BIOINSPIRED, BIOMIMETIC, DOUBLE-ENZYMATIC MINERALIZATION OF HYDROGELS FOR BONE REGENERATION WITH CALCIUM CARBONATE

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Introduction

Hydrogels are popular materials for tissue regeneration due to several advantages, which include the ease of incorporation of biologically active substances such as enzymes. Hydrogel mineralization is desirable for bone regeneration. Mineralization with calcium carbonate ($CaCO_3$) is a promising approach, and has led to superior bone healing *in vivo*. In this study, hydrogels of Gellan Gum (GG), a biocompatible polysaccharide, were mineralized biomimetically using a double enzymatic approach. The enzymes urease and carbonic anhydrase (CA) were incorporated in GG hydrogels. Urease and CA are used by bacteria and marine invertebrates, respectively, to cause mineralization with $CaCO_3$. Hydrogels were then incubated in a mineralization solution containing enzyme substrate (urea) and calcium ions. Urease converts urea to ammonia, which raises pH, and carbon dioxide (CO_2). CA catalyses the reaction of CO_2 with water to form bicarbonate ions, which in turn undergoes deprotonation to form carbonate ions. Subsequently, carbonate ions react with calcium ions to form insoluble $CaCO_3$ inside the hydrogel (FIG. 1).

Urease-catalysed reaction

$$Urea + 2H_2O \rightarrow 2NH_3 + CO_2$$

Formation of $CaCO_3$ from CO_2 and H_2O

$$CO_2\ (aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_{3^-} \leftrightarrow H^+ + CO_3^{2-}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \text{precipitates}$$

FIG.1. Process of enzymatic mineralization by urease.

Materials and Methods

GG hydrogel discs were incubated in 50 mg/ml urease solution containing 0, 0.625, 1.25 or 2.5 mg/ml CA for 1 h to allow the enzyme to diffuse into the hydrogel. Subsequently, discs were immersed in mineralization solution containing 0.27 M $CaCl_2$ and 0.17 M urea as applied by Rauner *et al* [1]. Physicochemical characterization was performed by measurement of dry mass percentage, defined as (Wd / Ww) x 100 where Wd is the weight after drying, Ww is the weight in the wet state before drying, which served as a measure of mineralization. ICP-OES, FTIR and XRD and compressive testing were also performed. MC3T3-E1 osteoblast-like cells were used for biological testing with the AlamarBlue assay and SEM.

Results and Discussion

All hydrogels containing both urease and CA were mineralized more strongly (FIG. 2) and were stiffer than hydrogels which only contained CA. $CaCO_3$ formed was appeared to be predominantly calcite. Autoclaving did not significantly decrease compression strength. Osteoblast-like cell proliferation after 1d, 3d and 8d was not hindered by mineralization with $CaCO_3$. Cell spreading after 8 d was superior on mineralized hydrogels (FIG. 3).

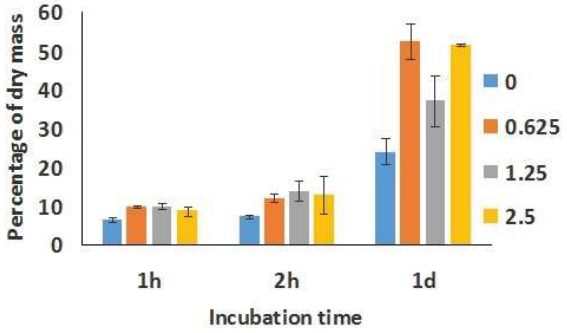

FIG. 2. Dry mass percentage of GG hydrogels preincubated in 50 mg/ml urease with differing carbonic anhydrase concentrations (mg/ml).

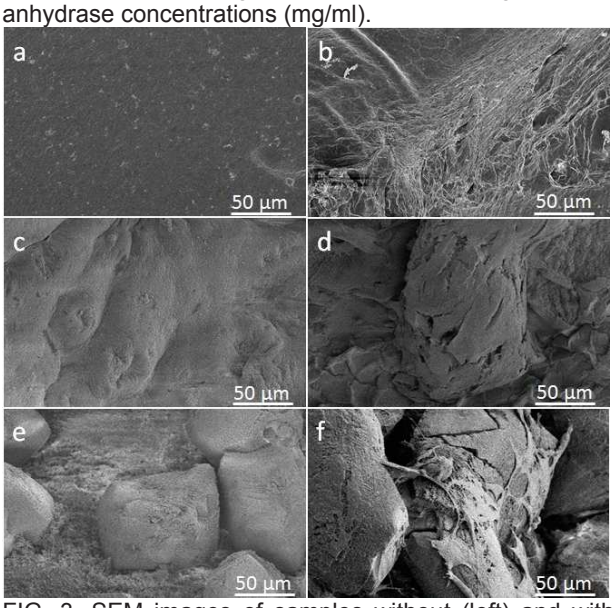

FIG. 3. SEM images of samples without (left) and with (right) MC3T3-E1 osteoblast-like cells 8 d after seeding on unmineralized hydrogels (GG, a&b), hydrogels mineralized for 1 d using 50 mg/ml urease (U, c&d) and using 50 mg/ml urease and 2.5 mg/ml CA (U+CA, e&f).

Conclusions

Double-enzymatic mineralization led to a higher amount of $CaCO_3$ in hydrogels and mineralization did not hinder cell proliferation.

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References

[1] Rauner et al. Acta Biomater. 2014 10(9):3942-51.