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Short Communication

Utilization of gelatin methacryloyl scaffolds in the regeneration of tooth and its supporting tissues

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ABSTRACT

Gelatin methacrylate (GelMA) due to its good biocompatibility, modifiable physical properties, and promotion of cellular adhesion, proliferation, and differentiation, serves as excellent scaffolds in tissue engineering and regeneration. These properties of GelMA make it a suitable choice for engineering of tooth and its supporting tissues. The complex spatial arrangement of the tooth along with the anisotropic nature of different tissues associated with it, make its regeneration challenging. Bioprinted GelMA scaffolds offer a great potential in mimicking the tooth architecture. In this study, GelMA scaffold was constructed by one pot method. The Fourier Transfer Infra red spectroscopy of GelMA confirmed the presence of amide bond at 1650 cm^{-1} (C=O stretching), 1550^{-1} (C-N stretching) and 1450^{-1}. As evident from previous studies, the application of GelMA in whole tooth regeneration has not been studied exhaustively. However, GelMA has produced effective results in the regeneration of individual tooth components as well as the tooth germ. Therefore, this scaffold is proposed to be used in the whole tooth regeneration process because of its cytocompatiblity, cellular adhesion and tuneable mechanical properties.

Keywords: Gelatin methacryloyl scaffolds, GelMA, Tooth regeneration, Tissue engineering

INTRODUCTION

The regeneration of tooth and its supporting tissues is one of the most challenging areas involving a host of different tissues such as the dentin-pulp complex, tooth root, periodontium, blood vessels and nerves, working synergistically with each other. This is usually achieved using biomaterials laden with pleuripotent stem cells and growth factors that interact with each other to stimulate the cellular function, thus inducing tissue formation.[1] The field of regenerative medicine has seen tremendous advancements in the recent era owing to the application of three dimensional (3D) bioprinting of biocompatible scaffolds that facilitatescellularadhesion, migration and proliferation with optimum cell-to-cell interaction.[2]

The selection of an appropriate scaffold plays a crucial role in the outcome of the biomimetic regeneration of tooth. A variety of factors need to be considered in the scaffolds such as theappropriate biomaterials, surface topology, porosity, and mechanical integrity. Recently in the field of tissue engineering of tooth, various scaffolds such as hydroxyapatite ceramics, collagen and chitosan, polyglycolic acid (PGA), polylactic acid (PLA), polyglycolic acid-poly-l-lactic acid (PGA-PLLA), as well as polylactic polyglycolic acid (PLGA) have been employed.[3] Additionally, Gelatin metharcyloyl (GelMA) hydrogel, which can be 3D bioprinted has shown favorable results in dental pulp and whole-tooth regeneration due to its excellent biocompatibility and optimum revascularization properties.[4,5] However, the area of research still remains in its early stages and further studies are required to assess the viability of such scaffolds for dental constructs.

The current study proposes to use GelMA as a scaffold for whole-tooth regeneration and to study the properties of the scaffold as an ideal choice for tooth regeneration. The mechanical and biological properties have been discussed in this preliminary study to assess the suitability of GelMA hydrogels in regeneration of tooth through tissue engineering.

METHODS

This study was carried out over a period of three months from April, 2022 up to July, 2022 in which GelMA scaffold was constructed, followed by Fourier transfer Infra-red spectroscopy characterization of the scaffold.

Preparation of GelMA

GelMA scaffold was prepared at Amity Institute of Molecular Medicine and Stem cell Research, Amity University, Noida. Gelatin (Bloom Strength 175, Type A, Sigma-Aldrich), and Methacrylic anhydride (MAAnh, Sigma-Aldrich) were used in the construction of the scaffold. Synthesis of GelMA was done using Methacrylic anhydride by one pot method. Briefly, 7.95g ofNa_2CO_3 and 14.65 g of $NaHCO_3$ were dissolved in 1 l distilled water to produce a 0.25M carbonate-bicarbonate (CB) buffer solution. About, 50g of Gelatin was dissolved into 500 ml CB buffer. About 1mL of Methacrylic anhydride (MAAnh) was added to the solution and reaction proceeded at 50°C for 3h. About 1ml of 1N HCl was added to stop the reaction and the product was filtered, dialyzed and the obtained GelMA was lyophilized to obtain a dried product and stored at−20°C. GelMA were characterized for the degree of substitution.

FTIR characterization

FTIR characterization of GelMA was carried out at Jamia Millia Islamia, New Delhi. It was performed to assess the linkage between the polymeric scaffold designs.

