299

## Mechanical properties of model composite resin containing nanosilver filler

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**Purpose:** Previous studies have successfully added nanosilver particles in commercial restorative composites to inhibit the growth of *Streptococcus mutans* and Lactobacillus. The aim of the present study was to evaluate the flexural strength (FS), flexural modulus (E), Knoop hardness (KH) and changes in surface roughness (Ra) and gloss after simulated toothbrushing of model composites resins containing nanosilver filler.

**Methods and materials:** A co-monomer blend of bis-GMA diluted in a 80:20 ratio with TEGDMA was used as model resin matrix. The photoinitiator system was composed by 1 mol% of camphorquinone, 2 mol% of ethyl-4-dimethylamino benzoate and 0.5 mol% of diphenyliodonium hexafluorphosphate. Hydroxy butyl toluene (0.1 mol%) was used as inhibitor. The composite resins were loaded according to the following groups: Control – 60 wt% of silanated Ba-Al-Si glass fillers; G1 – 59.94 wt% Ba-Al-Si glass fillers + 0.06 wt% of nanosilver fillers; G2 – 59.875 wt% Ba-Al-Si glass fillers + 0.125 wt% nanosilver fillers; G3 – 59.75 wt% Ba-Al-Si glass fillers + 0.25 wt% nanosilver fillers; G4 – 59.5 wt% Ba-Al-Si glass fillers + 0.5 wt% nanosilver fillers; G5 – 59.0 wt% Ba-Al-Si glass fillers + 1 wt% nanosilver fillers; and G6 – 58.0 wt% Ba-Al-Si glass fillers + 2 wt% nanosilver fillers. The FS and E tests were performed in a universal testing machine with a crosshead speed of 0.5 mm/min ($n = 10$); KH was performed with a load of 25 gf during 15 s ($n = 8$). Ra was measured with contact profilometer and the Gloss ($n = 8$) with a glossmeter before and after simulated toothbrushing with toothpaste slurry, load of 150 g and frequency of 4 Hz during 2 h. The data of E and KH were submitted to one-way ANOVA; FS data were compared by Kruskal–Wallis. The data of Ra and Gloss were analyzed by repeated-measure two-way ANOVA and t-test for paired specimens.

**Results:** Significant differences were not found among the groups for FS ($p = 0.377$), E ($p = 0.660$) and KH ($p = 0.542$). However, toothbrushing had a negative influence on the Ra and Gloss results of all tested groups ($p < 0.05$).

**Conclusion:** The incorporation of nanosilver fillers had no influence on the mechanical properties of the tested model composites.

*Keywords*: Resin composites; Filler particles; Physical and chemical properties

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300

## Effects of the exposition to dental alloys in human fibroblasts

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**Purpose:** Cast alloys used in dentistry are common source of metal ions released into the gingival and oral mucosa, which in turn often cause gingival and periodontal inflammation. The aim of this study was to evaluate effects of exposure to orthodontic materials on transglutaminases in cultured human gingival fibroblasts.

**Methods and materials:** The human gingival fibroblast cell line, HGF-1 (CRL 2014), was obtained from American Type Culture Collections. The incubation with Ni–Ti heat-activated (T3) or Ni–Ti super-elastic (T4), and with Ni–Cr–Co (T2) alloys produced respectively 2.5-fold and 8-fold increases in IL-6 release compared with control cultures. The biocompatibility of the five orthodontic samples after 24–48 h of incubation with the release medium from cast alloys was tested by MTT reduction assay. IL-6 release from treated and untreated cultures was measured using an ELISA test and confocal laser scanning microscopy and western blot analysis was performed to evaluate transglutaminases activity.

**Results:** A not significant loss of cell viability was observed in fibroblast cultures exposed for 24–48 h to the medium containing the release products of ceramics (Ce) and Ni–Ti (T1, T3, T4) orthodontic alloys compared to control cultures, as monitored by MTT reduction assay. The Ni–Cr–Co (T2) alloy was the most toxic material tested. Transglutaminases activity was significantly increased in cells exposed to T3 and T4 alloys (about 170% of control; $p < 0.05$), where it was mainly localized close to inner part of cell membrane. The exposure to T3 and T4 specimens significantly up-regulated also tTG expression compared with control cultures.

**Conclusion:** This study demonstrated that transglutaminases activity is significantly elevated in fibroblasts exposed to Ni–Ti alloys. These effects were associated with IL-6 release and were dependent on the presence of different elements in the cast alloy. It was shown an association between IL-6 release and tissue transglutaminases increases, suggesting that TGase-mediated reactions may play a major role in peri-

odontal inflammation.

