Synthesis and characterization of osteoinductive adhesive composites with antimicrobial properties

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Statement of Purpose: More than two million bone grafting procedures are performed worldwide each year. Conventional sources for bone grafts are hindered by their high cost, limited availability, immunogenicity, poor osseointegration, and comorbidities such as bacterial infections [1]. Bioresorbable bone grafting substitutes (BGSs) have emerged as alternatives for bone augmentation and skeletal repair [2]. Among these, bioactive glasses (BGs) have been widely used for bone tissue engineering (TE) owing to their high cytocompatibility, osteoinductivity, and antimicrobial properties [3]. Moreover, their incorporation into soft polymeric matrices holds great potential for the development of cytocompatible and bioactive scaffolds for bone repair. Here, we developed a multifunctional composite biomaterial for bone TE based on the incorporation of a novel Sr- and lithium (Li)-doped BG into gelatin methacryloyl (GelMA) hydrogels.

Methods: We first synthesized a new formulation of BG consisting of $60SiO_2$-$26CaO$-$4P_2O_5$-$5SrO$-$5Li_2O$ (mol. %) (i.e., BG-5/5) with high cytocompatibility and bioactivity *in vitro*. To form the composites, varying concentrations of BG-5/5 (i.e., 1, 5, and 10% (w/v)) were then mixed with 10% (w/v) GelMA precursor in a solution containing triethanolamine (TEA, 1.88% (w/v)), N-vinyl caprolactam (VC, 1.25% (w/v)) and Eosin Y disodium salt (0.5 mM). Photocrosslinking of the hydrogels was carried out at 100 mW cm^{-2}, using a xenon Genzyme Biosurgery light source (450 to 550 nm). We evaluated the physical properties of GelMA/BG-5/5 composites, such as their porosity, swellability, and degradability, as well as their mechanical and adhesive properties *in vitro*. We investigated the cytocompatibility of the composites using 2D and 3D cultures of MC3T3-M1 preosteoblasts via fluorescent F-actin/DAPI staining, and commercial LIVE/DEAD. The osteoinductivity of the composites was evaluated via Alizarin red staining of 3D encapsulated preosteoblasts. The antimicrobial properties were evaluated against methicillin-resistant *Staphylococcus Aureus* (MRSA) using a BacLight assay.

Results: Our results showed that the swelling ratio and the degradation rate of the composites increased consistently by increasing the concentration of BG-5/5. The compressive modulus of hydrogels also increased from 142.49 ± 10.29 kPa to 511.83 ± 32.25 kPa and 757 ± 17.75 kPa when the BG-5/5 concentration was increased from 0% to 10%, and 20% (w/v), respectively (**Fig. 1a**). Our results also showed that the incorporation of 20% (w/v) BG-5/5 increased the elastic modulus and the ultimate tensile strength of pristine hydrogels from 122.47 ± 14.37 kPa to 383.4 ± 16.11 kPa, and from 295.8 ± 10.29 kPa to 394.8 ± 21.01 kPa, respectively. Based on the adhesion test, the highest adhesion strength to *ex vivo* bone tissue (208.7 ± 9.97 kPa) corresponded to hydrogels with 10% BG-5/5 (**Fig. 1c**). In addition, the composites were shown to support the growth, spread, and proliferation of MC3T3-M1 preosteoblasts in both 2D and 3D cultures. Alizarin red staining showed that GelMA/BG-5/5 hydrogels exhibited significantly higher levels of calcium deposition, when compared to GelMA controls (**Fig. 1c**). Lastly, our results showed that GelMA/BG-5/5 hydrogels could effectively prevent the growth of MRSA bacteria, as demonstrated by fluorescent micrographs of the BacLight assay (**Fig. 1d**).

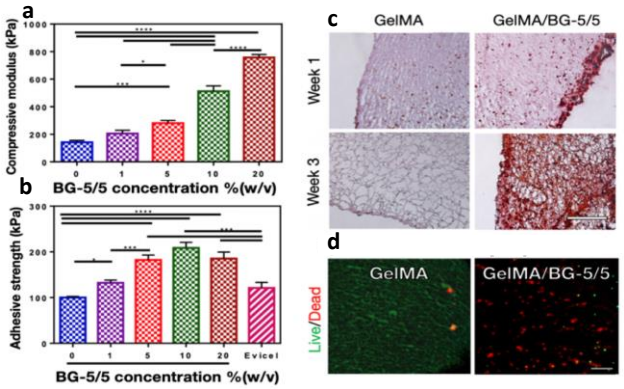

Figure 1. *In vitro* evaluation of GelMA/BG-5/5 composites. **(a)** Compressive modulus, and **(b)** *ex vivo* adhesion strength of composites formed by using 10% GelMA and various concentrations of BG-5/5. **(c)** Histological evaluation (Alizarin red) of the osteogenic differentiation of 3D encapsulated MC3T3-M1 cells in GelMA, and GelMA/BG-5/5 hydrogels at weeks 1, and 3 post-encapsulation (Scale bar: 500 µm). **(d)** Representative fluorescent micrographs of the BacLight viability assay. MRSA were grown on pure GelMA and GelMA/BG-5/5 hydrogels (Scale bar: 20 µm).

Conclusions: GelMA/BG composites present various technical advantages over conventional approaches for bone repair and orthopedic infections. The intrinsic bioactivity of the scaffolds could promote osteoblastic differentiation without the need for growth factors. The antimicrobial properties and high adhesive strength could aid in the prevention of bacterial infections while also promoting osseointegration.

References: [1] A. Wubneh, E.K. Tsekoura, C. Ayranci, H. Uludag, Acta Biomater (2018). [2] G. Fernandez de Grado, J Tissue Eng 9 (2018) 2041731418776819. [3] F. Baino, J Funct Biomater 9(1) (2018).

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