HYDROGEL MODIFICATION WITH THE USE OF $CaCO_3$ MICROPARTICLES ENRICHED WITH ANTIBACTERIAL PEPTIDES

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Introduction

Antibiotics have been largely produced and overused in many countries. This led to an increase of the resistance of bacteria and biofilms to commonly used antimicrobial agents [1]. Novel methods for efficient eradication of bacterial infections are needed. Antibacterial peptides (ABPs) – cationic molecules consisting of 12-45 amino acids, are promising alternatives for antibiotics. ABPs are able to hamper bacterial proliferation as effectively as antibiotics, whereby bacteria are not able to develop resistance for ABPs [2]. The aim of the present study was to fabricate ABPs-enriched calcium carbonate ($CaCO_3$) microparticles (MPs) and use them for modification of gellan gum (GG) hydrogel in order to develop an injectable composite material that can be used for the treatment of bacterial infections in bones. Bacitracin (BCT) was used as a model ABP.

Materials and Methods

$CaCO_3$ MPs were fabricated using co-precipitation method. In brief, 5 ml of aqueous 0.3M $CaCl_2$ solution was mixed with 5 ml of aqueous 0.3M Na_2CO_3 solution under magnetic stirring (1000 rpm, 30 s). For the preparation of ABPs-enriched MPs, BCT was added to Na_2CO_3 solution at different concentration ranging from 0 (control) to 2 mg/ml. The MPs were fabricated in the same way as unloaded MPs. Following the precipitation procedure, MPs were collected and purified using repetitive centrifugation (5000 rpm, 5 min) and rinsing with deionized water (3x). Finally, MPs were air dried at 37°C overnight. MP morphology was evaluated under scanning electron microscopy (SEM) and BCT loading efficacy was determined using bicinchoninic acid (BCA) assay.

GG was dissolved in water at 90°C at a concentration of 1.11% (w/v) and then cooled down to 60°C. Then 9 ml of GG solution was mixed with 0.5 ml of aqueous suspension of MPs (0-200 mg/ml giving final concentration of MPs of 0-2%, unloaded or loaded with BCT) and 0.5 ml of aqueous $CaCl_2$ solution (0.3%, cross-linking agent). A mixture was cast into a Petri dish (for mechanical testing, SEM analysis and *in vitro* testing) or inserted into 2 ml syringes (for injectability testing) and cooled down to 4°C. The morphology, Young's modulus, injectability and chemical composition (FTIR spectroscopy) of the samples were analysed together with *in vitro* performance of the materials in contact with MG-63 osteoblast-like cells.

Results and Discussion

Obtained MPs were highly porous and spherical in shape with the average particles size ranging from 2.4 – 5.9 µm (FIG. 1). BCT encapsulation efficiency (EE) was 18 – 25%, resulting in BCT loading in MPs between 4.6 to 44.4 µg BCT per 1 mg of MPs.

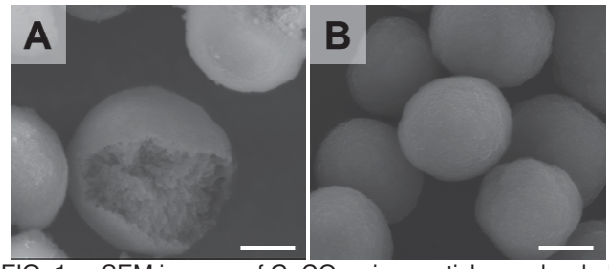

FIG. 1. – SEM images of $CaCO_3$ microparticles: unloaded (A) and loaded with BCT (B). Scale bar: 2 µm.

MPs were successfully loaded into GG hydrogels as confirmed by both SEM/EDX (FIG. 2) and FTIR spectroscopy. The MPs were distributed uniformly across the whole volume of GG and no signs of MP agglomeration were observed.

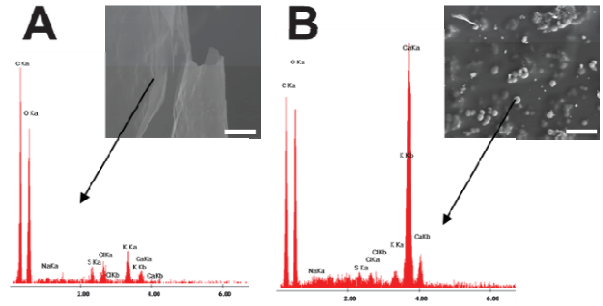

FIG. 2. SEM images (scale bar: 20 µm) and EDX analysis of GG: without MPs (A) and with 1% MPs (B).

The addition of up to 1% of MPs did not significantly influence mechanical properties of GG hydrogels (both in terms of Young's modulus and injectability). Also no differences were observed in the case of GG samples loaded with BCT-enriched MPs.

In vitro studies of GG-MPs composites showed increased proliferation of MG-63 cells in the presence of at least 0.25% of MPs in GG (FIG. 3).

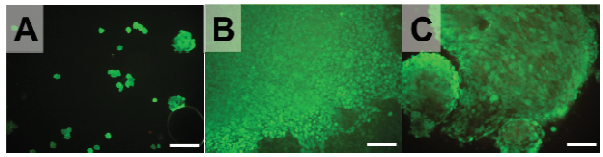

FIG. 3. Live/dead staining of MG-63 cells cultured for 14 days on GG-MP composites: without MPs (A), with 0.5% MPs (B) and with 1% of MPs (C). Scale bar: 100 µm.

Conclusions

Co-precipitation method allowed for fabrication of uniform spherical $CaCO_3$ MPs enriched with an exemplary ABP – bacitracin. MPs can be incorporated into GG hydrogel and addition of up to 1% of MPs does not decrease mechanical properties or injectability of GG, but it significantly improve *in vitro* performance of GG. Further studies will focus on evaluation of antibacterial properties of the developed materials.

Acknowledgments

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References

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