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

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Introduction

REP

Regenerative endodontic procedures (REPs) aim to regenerate the pulp-dentin complex in immature permanent teeth diagnosed with pulp necrosis.[1]

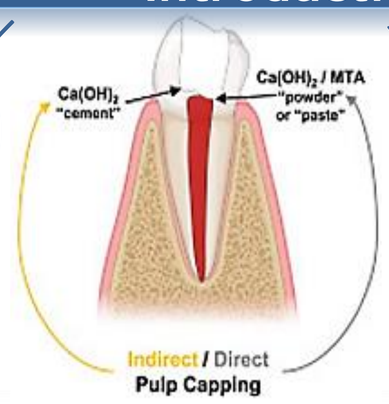
Divided into:

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]

2.Revascularisation: Novel approach for teeth with irreversible pulpitis or necrotic pulp and open apex.

-Involves inducing bleeding to provide a scaffold for stem cells to attach, proliferate, and differentiate into the vital components of the pulp-dentin complex[3]



MTA Biodetine Dycal

-Current materials used with these therapeutic techniques

-Disadvantages :

-Cost -Long setting time -Poor handling properties

-Inferior physical and mechanical properties

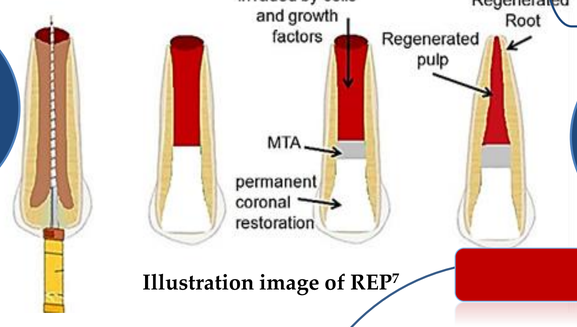


Illustration image of REP[7]

Calcium phosphate-based materials

-Biocompatible - Bioactive

-Bio-interactive (releases biologically relevant ions (Ca, P)

-Two of its Different phases are

Brushite : -Initial fast degradation rate by dissolution [4] 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 and70%wt respectively)

-Chemotactic ability: -Mediator in cell-to-cell interaction,

-Encourages pulpal fibroblasts to release alkaline phosphatase [5]

-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 antibacterial andantifungal[6] 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:

Powder : Liquid ratio 4:1

-**Powder:** equimolar Monocalcium phosphate monohydrate (MCPM) and β-Tricalcium Phosphate

Modification: β-TCP substitution by HA nanoparticles (25 or 5o wt%)

-**Liquid**: aq solution of 800mM citric acid

Modification : Addition of ε-PLS (20 or 40 wt%)

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

Formulation	Modification	Powder HA : TCP (wt%)	Liquid PolyLysine (wt%)
F1	F1.1	0	20
	F1.2	0	40
F2	F2.1	25	20
	F2.2	25	40
F3	F3.1	50	20
	F3.2	50	40

II-Setting Kinetics and chemistry

unset cement were examined using FTIR

n=3

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

III- 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 (hDPSC)**

-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

-Cellular proliferation 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)

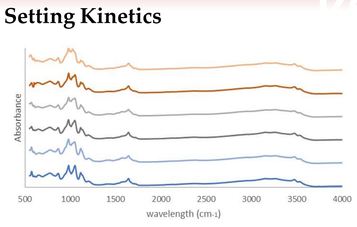
-Live/dead immunofluorescence staining

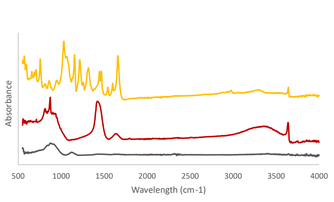
-Cells were cultured for 14 days in dilution 1:4

