HA - UNILEVER Travel Award (Hatton)

HA001 Effect of the isolation technique, donor age and cell passage on the proliferation rate and phenotype of stem cells from dental pulp

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The aim of this study was to investigate the effect of donor age, cell isolation technique and passage number on the proliferation rate and phenotype of dental pulp stem cells (DPSC). Pulp tissue was obtained from six molar teeth of healthy persons (16-60 years of age), and divided into two parts. DPSC were isolated using either enzymatic digestion (ED) or explant (EX) technique. To study the proliferation rate of different cell passages, it was determined the number of days needed for the cells to reach confluence. Immunophenotyping was performed for the 2nd, 5th and 8th passages, by immunofluorescence and flow cytometry analysis using antibodies specific for nestin (+), vimentin (+), CD44 (+), CD146 (+), Oct3/4 (+) and CD34 (-). Data from flow cytometry were subjected to ANOVA and t-tests ($p < 0.05$) and a desirability test was applied using a response optimizer. DPSCs presented a high proliferation rate from passages 2 to 5, while cells from passage 7 proliferated at a slower rate. For all markers, it was observed no statistical difference among passages, irrespective of the technique used or the donor's age. The mean fraction of specific antibody-reactive cells was 75%, 50%, 80%, 45%, 65% and 2% for CD44, OCT, Vimentin, Nestin, CD146 and CD34, respectively. The optimal desirability value, considering all positive markers, was obtained using ED technique and cells from younger subjects (d= 0,92).

It was concluded that neither isolation technique, donor age or cell passage significantly interfered on stem cells phenotype and proliferation rate. (Apoio: FAPESP - 2013/17758-3)

HA002 Novel easy-intracanal-adaptable 3D-Triple antibiotic paste mimic scaffold as an antimicrobial strategy for regenerative endodontics

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A novel three-dimensional triple antibiotic paste mimic scaffold (3D-TAPs) is proposed as a drug-delivery strategy for regenerative endodontics. Polydioxanone (PDS) polymer solution alone and loaded (35 wt.%) with metronidazole, ciprofloxacin and minocycline were electrospun into 3D fibrous scaffolds. The fibers were evaluated via scanning electron microscopy (SEM), mechanical testing, and Fourier-transform infrared spectroscopy (FTIR).Actinomyces naeslundii (ATCC 43146) were centrifuged to induce intra-tubular biofilm formation in a human root dentin slice model (1 mm thickness and 2.5 mm3 canal orifice). The infected slices ($n = 16$) were exposed to 3D-TAPs (~3.3 mg of each drug), TAP solution (50mg/mL of each drug), and pure PDS (drug-free). Biofilm elimination was quantitative and qualitative analyzed by confocal laser scanning microscopy (CLSM) and SEM, respectively. FTIR data demonstrated that the antibiotics were successfully incorporated into the submicron fibers. 3D-TAPs demonstrated significantly lower mechanical properties than PDS ($p \leq 0.040$). A dense penetration of Actinomyces naeslundii biofilm was observed by CLSM throughout the dentinal tubules. 3D-TAPs significantly reduced the percentage of viable bacteria compared to PDS ($p < .05$). TAP solution completely eliminated viable bacteria without differing from 3D-TAPs. SEM images showed similar results to CLSM.

Collectively, the proposed easy-intracanal adaptable 3D-TAPs holds biological and clinical potential as a disinfection strategy prior to regenerative endodontics. (Apoio: NIH grant and CAPES doctoral sandwich scholarship - #UL1 TR001108 and 99999.014311/2013-05)

HA003 Glide path management with two different mechanical systems in curved mandibular molars: anex vivo micro-ct study

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Securing a reproducible glide path prior to instrumentation is recommended to maintain the original geometry of the root canal system and to prevent file separation. Mechanical glide path management systems have been introduced to expedite this step and multi and single file systems are available. The aim of this study was to compare apical transportation, volume increase and working time during glide path management with ProGlider (PG) and PathFiles (PF). Forty curved mesial canals of mandibular molars were evenly allocated into two experimental groups ($n = 20$) according to the glide path management system: PG or PF. Glide path was achieved according to the manufacturers'' protocol. Micro-computed tomographic analysis was performed in order to assess apical transportation at 1, 3 and 5 mm and volume increase. The time required to achieve the glide path was measured. No significant difference was found in apical transportation between the PF and PG in all levels assessed ($p > .05$). Comparisons made among the levels occurred as follows for both groups: 1 mm ≥ 3 mm > 5 mm ($p < .05$). Significant increase in volume occurred after mechanical glide path management in both groups ($p < .05$). No significant difference in volume was found between PG and PF ($p > .05$). Significant difference in the length of time needed for glide path management was found between PG and PF ($p < .0001$).

Both apical transportation and volume increase occurred during glide path management with PG single-file and PF multi-file systems, with no difference between them, however PG achieved glide path faster than PF.