RESULTS

GelMA are processed and lyophilized until use. Further they are photocrosslinked using photoinitiator Lithium phenyl-2,4,6-trimethylbenzoylphosphinate and microbial transglutaminase before 3D printing. Figure 1 represents the steps involved in cross-linking and printing GelMA. The Fourier Transfer Infra-red spectroscopy of GelMA confirmed the presence of amide bond at 1650 cm^{-1} (C=O stretching), 1550^{-1} (C-N stretching) and 1450^{-1} (Figure 2).

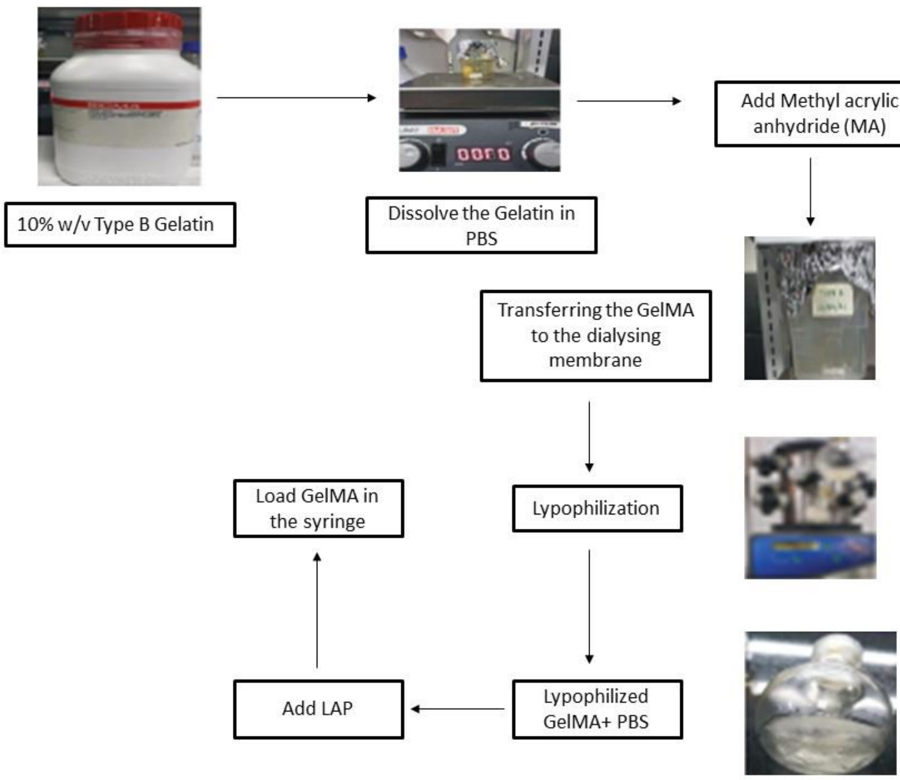

Figure 1: Processing of GelMA.

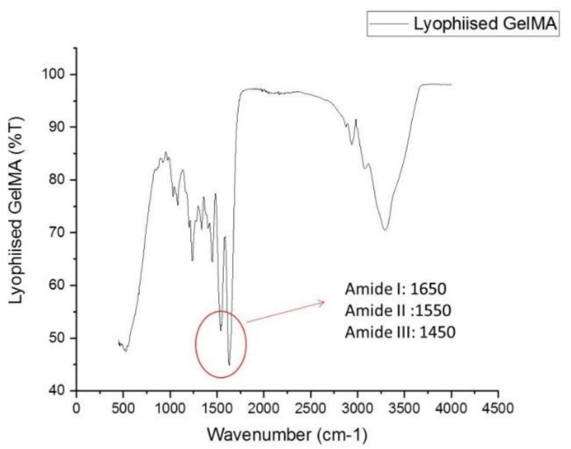

Figure 2: Fourier transfer infra-red spectroscopy of GelMA to assess linkage.

