Novel Bioactive Calcium Phosphate based endodontic cements with added Hydroxyapatite nanoparticles and antibacterial agent ε-Polylysine

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Introduction

Regenerative endodontic procedures (REPs) aim to regenerate the pulp–dentin complex in immature permanent teeth diagnosed with pulp necrosis. They are Divided into:
1. Vital pulp therapy: Aims to maintain the dental pulp’s vitality and stimulate regeneration of the pulp complex
   - Includes indirect and direct pulp capping/pulpotomy
2. Revascularisation: Novel approach for teeth with irreversible pulpitis or necrotic pulp and open apices
   - Involves inducing bleeding to provide a scaffold for stem cells to attach, proliferate, and differentiate into the vital components of the pulp–dentin complex

Calcium phosphate-based materials

<table>
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<tr>
<th>MTA</th>
<th>Biodentine</th>
<th>Dycal</th>
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</table>
| Biocompatible | - Bioactive | - Bio-interactive (releases biologically relevant ions (Ca, P))
| Two of its Different phases are
| Brushite: Initial fast degradation rate by dissolution and releasing Ca and P
| Can form hydroxyapatite by phase transformation resulting into a slower biodegradation
| Hydroxyapatite: Main constituent of the inorganic matrix of Enamel and Dentine (96% wt and 70% wt respectively)
| -Chemotactic ability: Mediator in cell-to-cell interaction,
| -Encourages pulpal fibroblasts to release alkaline phosphatase
| -Promotes repair by pulpal calcification and osteoid deposition
| -Osteo-conductive: Encourages the differentiation and recruitment of Osteoblasts.

ε-Polylysine

- FDA approved natural homopolymer
- Deemed GRAS antimicrobial agent
- Potent Broad spectrum antibiotic and antifungal including MRSA

Aim

To improve the bioactivity of a Brushite forming novel calcium phosphate cement, by addition of Hydroxyapatite (HA) nanoparticles in the presence of ε polylysine (PLS) as an antibacterial agent and comparing it to Mineral Trioxide Aggregate (MTA), Biodentine and Dycal.

Materials & Methods

I. Preparation of Brushite Formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Powder : Liquid ratio 4:1</th>
<th>Powder</th>
<th>Liquid</th>
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<tbody>
<tr>
<td>Powder: equimolar Monocalcium phosphate monohydrate (MCPM) and β-Tricalcium Phosphate</td>
<td>TCP</td>
<td>Polylysine</td>
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<td>Modification: β-TCP substitution by HA nanoparticles (25 µg or 50 µg wt%)</td>
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⋅ = Liquid: solution of 80 mM citric acid
⋅ Modification: Addition of ε-PLS (20 or 40 wt%)

Formulations were compared to 3 commercial alternatives, MTA, Biodentine and Dycal

II. Setting Kinetics and chemistry

Cement discs were prepared using washer moulds diameter 10mm & thickness 1mm

II-Physical & Mechanical Properties

1. Biaxial flexural strength using a ball on ring jig in dry and wet conditions n=6
2. Dissolution kinetics was analysed gravimetrically after immersion in deionized water for up to 4 weeks n=3

IV- Biocompatibility in vitro

Culture of Human dental pulp stem cells (hDPSCs)

- Different dilutions of eluates were prepared from discs of all experimental and commercial formulations (1:2, 1:4, 1:8, 1:16)
- Cells were cultured and incubated for 1, 3, and 7 days with different dilutions
- Cell proliferation was assessed by MTT assay
- To assess direct adhesion, cells were cultured on the discs in direct contact with the surface of each material then analysed by:
  - Scanning Electron Microscopy (SEM)
  - Live/dead immunofluorescence staining
- Cells were cultured for 14 days in dilution 1:4
  - Calcification assessed by Alizarin red staining
  - Alizarin red staining D14

Results

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<tr>
<th>F1</th>
<th>F2</th>
<th>F3</th>
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<tr>
<td>25</td>
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Discussion & Conclusion

- CaP based cement formulations have a faster setting time compared to MTA and Biodentine.
- Early Brushite dissolution was accelerated by the increase in ε-PLS concentration, unlike commercial cements that initially increased in mass before dissolution.
- Addition of ε-PLS reduced the flexural strength of the CaP formulations, yet they remain significantly higher than tested commercial cements even after 24hr submersion in deionised water.
- CaP based formulations modified with HA increased the hDPSCs proliferation rate, viability and adhesion to its surface compared to HA free formulations and commercial cements.
- Addition of Hydroxyapatite nanoparticles improved the bioactivity of the Brushite formulations as evident by the earlier calcific deposition by hDPSCs after 14 days.
- Brushite forming CaP based cements modified with hydroxyapatite nanoparticles and ε polylysine may be a useful candidate as an endodontic cement.

References