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Influence of a bioceramic root end material and mineral trioxide aggregates on fibroblasts and osteoblasts

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ABSTRACT

Objective: The biocompatibility of materials used in endodontic treatment is of high importance, because they can come in contact with periradicular tissues and there is a risk of possible systemic toxicity. The aim of the present study was to investigate the in vitro reaction to a bioceramic based root end material in comparison to mineral trioxide aggregates (MTA) as the established gold standard.

Design: The root end materials grey MTA Angelus (GMTA), white MTA Angelus (WMTA), ProRoot MTA, and EndoSequence Root Repair Material (ERRM) were incubated with human periodontal ligament fibroblasts and osteoblasts (10^4 cells/ml) for up to 96 h. Cell proliferation (RFU) was determined by means of the Alamar Blue assay. In addition, fluorescence staining was carried out to visually monitor cell growth and morphology.

Results: For most of the observational time period of up to 96 h, there was no statistically significant difference between the proliferation rates of the control cells and those in contact with ERRM. In contrast, the mineral trioxide based materials caused from 24 to 96 h significantly lower proliferation rates in comparison to the controls ($p < 0.001$). For proliferation rates of cells in contact with MTAs and ERRM significant differences were observed throughout the whole observation time for the osteoblasts, but only up to 24 h for the human periodontal ligament fibroblasts.

Conclusion: Within the limits of this study the results suggest that the bioceramic root end material is biocompatible, but needs to be investigated in clinical studies before it can be recommended as retrograde sealer in endodontic practice.

1. Introduction

Mineral trioxide aggregate (MTA) is widely used in endodontic treatment for indications such as apical root-end surgery, perforations, pulp capping or root-end fillings.[1–3] While MTA is considered to be the gold standard for the above mentioned indications,[4] new root end filling materials with similar properties, mainly composed of zirconium oxide, calcium silicates, calcium phosphate and calcium hydroxide, have been developed. MTA has received favourable reports in the literature[5–7]; yet, these new materials have been mainly designed to reduce some short-comings, such as prolonged setting time and difficult manipulation of MTA.

In general, dental materials can be distinguished by parameters such as pH, solubility, setting time, radiopacity

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and compressive strength.[4] For the clinical application variables such as adherence to dentine, adequate working and setting time, easy manipulation, a sufficient seal and long-term stability are required, which is the case also for conventional root canal sealers.[8] When using dental materials in humans, a sufficient biocompatibility is essential, and thus a detailed analysis of their respective genotoxicity and cytotoxicity has to be considered prior to clinical application. The term biocompatibility has been redefined as the ability of a material to perform with an appropriate host response in a specific situation.[9]

Many in vivo studies of the biocompatibility of MTA have been carried out.[1,5,10,11] Torabinejad et al. have described MTA as a valuable addition to the restorative materials presently used in endodontics.[12] A number of in vitro and in vivo studies have shown that MTA prevents microleakage, is biocompatible, and promotes tissue regeneration when placed in contact with the dental pulp or periradicular tissues.[5–7,11] However, so far there are relatively few studies comparing the biocompatibilities of the root end material EndoSequence Root Repair Material (ERRM) and mineral trioxide based materials as the established gold standard.[10,11,14–17]

The biocompatibility of the root end material ERRM is discussed controversially in the literature. While in one study the authors claim that the cytotoxicity of ERRM Paste is comparable to that of MTA,[18] Ma et al. observed a slightly lower cell viability with fresh samples of ERRM than with MTA.[13]

After investigating the same materials in a similar study design, Damas et al. report contradictory results.[11] They found no statistically significant differences in cell viability of human dermal fibroblasts after exposure to ERRM paste and other mineral trioxide based materials; however, they report a negative influence of EndoSequence Root Repair Putty.

The biocompatibility and cytotoxicity of EndoSequence is discussed controversially. The aim of the present study was to investigate the biocompatibility and cytotoxicity of this material in comparison with established mineral trioxide based materials, using as in vitro model cell cultures of human periodontal ligament fibroblasts and human osteoblasts.

2. Materials and methods

2.1. Cell culture

Clonetics® HPdLF (human periodontal ligament fibroblasts) and human osteoblasts were purchased from Promocell, Heidelberg, Germany. The cells were passaged at a 1:2 split ratio following trypsinization with 0.05% trypsin/0.02% EDTA (Lonza, BioWhittaker, Kerviers, Belgium). The fibroblasts were cultured in Dulbecco's Modified Eagle Medium (Gibco/Life technologies, Paisley, UK), supplemented with 10% foetal bovine serum, 2 mM L-glutamine and 100 U/100 μg/ml penicillin/streptomycin (Invitrogen, Germany), the osteoblasts in Osteoblast Growth Medium C-27001 and SupplementMix C-39615 (Promocell, Heidelberg, Germany), and incubated at standard cultivation conditions.

2.2. Root end materials

EndoSequence Root Repair Material paste (Brassler, Savannah, USA) is composed of zirconium oxide, calcium silicates, monobasic calcium phosphate, tantalum oxide, proprietary fillers and thickening agents. Premixed and available as an injectable form or in putty consistency, this is a highly radiopaque and hydrophilic material with a particle size of less than 2 μm[17] and a unique composition that allows bonding to dentine.

ProRoot® MTA (Dentsply/Maillefer, Ballaigues, Switzerland) is mainly composed of calcium silicates, calcium sulfate, bismuth oxide and tricalcium aluminate. This powder sets in the presence of water, creating a colloidal gel that solidifies to form a strong impermeable barrier. It is indicated for different clinical applications such as repair of root perforations/resorption, pulp capping, apexification and root-end filling.

MTA-Angelus (Angelus, Londrina, PR, Brazil) is composed of similar mineral oxides like tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium sulfate and bismuth oxide. The difference between grey MTA-Angelus (GMTA) and white MTA-Angelus (WMTA) is the amount of iron oxide (Fe_2O_3), which is responsible for the grey colour in the material and which is reduced in WMTA. This reduction does not interfere with any properties of WMTA.

All root end materials were mixed according to the manufacturers' recommendations.

For the cell culture experiments, the freshly mixed materials (1.5 mg/well) were placed into multi-well cylinders (16 mm diameter; Greiner Bio-One, Frickenhausen, Germany). The amount of root end materials was determined according to previous experiments by weighing them with an analytical balance (Pioneer PA64, Ohaus, Pine Brook, USA). All materials were kept for 24 h at room temperature to allow them to set.

2.3. Alamar Blue® cell viability reagent

The Alamar Blue® assay (Biozol, Eching, Germany) was used to determine the influence of the different root end materials on the viability and proliferation of human periodontal fibroblasts or human osteoblasts. After the materials had set, they were incubated with human periodontal fibroblasts (10^4 cells/ml, $n = 6$ for each material) or with osteoblasts (10^4 cells/well, $n = 6$ for each material). Cells without root end material served as control ($n = 6$ per cell-line), amounting to an overall sample size of 60 (30 per cell line). After 6, 24, 72 and 96 h of incubation with 10% Alamar Blue®, the fluorescence was measured at a wavelength of 560/20 and 620/40 nm with a fluorescence reader (Synergy HT-Reader, Biotek, Winooski, VT, USA) and analysed with Gen 5 (BioTek, Winooski, VT, USA). Logarithmic signals were converted to a linear scale and expressed as relative fluorescence units (RFU).

2.4. Cell visualization

After 24 h the cells were stained for visualization with the fluorescent dyes phallacidin (BODIPY® FL phallacidin; Invitrogen, Oregon, USA) and DAPI (Roche Diagnostics GmbH, Mannheim, Germany) and viewed with an inverted