-Calcification assessed by Alizarin red staining

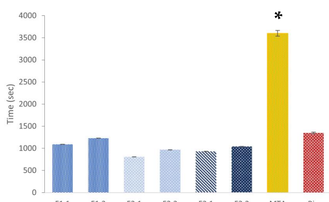
Results

Setting Kinetics



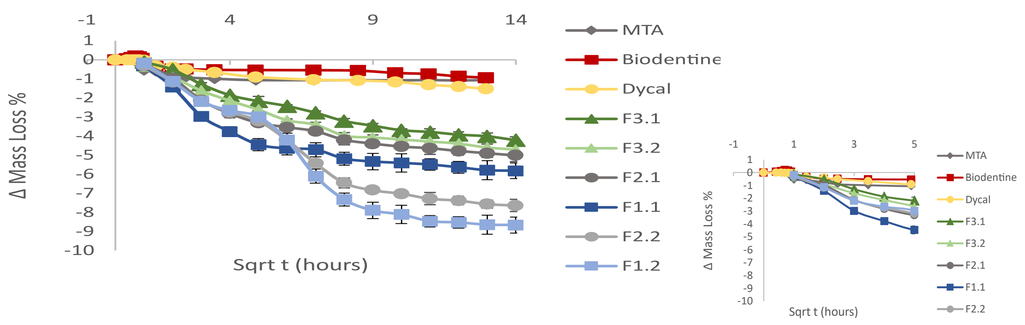


FTIR spectra of experimental and commercial formulations



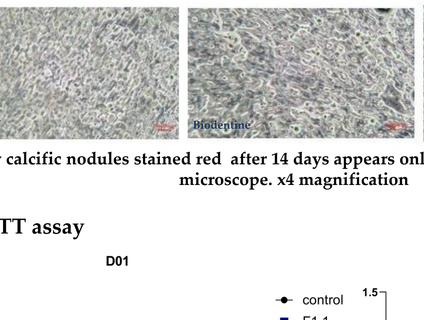
Half reaction time-T50

Dissolution Kinetics



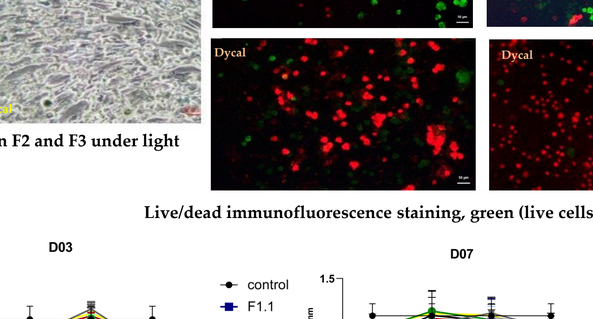
ΔMass loss with initial burst release, followed by sustained slow release
Mean ±SD, n=3

Biaxial Flexural Strength

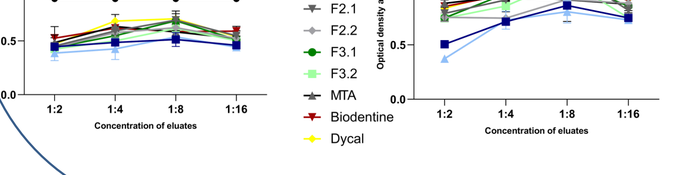


Mean ± SD, n=6

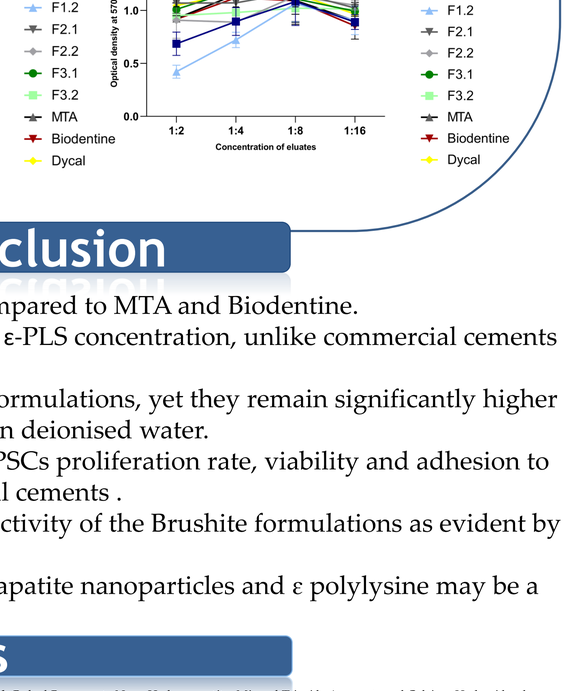
hDPSCs Culture



Cell adhesion on disc surface

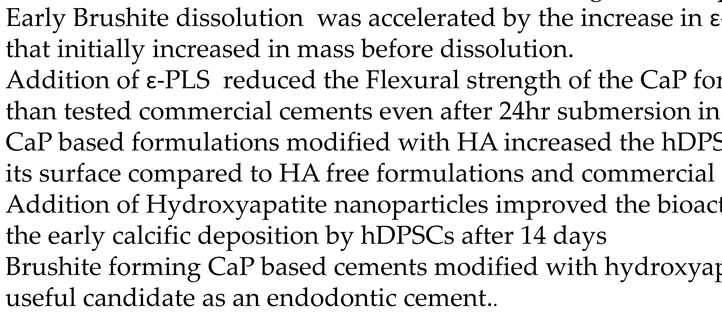


SEM micrographs at x700 mag showing cell adhesion to all formulations except Dycal



Live/Dead cell Count. Mean ±SD, n=3

Alizarin red Staining D14



Early calcific nodules stained red after 14 days appears only in F2 and F3 under light microscope. x4 magnification

Live/dead immunofluorescence staining, green (live cells) and red (dead cells)

MTT assay

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 early 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..

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