methods. Use of curettes resulted in improved probing depth (PD) (WME = 2.14 mm), but the results in terms of marginal bone levels (MBL) and percentage of disease resolution were unsatisfactory. Moreover, the potential benefit of adjunctive systemic antimicrobials over placebo was evaluated in two studies, representing a total of 178 implants. The meta-analyses identified a significantly larger improvement in MBL (WMD = 1.17 mm) and disease resolution (RR = 1.48) for test procedures but found no differences for PD reduction. None of the included studies addressed patient-reported outcome measures.

Conclusions: Although protocol heterogeneity was high, a combination of mechanical and chemical implant surface decontamination is recommendable, with titanium brushes and local delivery of minocycline showing encouraging results. Furthermore, there is evidence to support adjunctive usage of systemic antimicrobials together with the surgical treatment of peri-implantitis.

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TITLE: Bacterial adhesion on laser and milled micro-texturized zirconia implant surfaces
ABSTRACT BODY:

Objectives: Implant surface modification has been widely used to increase osseointegration and more recently to reduce bacteria adhesion and biofilm formation. The aim of this pilot in vitro study was to evaluate and compare the initial adhesion of Streptococcus oralis on Zirconia implant surface micro-texturized by laser machining and conventional milling.

Methods: A total of 12 Zirconia discs with 8 mm in diameter and 2 mm in thickness were prepared using the pressing and sintering technique. Ultra-high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM)substrate micrographs and roughness using contact profilometry were obtained for the tested discs before bacterial culture. Discs were randomly distributed into two groups according to the micro-texturing technique performed: conventional milling (Conventional) and Nd: YAG laser machining (Laser). Streptococcus oralis CECT 907T strain were seeded on the discs at exponential phase and cultured at 37°C in anaerobic condition. Biomass was evaluated after 1 and 5 hours of incubation with 0.1% violet crystal (v / v), using a direct reading of the absorbance units (AU) at 595 nm. Viability was determined after 1 hour of culture with a bacterial viability assay kit (SYTO / Propidium Iodide), using Confocal Laser Scanning Microscopy (CLSM). The CLSM images were analyzed by FIJI software and the viability results expressed as a mean percentage. Appropriate statistical software was used to perform the analyses and the significance was set at $p < 0,05$.

Results: Biomass increased over time in both groups. The comparison of biomass between the groups did not reveal statistically significant differences ($p > 0,05$),). Values of bacterial viability based on CLSM image analysis were similar in the Laser group compared to the conventional group

Conclusions: Streptococcus oralis adhered to all zirconia textured samples after 1h and 5h of incubation. The type of texturization (Laser or milling) did not appear to affect the initial colonization by Streptococcus oralis.

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TITLE: Shear Bond Strengths of Two Newly Marketed Self-Adhesive Resin Cements

ABSTRACT BODY:

Objectives: The purpose of this in-vitro study was to compare the shear bond strength (SBS)s of two newly marketed self-adhesive resin cements to enamel, dentin and lithium disilicate (Lisi) glass ceramic block.

Methods: Forty-eight sound human molars were sectioned mesiodistally and 48 enamel and 48 dentin substrates were obtained. Additionally, 6x7x5mm -sized 24 specimens were produced from initial LiSi blocks. The prepared tooth specimens were randomly assigned into four groups(n=12) according to the surface treatments: (1) G-CEM ONE [GCO], (2)G-CEM ONE Adhesive Enhancing Primer [GCO-AEP]+GCO, (3)RelyX Universal [RXU], (4)Scotchbond Universal Plus [SUP]+RXU. LiSi specimens were randomly divided into two groups (n=12), as follows: (1)G-Multi Primer [GMP]+GCO, (2)Scotchbond Universal Plus+RXU. The resin cements were applied by a bonding jig with a cylindrical mold (Φ=2.38 mm). All specimens were kept in 100% humidity at 37°C for 24 h and then submitted for SBS testing in a universal testing machine(1mm/min). Data were analyzed by Welch's, one-way ANOVA and two independent samples t-tests. The nature of failures was examined under a light microscope. One representative specimen from each group was evaluated for the bonding interface and failure mode using Scanning Electron Microscope.

Results: GCO and RXU showed similar SBS to enamel (p>0.05) and the use of GCO-AEP and SUP resulted in improved SBS(p<0.05). No difference was detected between GCO-AEP+GCO and SUP+RXU. The GCO-AEP+GCO exhibited the highest SBS to dentin(p<0.05), followed by GCOSUP+RXU>RXU(p<0.05). There was no significant difference between SBSs of two resin cements to LiSi blocks(p>0.05). No cohesive failure was observed for both groups.

Conclusions: The use of adhesives prior to the application of self-adhesive resin cements improved their bonding to tooth tissues. GCO demonstrated superior SBS to dentin, whereas both self-adhesive resin cements generated similar SBS to enamel and LiSi glass ceramic surfaces.

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TITLE: Bone-forming Genes Expression of Osteoblasts Cultured on Polymeric Nanosctructured Membranes.

ABSTRACT BODY:

Objectives: To evaluate the effect of polymeric nanostructured membranes in osteoblasts differentiation.