*Keywords*: Transglutaminases; Gingiva; Fibroblast

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301

## Cytokine secretion screening of odontoblasts treated with chlorhexidine and epigallocatechin-gallate

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**Purpose:** Cytokines are small and potent signaling proteins secreted by a range of cells in response to diverse types of stress. The aim of this study was to screen the effect of two compounds, chlorhexidine diacetate (CHX) or epigallocatechin-gallate (EGCg), on the secretion of cytokines by odontoblast-like cells (MDPC-23).

**Methods and materials:** Odontoblast-like cells were seeded for 48 h with a serial dilution of the compounds to determine cell metabolic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay ($n = 3$). At tolerable compound levels determined by MTT, total protein concentration was measured using a Pierce bicinchoninic acid (BCA) assay ($n = 3$), and cytokine secretions were analyzed using the Bio-Plex Pro mouse cytokine 23-plex assay ($n = 2$). Data were calculated by 1-way ANOVA and Tukey's test ($a = 5\%$).

**Results:** The MTT revealed that CHX or EGCg did not reduce cell metabolic activity at concentrations of 2.5–20 μM (CHX) and 2.5–160 μM (EGCg), respectively ($p > 0.05$). Total protein levels were consistent across all groups at 20 μM compound dilution (cells cultured without the compounds: 1.04 mg/mL; CHX: 0.98 mg/mL; and EGCg: 1.06 mg/mL), indicating that cell growth was uniform ($p > 0.05$). At 20 μM dilution, both CHX and EGCg significantly increased the secretion of IL-1b, IL-10, IL-12, TNF-α, MIP-1α, KC, Rantes, IFN-γ and GM-CSF ($p < 0.05$), and significantly reduced the secretion of IL-17 ($p < 0.05$). EGCg induced an increase on the secretion of IL-2, IL-6 and Eotaxin, and CHX induced an increase of IL-4 ($p < 0.05$).

**Conclusion:** Both CHX and EGCg had various effects on cytokine secretion of odontoblast-like cells. The findings may provide insights into mechanisms of dental tissue remodeling, as inflammatory signaling may relate to tooth tissue repair. For instance, the cytokine TNF-α (tumor necrosis factor α) was shown to stimulate dental pulp stem cell differentiation towards an odontoblast-like cell phenotype, promoting mineralized secretory activity.

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302

## Transdentinal cytotoxicity of glutaraldehyde-containing solutions/materials

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**Purpose:** The treatment of acid-etched dentin with glutaraldehyde (GA) may increase collagen mechanical properties, reduce MMP activity and slow down resin-dentin bond degradation. However the possible cytotoxicity of this organic compound is still disquieting. Therefore, this study aimed to investigate the transdentinal cytotoxicity of glutaraldehyde-containing solutions/materials on odontoblast-like cells.

**Methods and materials:** Thirty-six 0.4 mm-thick dentin disks obtained from sound human molars were adapted to artificial pulp chambers. Using DMEM, odontoblast-like MDPC-23 cells were seeded on the pulpal surface of the disks ($3 \times 10^4$ cells) and incubated for 48 h. Then, the occlusal dentin surface was acid-etched and treated with the following solutions ($n = 6$): deionized water (control), 2% glutaraldehyde (GA), 5% GA, 10% GA, Gluma Comfort Bond + Desensitizer (GCB + De) or Gluma Desensitizer (GDe). Cell viability and morphology were assessed by Alamar Blue assay and SEM, respectively. The eluates (DMEM + products that diffused through the disks) were collected and applied on MDPC-23 cells previously seeded in wells of 24-well plates. After 7 or 14 days in contact with the eluates, it was assessed the total protein (TP) production, alkaline phosphatase activity (ALP) and deposition of mineralized nodules by the MDPC-23 cells. Data were analyzed by Kruskal–Wallis and Mann–Whitney tests ($p < 0.05$).

**Results:** The highest reduction in cell viability was observed for GCB + De (85.1%) and GDe (77.2%), as well as a significant reduction in total protein production and ALP activity in both 7- and 14-day periods. After 14 days, the cells exposed to GCB + De and GDe produced significantly less mineralized nodules. The cytotoxic effects of these materials were confirmed by the cell morphological analysis. Affected MDPC-23 cells presented deformation of the cytoskeleton (round-shaped cells) and significant reduction of cellular projections.

**Conclusion:** The treatment of acid-etched dentin with 2.5%, 5% and 10% GA was not harmful to odontoblast-like cells. Conversely, when GA was combined with other components like HEMA, the final glutaraldehyde-containing material became cytotoxic.

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*Keywords*: Cytotoxicity; Glutaraldehyde; Odontoblast-like cells

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