

Project Report - ICMR-RA

Summary Sheet

Title: DEVELOPMENT OF BIOMINERAL BASED SELF-SETTING FORMULATIONS FOR ENDODONTIC TISSUE REGENERATION.

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Reporting period – 10.08.2020 to 09.08.2023 (3 years)

Introduction

The proposed project is aimed at the preparation, optimization and evaluation of an inorganic self-setting cement formulation as a near-ideal, multipurpose endodontic regenerative biomaterial ideal for regenerative endodontic treatment approaches such as (i) Dental Pulp Capping, (ii) Pulpotomy, (iii) Apexification and/or apexogenesis and (iv) Revascularisation/revitalization. The formulation is composed of a combination of specific biominerals consisting of sulfates and phosphates of calcium.

Rationale of the proposed work

The rationale of the project work is to develop an optimal combination of the biominerals which can be converted into appropriate formulations fit for dental pulp capping and/or dental pulp regeneration following pulpotomy or pulpectomy and apexification or revascularization. The study plan was to utilise the in-house know-how to synthesize medical grade calcium sulfate hemihydrate with uniformly nano sized powder particles to formulate an injectable self-setting matrix with the possibility of addition of phosphates of calcium, sodium, potassium, etc., to impart increased bioactivity. The material is so selected that it is cost-effective and have most ideal handling properties.

Objectives

1. Designing of biomineral based self-setting formulations containing phosphates and sulfates of calcium.
2. Optimizing the formulations for pulp capping, pulp regeneration after pulpotomy and apexification.
3. *In vitro* evaluation of the cements using the cell culture system using dental pulp cells.
4. *In vivo* evaluation of pulp and dentin regeneration using suitable small animal model.

Outcome of the project

1. Objectives achieved during the project duration.

The four objectives laid down in the proposed project are completed. The first three objectives are completely carried out and documented. The animal study as part of the fourth objective is partially completed with the tissue specimen with implantation submitted for histopathology evaluation. The results of the histopathology evaluations are due in a couple of months. The reason for the delay in *in-vivo* was a temporary halt in the breeding of animals at the *in vivo* facility during the Covid-19 pandemic, to contain and prevent the spread of virus to laboratory animals and personnel.

2. Patent Filing.

The Intellectual Property of this work has been patented through ICMR, for the invention titled **“A SELF-GELLING AND SELF-SETTING BIOACTIVE CEMENT FORMULATION FOR DENTAL TISSUE REGENERATION”**. ICMR is the Co-Patentee along with the inventors’ institution, SCTIMST. The duly signed form 26 is also submitted to ICMR. Patent was filed on 27 January 2023. The application number is **202311005516**.

3. Details of publications highlighting ICMR acknowledgement.

- (i) Das, Eva C., PR Anil Kumar, D. Vinod, Jolly Mary Varughese, and Manoj Komath. "Isolation and Characterization of Stem Cells from Human Exfoliated Deciduous Teeth (SHED) and Their Potential Role in the Cytocompatibility Evaluation of Materials for Regenerative Dentistry." Trends in Biomaterials and Artificial Organs. 2022;36 (1):4-10.
- (ii) Adarsh RK, Das Eva C, Gopan GV, Selvam S, Komath M. Functionally Graded Bioactive Composites Based on Poly (vinyl alcohol) Made through Thiol–Ene Click Reaction. ACS Omega. 2022;7(33):29246-55.

(Copies attached separately)

4. Details on technical advancement over the existing products/technologies.

The technical advancements of the current research involve the development of a biocompatible mineral based formulation with uniformly low-sized particles that possess self-gelling and self-setting properties. The calcium sulfate hemihydrate powder of uniformly low dimensional (~5 microns length) particle size was successfully synthesized and characterized. The particle size was confirmed by SEM and the chemical phases were tested with XRD and FTIR.