DISCUSSION

GelMA hydrogels present a promising front as a biomaterial to engineer cellular growth, stem-cell interaction and tooth regeneration. GelMA consist of covalently crosslinked hydrogels that is formed by photoinitiated radical polymerization. They closely resemble the natural extracellular matrix components and the presence of gelatin, a hydrolysed product of collagen promote cellular adhesion because of their arginine-glycine-aspartic acid motifs, and the target sequences of matrix metalloproteinases that allow cellular proliferation and remodeling.[6,7] The controlled photopolymerization reaction with alterations in temperature, pH, and aqueous environment, along with variation in GelMA and/or photoinitiator concentrations could be utilized to create unique cellular patterns and structures that allow study of cellular behaviors and cellular-biomaterial interactions at different conditions.[8] The physical properties of GelMA such as the porosity, degradation rate, compressive modulus, water swelling ratio, and biological properties with respect to cellular viability, proliferation, and spreading are essential in determining the suitability of the hydrogel in the tooth or any other tissue engineering techniques.

There is currently little evidence of GelMA hydrogels being used in whole-tooth regeneration process. Previous literature discusses the applications of GelMA in the engineering of individual components such as pulp revascularization, and neurotization, and the periodontium.[9-11] Khayat et al studied three groups in which root segments of the first group were injected with human dental pulp stem cells and human umbilical vein endothelial cells in 5% GelMA hydrogel. The other group was injected with acellular GelMA alone and the third group was kept as control. It was found that the first group with GelMA encapsulated stem cells exhibited well-organized neovascularizationresembling pulp-like tissue whereas less cellularized host cell derived pulp-like tissue was observed in the second and third groups.[9] Additionally, well vascularization through GelMA was obtained in a study, thus furthering the promotion of GelMA hydrogels in pulp revascularization.[12] For reinnervation of the pulp, Hu et al utilized a 3D printed cryopolymerized GelMA gel with adipose-derived stem cells. The biocompatibility, biodegradability, and absorbability of the gel was found promising along with proliferation and survival of the stem cells, and upregulation of miRNA expression of neurotrophic factors, thus supporting reinnervation.[10]

GelMA have also been used for periodontal regeneration and osteogenic differentiation of tooth supporting structures. In a study, the viability, proliferation, and in vivo osteogenesis of human periodontal ligament stem cells encapsulated in GelMA and novel GelMA/nanohydroxylapatite microgel arrays were evaluated. It was found that the microgels exhibited adequate mechanical strength, surface roughness, and microarchitecture, and produced abundant mineralization and vascularization, thus promoting periodontal tissue repair and regeneration.[11] Apart from regeneration of the pulp complex and periodontium, few studies have also studied the application of GelMA in bioengineering the tooth germ. Biomimetic tooth bud models were constructed by the progenitor dental epithelial and dental mesenchymal cell sheets encapsulated within the GelMA hydrogel mimicking the enamel and pulp organ layers, aimed to facilitate epithelial-mesenchymal interaction occurring in the early stages of tooth development. After *in-vivo* implantation of the models, presence of mineralized tissues of specific size and shape was confirmed.[4,5]

Besides multiple advantages of GelMa such as biocompatibility, cellular adhesion and proliferation, the major drawback of GelMA hydrogels is its inferior mechanical properties owing to the physical cross-linking of unsubstituted gelatin at specific temperature and concentration. This is usually overcome by addition of carbon-based or inorganic nanomaterials to GelMA which enhances its mechanical as well as cellular properties.[13] Secondly, as with any hydrogel, GelMA possess low viscosity which affects the resolution of printed scaffolds and sometimes might result in complete loss of printability at lower concentrations.[14] Moreover, the complex spatial arrangement of tooth with different structural and functional components itself presents a much greater challenge that requires a scaffold with excellent and mechanical, and biochemical properties along with anisotropic nature of the scaffold to efficiently engineer the variable composition of human tooth.

Limitations

GelMA as a potential scaffold for tooth regeneration has been explored to a less extent. The available literature involves non-human subjects which could affect the quality of evidence. This study discusses *in-vitro* and *in-vivo* studies involving non-human subjects for application of GelMA as a biocompatible scaffold for tooth regeneration. Therefore, more *in-vitro* and further *in-vivo* studies involving human participants would be required to establish conclusive evidence on usage of GelMA scaffolds in the field of tooth tissue engineering.

CONCLUSION

Tooth regeneration studies involving human participants are currently lacking to enable translation of this tissue engineered approach into clinical practice. Further studies are required to assess the viability of such scaffolds *in-vivo*. However, the existing *in-vitro* studies and *in-vivo* studies involving animal models have promoted the application of GelMA encapsulated stem cells in the regeneration of tooth and supporting structures such as the pulp-dentin complex, periodontium, and tooth germ, owing to their excellent biocompatibility, cellular adhesion, and tuneable mechanical properties.

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