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Complement C5a-loaded scaffold for dentin-pulp regeneration

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1. Introduction

In vital pulp therapy (VPT), materials are applied directly onto the exposed pulp to restore the tooth and stimulate tertiary dentin production. However, this reparative process often occurs at the expense of pulp volume, potentially compromising the long-term vitality and physiological function of the pulp tissue. Traditional capping materials, while promoting dentin bridge formation, do not support full regeneration of the pulp-dentin complex. For example, materials like MTA/Biodentine are widely used but mainly act as reparative agents rather than enabling complete regeneration. Hydrogels and scaffolds have been explored to improve regeneration but often face limitations in controlling the release of bioactive factors. To address this limitation, a novel biomaterial was developed: a resorbable collagen scaffold integrated with Complement C5a-loaded PLGA microspheres. Collagen provides a biocompatible, porous matrix that supports cell growth, while C5a guides stem cell migration through their specific receptor (C5aR). This approach promotes complete dentin-pulp regeneration without volume loss by enhancing stem cell recruitment and proliferation. C5a, through immune modulation and chemotaxis, guides regeneration. This study evaluates the scaffold's ability to support pulp and endothelial cell colonization for tissue reconstruction and vascularization.

2. Methods

To fabricate the collagen-based scaffold, a lyophilization technique was used with a collagen hydrogel to create a porous, cylindrical 3D structure. This structure is ideal for facilitating tissue regeneration by allowing cell infiltration and promoting the formation of new tissue. Type I collagen used in this study is of bovine origin and certified for pharmaceutical application. It was selected for its biocompatibility, biodegradability, and its ability to support the adhesion, migration, and proliferation of mesenchymal stem cells, making it a material of choice for tissue engineering applications. C5a was encapsulated within PLGA microspheres (0.10 µg of C5a per microsphere) using a double-emulsion technique, chosen for its efficiency in encapsulating the bioactive molecule and allowing prolonged, controlled release. The microspheres, with an average diameter of 25 µm, were strategically embedded on one side of the scaffold to create a C5a gradient guiding stem cell migration toward the loaded side. The opposite side, without microspheres, served as a control. Human dental pulp stem cells positive for STRO-1 were isolated from healthy immature third molars and selected by magnetic-activated cell sorting. Experiments were performed using cells between passages 2 and 4. To assess the scaffold's properties, scanning electron microscopy (SEM) was used to examine the scaffold's morphology and the distribution of the microspheres. The biocompatibility of the scaffold was evaluated using MTT

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assays, which measured cell viability in response to extracts from the scaffold. The release kinetics of C5a from the microspheres were determined by ELISA, after incubating the biomaterial in complete MEM medium and collecting the supernatant daily to quantify the released C5a. The effects of scaffold extracts on pulp stem cells and endothelial cells were assessed using MTT assays. Cell recruitment was analyzed with Boyden chambers to quantify chemotactic movement toward the C5a-loaded side. HUVECs were labeled with a red fluorescent probe to visualize scaffold colonization.

3. Results and discussion

The encapsulation of C5a in PLGA microspheres was successfully achieved. The collagen scaffold exhibited a porous structure, allowing for cell infiltration and growth. Microspheres were clearly visible on one side of the scaffold, while the other side remained microsphere-free, validating the creation of a spatial gradient for cell recruitment. C5a release from the microspheres was found to be biphasic, with an initial burst release phase followed by a more linear and constant release phase over a period of 52 days. C5a concentrations in the released extracts reached 50 ng/mL. Furthermore, this biomaterial was found to be non-toxic to our cells. These released C5a extracts significantly enhanced pulp stem cell proliferation and migration toward the C5a gradient in Boyden Chamber assay. The media used were biomaterial extracts corresponding to the incubation of our biomaterial for 24 hours with full MEM media, with the C5a released into the MEM acting as a chemotactic agent. C5a-loaded extracts induced a significantly stronger chemotactic response than controls. Endothelial cell proliferation and recruitment were also enhanced with C5a-loaded matrices, suggesting that C5a promotes both stem cell recruitment and endothelial organization - key processes for dentin-pulp complex regeneration.

4. Conclusions

The collagen-based scaffold, loaded with C5a, presents a promising approach for dentin-pulp regeneration due to its biocompatibility, porous structure, and sustained release of biologically active C5a. The controlled release of C5a significantly enhanced the proliferation and recruitment of pulp stem cells within the scaffold.

To explore the biomaterial's potential, an ex vivo whole tooth model with pulp exposure treated by the C5a-loaded scaffold and Biodentine could be used. After one month, histology would assess pulp regeneration and vascularization. In vitro, angiogenesis could be evaluated via endothelial tube formation on Matrigel.

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Conflict of Interest Statement

None.

Contributor Roles

Amiri Bilel: Investigation, Writing- original draft; **Jeanneau Charlotte:** Investigation, Supervision, Writing- review & editing; **Iasio Romain:** Supervision, Writing Review; **About Imad:** Conceptualization, Methodology, Supervision, Validation, Writing- review & editing.