HA004 Transcriptomic analysis of Bifidobacteria in root caries

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Bifidobacteria is regarded as caries-associated organism due to its aciduricity, although little is known about its metabolism in its natural site. This study aimed to evaluate the gene expression profiles of Bifidobacteria present in sound root surface biofilms (SRS) and in root caries (RC). Root surface biofilms were collected from 10 volunteers for the SRS group. Carious dentin was collected from 30 volunteers with active RC. Bacterial RNA was extracted and cDNA libraries were prepared and sequenced. Sequence reads were mapped to 255 strains, including 6 Bifidobacteria (B. kashiwanohense, B. thermophilum, B. breve, B. dentium, B. longum ss. infantis, B. longum). Count data were obtained and normalized to obtain gene expression (GE) and differential expression. Bifidobacteria presented low gene expression in both groups (transcripts= 5.1% and 28.1% in SRS and RC, respectively). B. kashiwanohense had the highest GE for both groups. It was found 1,062 genes with differential expression (foldchange>2; $p < 10\text{-}3$), all of them up-regulated in RC, except for the one that encode alkyl hydroperoxide reductase C in B. longum ss. infantis. Genes that encode 16S ribosomal RNA, TetR family transcriptional regulator, ABC transporter permease and macrolide ABC transporter ATP-binding protein showed the highest differential expression (foldchange>10.0; $p < 10\text{-}3$). There was high differential expression for genes coding for transport proteins.

Results suggest limited functions of bifidobacteria in root surfaces biofilms in both, carious and sound surfaces. A higher gene expression in root caries than in sound root surfaces biofilms were observed. (Apoio: Leeds Teaching Hospitals Charitable Foundation and Dunhill Medical Trust - R&D/PP/12011)

HA005 The extracellular matrix of fluconazole-susceptible and -resistantCandida albicans and Candida glabrata biofilms

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Infections caused by Candida spp. biofilms are worrying, especially in immunocompromised individuals. The resistance of Candida spp. biofilms to medications may be associated with the protective effect of the extracellular matrix (ECM) components. The aim of this study was to characterize the biofilms ECM of fluconazole-susceptible and -resistant Candida albicans and Candida glabrata strains. Single-species biofilms of C. albicans ATCC 90028 (fluconazole-susceptible- CaS), C. albicans ATCC 96901 (fluconazole-resistant- CaR), C. glabrata ATCC 2001 (fluconazole-susceptible- CgS) and C. glabrata ATCC 200918 (fluconazole-resistant– CgR) were grown in RPMI medium at 37º C for 48 h. Biofilms were processed for evaluation of colony forming units (CFU), total dry weight, and ECM components (water-soluble polysaccharides, alkali soluble polysaccharide, proteins and extracellular DNA - eDNA). Data were statistically analyzed by one-way ANOVA with Games-Howell post-hoc test ($\alpha = 0.05$). Significant differences were found for CFU ($p < 0.001$): CaS (6,84±0,20 log10), CaR (7,06±0,14 log10), CgS (7,25±0,19 log10), CgR (7,69±0,19 log10). No significant differences ($p > 0.05$) were observed for the other parameters tested. Interestingly, the most prevalent ECM component was eDNA (~22.7 µg/mg of total dry weight).

The biofilms ECM of fluconazole-susceptible and -resistantC. albicans and C. glabrata strains are similar, and eDNA is its major component. eDNA may confer negative charge to the ECM, thus, should be considered when designing biofilm control strategies.

HA007 1H NMR-based metabolomics from children with chronic kidney disease before and after hemodialysis: saliva, serum and plasma analysis

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This study evaluated the low molecular weight metabolites in the serum, plasma and saliva from children with chronic kidney disease (CKD) before and after hemodialysis, using the metabolomic strategy. Thirty patients with CKD undergoing to hemodialysis therapy (12.3 years old ±3.5) were recruited (HD). Blood and saliva were collected before (HD-B) and after (HD-A) hemodialysis. For the healthy group, 40 subjects (13.03 years old ±3.2) were selected, only saliva was collected. Dental caries and calculus were evaluated.1H-NMR spectra of biofluids were acquired and processed on a Bruker 600 MHz Advance spectrometer. The Partial Least Squared discriminant analysis (PLS-DA) and multilevel PLS-DA (M-PLS-DA) were used for multilevel statistical analyses, $\chi 2$ for clinical data, the Mann-Whitney and Wilcoxon tests ($p < 0.05$) to evaluate each metabolite. The caries status was similar between the healthy (dmft 0.67±2.1, DMFT 0.90±1.7) and HD (dmft 0.87±2.2, DMFT 0.79±1.3) groups ($p = 0.49$, $\chi 2$). HD group presented higher prevalence of calculus ($p < 0.001$). The M-PLS-DA distinguished the metabolomics profile between HD-B and HD-A, PLS-DA showed slight difference between saliva from HD-A and healthy group. There was a reduction in the levels of creatinine in blood and saliva after the hemodialysis ($p < 0.05$). Metabolites such as choline, TMAO, and urea also reduced in blood after HD, and presented a slight decreasing in saliva.

It is possible to conclude that HD procedure change the metabolites in blood and similar alterations were observed in saliva. (Apoio: CNPq)