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Cellular differentiation, bioactive and mechanical properties of experimental light-curing pulp protection materials

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ABSTRACT

Objective. Materials for pulp protection should have therapeutic properties in order to stimulate remineralization and pulp reparative processes. The aim of this study was to evaluate the mechanical properties, biocompatibility, cell differentiation and bioactivity of experimental light-curable resin-based materials containing bioactive micro-fillers.

Methods. Four calcium-phosphosilicate micro-fillers were prepared and incorporated into a resin blend: 1) Bioglass 45S5 (BAG); 2) zinc-doped bioglass (BAG-Zn); 3) βTCP-modified calcium silicate (β-CS); 4) zinc-doped β-CS (β-CS-Zn). These experimental resins were tested for flexural strength (FS) and fracture toughness (FT) after 24 h and 30-day storage in simulated body fluid (SBF). Cytotoxicity was evaluated using MTT assay, while bioactivity was evaluated using mineralization and gene expression assays (Runx-2 & ALP).

Results. The lowest FS and FT at 24 h was attained with β-CS resin, while all the other tested materials exhibited a decrease in FS after prolonged storage in SBF. β-CS-Zn maintained a stable FT after 30-day SBF aging. Incorporation of bioactive micro-fillers had no negative effect on the biocompatibility of the experimental materials tested in this study. The inclusion of zinc-doped fillers significantly increased the cellular remineralization potential and expression of the osteogenic genes Runx2 and ALP ($p < 0.05$).

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Significance. The innovative materials tested in this study, in particular those containing β-CS-Zn and BAG-Zn may promote cell differentiation and mineralization. Thus, these materials might represent suitable therapeutic pulp protection materials for minimally invasive and atraumatic restorative treatments.

1. Introduction

The operative treatment of deep carious lesions can be challenging especially when approaching the pulp as the increased risk of pulp exposure during excavation can reduce the probability of pulp survival [1]. An ideal pulp protection material for such scenarios should be highly biocompatible and bioactive [2,3]. This is especially relevant with the contemporary minimally invasive restorative philosophy where contaminated (caries-infected) dentin is removed from deep cavity selectively, retaining most of the demineralized but repairable (caries-affected) dentin for potential remineralization as well as avoiding pulp exposure [4,5]. Indeed, clinicians are increasingly relying on restorative ion-releasing materials such calcium silicate cements to seal the restored interfaces in order to help remineralize the caries-affected tissues [6].

In certain clinical cases, indirect and/or direct pulp protection may help maintain pulp sensibility by facilitating healing/repair. Materials used for this purpose should interact with the pulp cells to stimulate the formation of reparative dentin [7–9]. Calcium silicate and MTA-like cements have been used as they can encourage remineralization and dentin bridge formation with no/minimal inflammatory pulp response [9–11]. However, their use as a bio-interactive restorative material is limited due to shortcomings in their mechanical properties, setting time and dissolution rate [12,13]. It is hypothesized that the formulation of resin-modified bioactive cements might present a solution to combine the best of both resin-based technology with the bioactivity of such cements. Indeed, resin-modified calcium silicate cement–based materials such as Theracal LC (BISCO, Chicago, IL, USA), a light-curable material advocated for direct and indirect pulp protection, showed greater compressive and flexural strengths compared to conventional calcium silicate cements, being more able to resist fracture during immediate placement of a definitive overlying restoration [14]. Nevertheless, such a resin-modified calcium silicate cement–based material demonstrated a reduction in cellular metabolism and protein expression. It was also exhibited greater cytotoxicity compared to conventional calcium silicate cements [15].

Sodium calcium phosphosilicates (e.g. Bioglass 45S5, BAG) have been used successfully in orthopedics as regenerative materials for bone [8,16]. In particular, BAG has been shown to induce calcium-phosphate precipitation, subsequently converting to hydroxyapatite-like crystallites [8,17]. Within dentistry, BAG is used in toothpastes and also as powders for dental air-abrasion/polishing to remineralize the dental hard tissues as well as treating dentin hypersensitivity [18,19]. Bioactive glasses are so called due to their in loco remineralization of tissues, but they cannot be used in as a definitive dental restorative material unless incorporated into a resin-based matrix [20,21].

The aim of this study was to evaluate the mechanical properties, biocompatibility, cell differentiation and bioactivity of experimental light-curable resin-based materials containing bioactive micro-fillers for their potential use as indirect pulp protection materials, after storage in simulated body fluid (SBF). The mechanical properties were assessed through the evaluation of their flexural strength (FS) and fracture toughness (FT). The cytotoxicity of the tested materials was tested using MTT assay, while cell differentiation and mineralization were assessed using gene expression assays (Runx-2 and ALP). The bioactivity of the tested materials was evaluated using Raman spectroscopy and scanning electron microscopy (SEM). The null hypotheses tested in this study were that the addition of bioactive micro-fillers: 1) would have no effect on mechanical properties of the experimental resin-based materials tested; 2) would neither increase cytotoxicity nor induce differentiation in primary human mesenchymal stem cells (MSCs).

2. Materials & methods

2.1. *Formulation of the resin-based bioactive materials*

A control filler-free resin (RES) was made using three hydrophobic monomers (55 wt% urethane dimethacrylate and, 4.5 wt% bisphenol A diglycidildimethacrylate, 10.5 wt% triethylene glycol dimethacrylate, Sigma–Aldrich, Gillingham, UK), 20 wt% 2-hydroxyethyl methacrylate (Sigma–Aldrich), 8.5 wt% absolute ethanol (Sigma–Aldrich) and a photo-initiating complex comprising 0.5 wt% camphorquinone/1.0 wt% ethyl 4-dimethylaminobenzoate (Sigma–Aldrich). The experimental ion-releasing resins were formulated using 40 vol% micro-filler and 60 vol% resin blend [21]. Four experimental light-curable resin-based materials containing tailored bioactive micro-fillers were formulated as described by Sauro et al. [21]. In brief, Bioglass 45S5 (BAG) micro-filler (<20 μm size) was sintered and incorporated within the composition of the control light-curable resin blend. The second micro-filler, BAG-Zn (<20 μm), was created by modifying the composition of the BAG with 20 wt% zinc oxide (ZnO: Sigma–Aldrich) and a 10 wt% polycarboxylic acid solution (PAA: M_w 1800; Sigma–Aldrich) and finally incorporated into the light-curable resin blend. The third micro-filler (β-CS) was formulated by modifying the composition of a type I ordinary Portland cement (OPC: Italcementi Group, Cesena, Italy) by adding 10 wt% β-tri-calcium phosphate [βTCP: $Ca_3(PO_4)_2$ (Sigma–Aldrich)]. The cement was mixed in deionized water (ratio 2:1 powder/liquid) and allowed to set

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