The injectability is an inherent property, without the addition of any organic gelling agent which can alter the cytocompatibility and biological properties of the cement formulation. The incorporation of phosphate ions in the form of hydrogen orthophosphate salts of Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Sr^{2+} , etc., which can further enhance the biological and handling properties when added at specific ratios. The injectability of the cement within the initial setting time was tested and optimised. The cement is fully injectable within a clinically acceptable duration of less than 5 minutes.

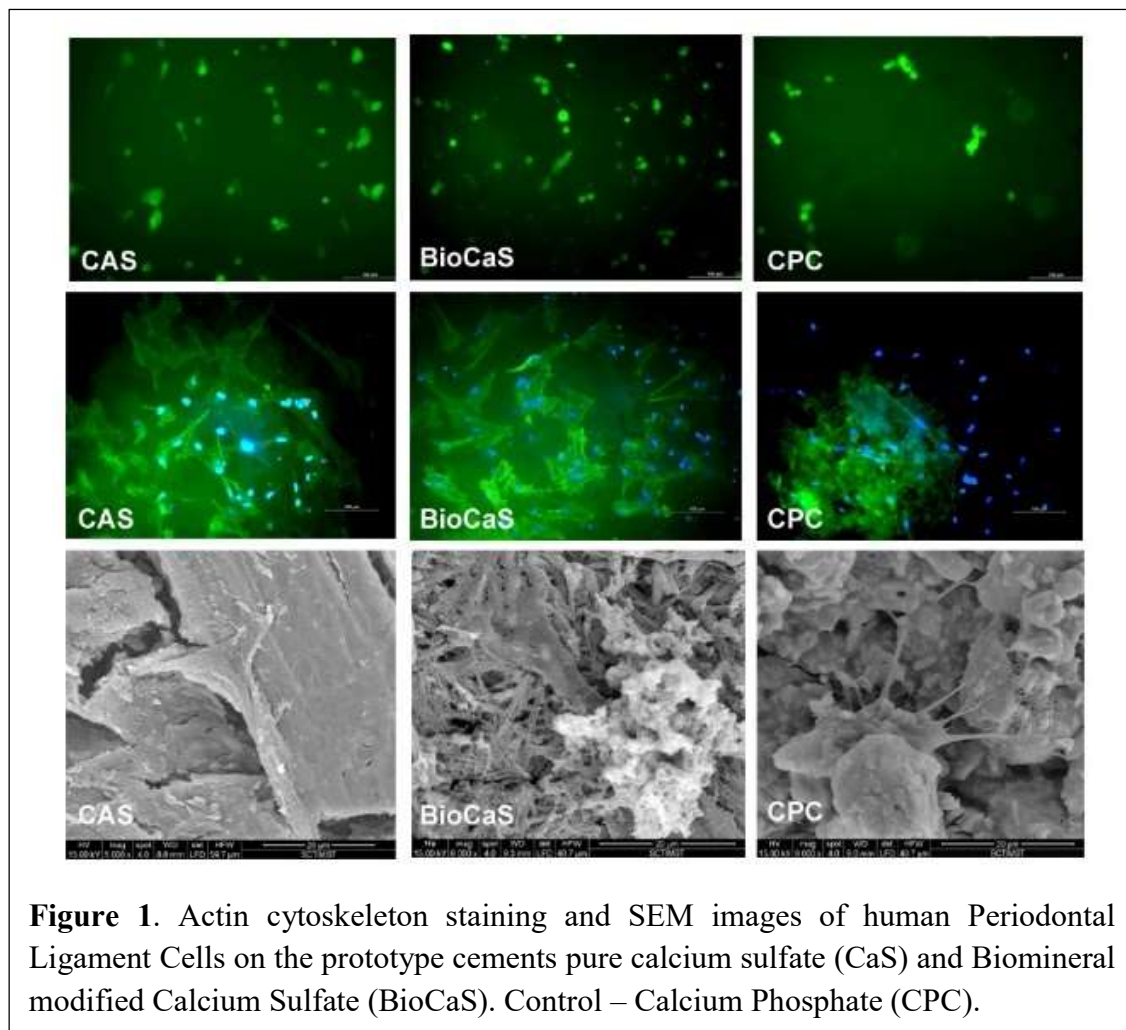
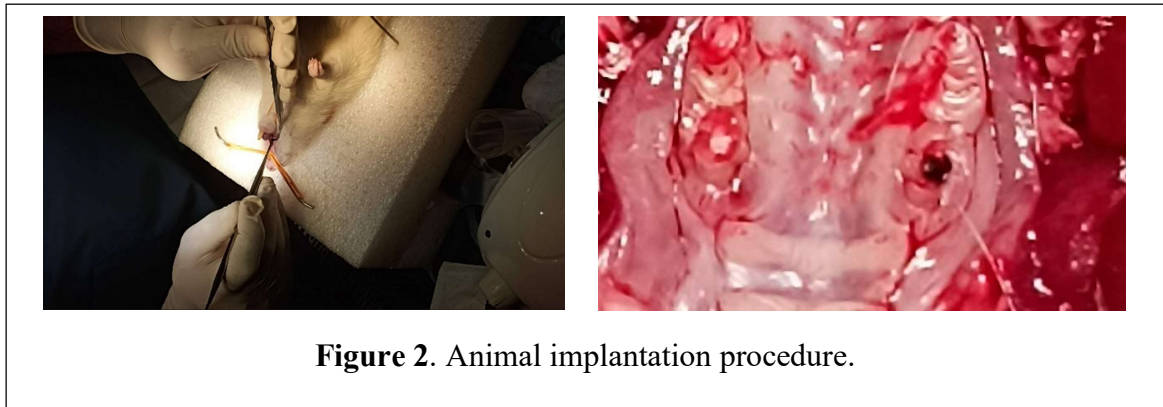


