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Thermo-setting glass ionomer cements promote variable biological responses of human dental pulp stem cells

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ABSTRACT

Objective. To evaluate the *in vitro* cytotoxicity of Equia Forte (GC, Tokyo, Japan) and Ionostar Molar (Voco, Cuxhaven, Germany) on human dental pulp stem cells (*h*DPSCs).

Methods. *h*DPSCs isolated from third molars were exposed to several dilutions of Equia Forte and Ionostar Molar eluates (1/1, 1/2 and 1/4). These eluates were obtained by storing material samples in respective cell culture medium for 24 h (n = 40). *h*DPSCs in basal growth culture medium were the control. Cell viability and cell migration assays were performed using the MTT and wound-healing assays, respectively. Also, induction of apoptosis and changes in cell phenotype were evaluated by flow cytometry. Changes in cell morphology were analysed by immunocytofluorescence staining. To evaluate cell attachment to the different materials, *h*DPSCs were directly seeded onto the material surfaces and analyzed by scanning electron microscopy (SEM). The chemical composition of the materials was determined by energy dispersive X-ray (EDX) and eluates were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). Statistical analysis was performed with analysis of variance (ANOVA) and Student's t-test ($\alpha < 0.05$).

Results. Undiluted Equia Forte extracts led to a similar cell proliferation rates than the control group from 72 h onwards. There were no significance differences between Equia Forte and Ionostar Molar in terms of cell apoptosis and phenotype. However, in presence of Equia extracts the migration capacity of *h*DPSCs was higher than in presence of Ionostar Molar ($p < 0.05$). Also, SEM studies showed a higher degree of cell attachment when Equia Forte extracts were used. Finally, EDX analysis pointed to different weight percentages of C, O and Ca ions in glass ionomer cements, while other elements such as La, Al, Si, W, Mo and F were also detected.

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Significance. In summary, Equia Forte promoted better biological responses in *h*DPSCs than Ionostar Molar.

1. Introduction

Glass ionomer cements (GICs) are dental restorative materials, which clinical use has increased significantly during the last years [1]. Their clinical use ranges from luting cements, cavity liners or bases under composites, to restore Class V cavities, small Class I cavities, deciduous teeth, long-term temporaries and core build-ups [2,3].

The biocompatibility of novel materials such as GICs is very important due that they come into contact with dental tissues when compacting the filling core material. The tissue response to the GICs may influence the success of the dental treatment. In an attempt to find an ideal GIC for each clinical situation, the GIC composition has become a key element for their classification into conventional, metal-reinforced, high viscosity and resin-modified [4]. Most of them have shown inadequate biological activity and have been exhibited a variable degree of toxicity depending on their chemical composition in several studies [4,5].

Recently, new GICs called Thermo-setting GICs have been developed such as Equia Forte (GC, Tokyo, Japan) and Ionostar Molar (Voco, Cuxhaven, Germany). Equia Forte uses a radiant heat to accelerate the set. Equia Forte is a complete glass ionomer based, bulk-fill, rapid restorative system, that is easy to use and allows a quick placement [6]. Ionostar Molar is a glass ionomer restorative material, distributed in capsules. The material is applied without conditioner or adhesive and scores particularly highly thanks to its non-sticky consistency and easy manipulation [7]. However, there is not yet evidence about their biocompatibility.

Cytotoxic studies represent an useful tool in order to assess the biological effects of new materials on different type cells. In fact, previous studies have shown that monomers and other derivatives can diffuse into pulp tissue through open dentinal tubules as a result of the capillary action and osmotic pressure of dentinal fluid, potentially inducing adverse effects in pulp tissue [8,9]. Some studies have focused on the cytotoxicity of GICs on fibroblasts and odontoblast [10,11]. However, there is no information available about the biological effects of GICs on human dental pulp stem cells.

Human dental pulp stem cells (*h*DPSCs), which are involved in dentin regeneration, are adversely affected by the chemicals present in glass ionomers which may induce cell death or cause alterations in cell viability, involving numerous pro-inflammatory cytokines/chemokines, which increase the likelihood of irreversible pulp inflammation [12,13].

The aim of the present study was to compare and assess the cytotoxicity of two glass ionomer-based materials: Equia Forte and white Ionostar Molar in contact with human dental pulp stem cells. The null hypothesis was that there would be no significant cytotoxic effects between the materials.

2. Materials and methods

2.1. Glass ionomer material extracts

The materials tested in this study were Equia Forte (GC, Tokyo, Japan), which has a powder/liquid ratio equals to 3.08 g/mL and Ionostar Molar (Voco, Cuxhaven, Germany), whose powder/liquid ratio is 2.50 g/mL. Their complete compositions are described in Table 1.

The materials were mixed for 10 s according to the manufacturers' instructions. Forty discs of each material were shaped under aseptic conditions in sterile cylindrical rubber molds, 1.5-cm in diameter and a 0.2 cm in height and pressed by a glass slide for 6 min at room temperature. After, discs were sterilized using ultraviolet irradiation for 15 min and stored in an incubator at 37 °C for 48 h to achieve their complete setting. To simulate the clinical situation where cells are in contact with the glass ionomer materials, we obtained extracts or eluates of the materials, according to the International Standard ISO 10993-5. The eluates of the different materials were extracted in sterile conditions, using DMEM culture medium (Gibco, Gaithersburg, MD, USA) as extraction vehicle. The extraction procedure was as follows; the materials were immersed in the culture medium for 24 h at 37 °C in a humid atmosphere containing 5% CO_2. In accordance with the International Organization for Standardization (ISO) standards, the ratio between the surface of the sample and the volume of the medium was 1.5-cm^2/mL. The extraction media was collected at the end of this period and passed through a 0.22-μm syringe filter (Merck Millipore, Billerica, MA, USA). Then, in order to study the effect of concentration of each material, various dilutions (1/1, 1/2 and 1/4 vol/vol) of these extraction media were prepared using fresh complete DMEM medium.

2.2. Assessment of pH, osmotic pressure and Inductively coupled plasma-mass spectrometry (ICP-MS) of extracts

Three discs from each material type were immersed in 5 mL of the milli-Q water to measure the pH, the osmolality and ion leaching. The pH was measure before and after soaking the specimen for 24 h by using a pH-meter (GLP21+, Crison, Barcelona, Spain) and the results are expressed as the mean ± standard deviation. The osmolality of 10 μL of the fresh MilliQ water and the MilliQ water after immersion of the test materials was determined by using an osmometer (OsmometerVapro 5520, Wescor, Utah, USA). These values are expressed as mmol/kg. Finally, the analysis of the presence of silicon, phosphorus, calcium and strontium was assessed using inductively coupled plasma-mass spectrometry techniques (ICP-MS- Agilent 7900, Stockport, UK).

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