Methods: Nanostructured membranes were produced by electrospinning and functionalized with SiO_2-NPs (NanoMyP). They were then doped with doxycycline by immersion in an aqueous doxycycline solution. The following groups were established: 1) Undoped membranes (HOOC-M), 2) SiO_2-NPs functionalized membranes (HOOC-Si-M), 3) SiO_2-NPs functionalized membranes and doped with doxycycline (Dox-HOOC-Si-M). Membranes were subjected to MG63 osteoblast-like cells culturing (ATCC, Manassas, VA, USA) during 48h. Differentiation was assessed by real-time quantitative polymerase chain reaction (RT-qPCR) and Field Emission Scanning Electron Microscopy. In the RT-qPCR; TGF-β1, Runx-2, ALP, OPG, RANKL and BMP-2 were studied. Three membranes of each group were subjected to each test and both tests were performed in triplicate. Mean comparisons were conducted by one-way ANOVA and Tukey tests ($p < 0.05$).

Results: The RT-qPCR results are in the Table -means (nG of mRNA per nG of House Keeper gene) and standard deviations-. Letters indicate differences between membranes. All the studied genes were overexpressed in the Dox-HOOC-Si-M group, except RANKL which was downregulated, when compared with HOOC-M. The OPG/RANKL ratio, which expresses the bone-building activity of osteoblasts, was up-regulated in 28-fold change by the Dox-HOOC-Si-M when compared with HOOC-M group. The osteoblasts cultured on the modified membranes, showed an elongated spindle-shaped morphology, which has been associated with a more differentiated state.

Conclusions: The functionalization of the polymeric membranes with SiO2-NPs and Dox produced an increase of osteogenic gene expression on cells. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

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TITLE: Cytotoxicity of commercial hyaluronate-based aphthae treatment gels

ABSTRACT BODY:

Objectives: To evaluate the in vitro cytotoxic effects of topical hyaluronic acid-based aphthae treatment gels on human gingival fibroblasts.

Methods: Human gingival fibroblasts were seeded in 48-well plates and grown for 24 hours according to previously described and tested procedures. Four different hyaluronate-based gels (A - Bexident®AFT, B - GUM®AftaClear , C - GENGIGEL®, D - Aloclair®Plus) were tested. Five different gel/culture medium concentrations of each gel were incubated with cells for 15 minutes or 4 hours (n=8). Negative (medium) and positive (DMSO) controls were used. Cell viability was evaluated at 1 and 2 days after exposure, using a resazurin-based assay and results were converted to % of the negative control. Cell morphology was assessed through phase-contrast microscopy (PCM) and alterations were graded according to ISO10993-5:2009(E). Comparisons between groups were made through ANOVA with Tukey post-hoc tests, statistical significance was defined at $p < 0,05$.

Results: In 15-minute evaluation Gels A and B presented higher viability than the other groups ($p < 0.05$) with no cytotoxic effects. Gel C presented intermediate results for lower concentrations and was cytotoxic for 75% dilution. When exposed for 4h, all gels presented cytotoxicity for the 75% dilution. At lower concentrations (50%, 25% and 12.5%) gels A, B and C respectively, recovered viability up to 70%. Gel D had a significant cytotoxic effect at all timepoints and concentrations which was significantly lower than all other groups ($p < 0.05$). PCM images confirmed viability results moderate to severe alterations in cells subjected to Gel D for all times, while for gels A-C only slight to mild changes were observed.

Conclusions: Of the tested products, Bexident®AFT and GENGIGEL® showed low to no cytotoxicity. GUM®AftaClear had intermediate cytotoxicity and Aloclair®, containing aloe barbadensis extract, showed a significantly higher cytotoxic potential in gingival fibroblasts comparing to the other tested gels.

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TITLE: Accuracy of virtually-guided access cavity preparation through Augmented Reality

ABSTRACT BODY:

Objectives: The aim of this study is to evaluate the accuracy of augmented reality (AR) for guided access cavity preparation in 3D printed jaws.

Methods: Two operators with different levels of experience in endodontics performed pre-planned virtually-guided access cavities through markerless AR in 3 sets (upper and lower) of 3D-printed jaw models. The models were mounted on a phantom to mimic a real clinical situation. After treatment, a post-operative high resolution CBCT scan (NewTom, Verona, Italy) was taken for each model and registered to the pre-operative model. All access cavities were then segmented using 3-Matic Medical software 15.0 (Materialise, Leuven, Belgium). For anterior teeth and premolars the distance deviation in mm at the coronal entry point, apical point, and angular deviation of the access cavity was compared to the virtual planning. For the molars the distance deviation in mm at the coronal entry point was compared. Additionally, the surface area of all access cavities at the entry point was measured and compared to the planning. Descriptive statistics for each parameter were performed. A 95% confidence interval was calculated for both operators and each parameter.

Results: A total of 84 teeth and 90 access cavities (45 per operator) were drilled up to 4 mm depth inside the tooth. The mean deviation for the front teeth and premolars at the entry point was 0.51 mm and 0.77 mm at the apical point, with a mean angular deviation of 8.48° and a mean surface overlap of 57%. The mean deviation for molars at the entry point was 0.63 mm, with a mean surface overlap of 82%.

Conclusions: The use of AR for the drilling of access cavities on teeth teeth showed promising results and might have potential for clinical use. However, further development and research is needed before using it in-vivo.

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