Figure 1. Actin cytoskeleton staining and SEM images of human Periodontal Ligament Cells on the prototype cements pure calcium sulfate (CaS) and Biomineral modified Calcium Sulfate (BioCaS). Control – Calcium Phosphate (CPC).

The cement placed within a dentin slice model showed *in situ* physicochemical deposition of hydroxyapatite in simulated body fluid in 24 hours and 4 days durations. The response to cells was tested *in vitro* using human dental pulp and periodontal ligament cells (Figure 1).

Animal implantation of the material has been done in the maxillary molar teeth of Sprague Dawley Rat (Figure 2). Histological analysis is going on.



Since the main component of the cement is calcium sulfate, the cement is cost-effective and non-cytotoxic. The injectability makes the cement appropriate for pulp-capping.

5. Details of deliverables.

The proposed product is deliverable as a powder-liquid system with adequate instructions regarding the wetting ratios, mixing time, working time and setting time. The powder-liquid packing delivered will have adequate shelf life and will be provided as a kit (Figure 3).



The injectability of cement is found below 50 N which is less than the finger pressure excretable on a syringe. This injectability is adequate for placement into prepared dental pulp space, when done within the setting time, or ~4 minutes.

6. Plan of action to convert lead into deliverables.

The product prototype is ready and we are actively discussing with potential industry partnership to take up the technology (Figure 4). Clinical trials are to be conducted. The technology translation will enable the industrial partner to take up the production and distribution.

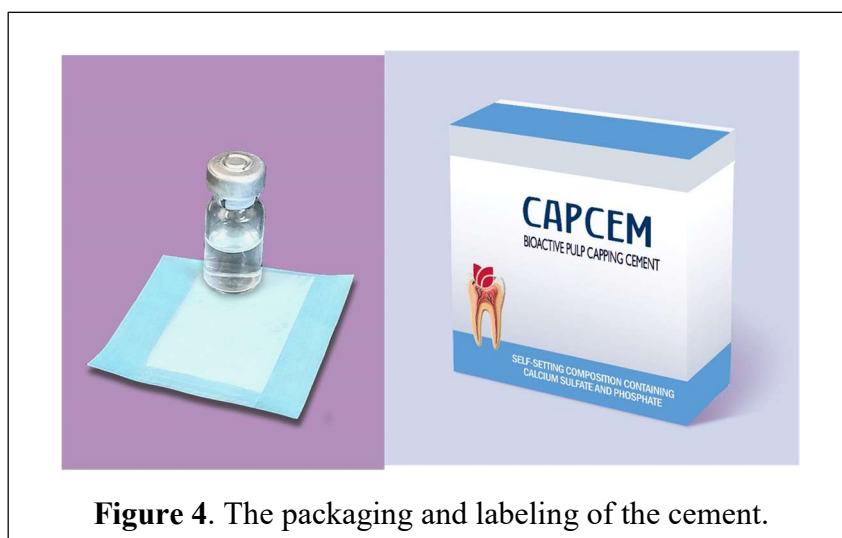


Figure 4. The packaging and labeling of the cement.

7. Details on Translational Potential.

The investigators have developed a self-gelling and self-setting cement which can achieve marginal sealing *in vivo* through its mineralization potential. The physicochemical properties of the cement are appropriate for the use as a dental pulp capping agent. Adequate flow properties within the initial setting time allows for the injectability of the cement to prepared root canal and dental pulp spaces, to conformally fill the defects. The specific biological responses of human dental pulp cells (the host cells to which the cement is intended to come to contact with) showed that the cells adhered to the cement surfaces, without any change in the cytoskeletal organization. Positive expression of certain osteogenic or dentinogenic markers such as the alkaline phosphatase enzyme, osteonectin and Dentin Matrix Protein 1 (DMP 1) are seen. *In vivo* experiments did not elicit any pain or difficulty in mastication in the laboratory animals following the dental pulp capping procedures. These data suggest the usefulness of the proposed dental pulp capping agent with good translational potential.

8. Applied value of the research

The technology under evaluation can be applied in dentistry for the regeneration of dental pulp where the coronal part of the dental pulp needs to be removed and when remaining healthy dental pulp tissue is present in the tooth roots. The cement formulations are promising candidate biomaterials, with proven capability of mineralization, injectability and inducing differentiation of dental pulp cells *in vitro*.

In addition, the cement can be used as an intra-canal cement to promote the continued tooth-end formation of incompletely formed young permanent teeth by means of apexification or revascularization procedures.

9. Status of Validation.

In-house validation done on the prototype, in the test labs in the Investigators' institution.

10. Status of prototype development.

A prototype is developed with the cement deliverable as a Powder – Liquid system with instructions regarding the storage, mixing and handling of the material. The injectability within the initial setting time is confirmed.

11. Unique Selling point.

The study provides promising results towards the utilization of the newly designed cement formulation as a dental pulp capping agent. In addition, the proposed cement can be modified to extend its application towards revascularization or revitalization approaches that attempt de novo regeneration of dental pulp or dental pulp-like tissues in case of total pulp necrosis. Therefore, the proposed bioactive cement can cater to the growing market of regenerative endodontic biomaterials in the near future.

12. Conference Presentations and Publications.

1. The poster titled “**Investigation of Osteogenic Potential of Periodontal Ligament Cells on Phosphate Incorporated Calcium Sulfate Cement – An In Vitro Study**” was presented at the National Conference on Recent Trends in Materials Science and Technology (NCMST 2020) held on 7th – 9th December 2020 and was awarded the best poster in the Biomaterials category
2. The paper titled “**Isolation and Culture of Tooth - Derived Cells for Tissue Regeneration in Dentistry**” was presented in the young dynamic speaker category at the first virtual National conference by the Indian Society of Prosthodontics, Restorative and Periodontics, held on 9th and 10th January, 2021.