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Enzymatic, urease-mediated mineralization of gellan gum hydrogel with calcium carbonate, magnesium-enriched calcium carbonate and magnesium carbonate for bone regeneration applications

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Introduction: Mineralization of hydrogel biomaterials is considered desirable to improve their suitability as materials for bone regeneration[1,2]. Hydrogels have been most commonly mineralized with calcium phosphate (CaP), but hydrogel-CaCO₃ composites have received less attention. Magnesium (Mg) has been added to CaP to stimulate cell adhesion and proliferation and bone regeneration in vivo, but its effect as a component of carbonate-based biomaterials remains uninvestigated. In this study, gellan gum (GG) hydrogels were mineralized enzymatically with (CaCO₃), Mg-enriched CaCO₃ and magnesium carbonate to generate composite biomaterials for bone regeneration. GG is an inexpensive, biotechnologically produced anionic polysaccharide, from which hydrogels for cartilage regeneration have been formed by crosslinking with divalent ions[3].

Methods: GG hydrogels were loaded with the enzyme urease by incubation in 5% (w/v) urease solution and mineralized for 5 days in five different media denoted as UA, UB, UC, UD and UE, which contained urea (0.17 M) and different concentrations of CaCl₂ and MgCl₂ (270:0, 202.5:67.5, 135:135, 67.5:202.5 and 0:250, respectively (mmol dm⁻³)). Discs were autoclaved and subjected to physiochemical, mechanical and cell biological characterization.

Results: FTIR, SEM, TGA and XRD analysis revealed that increasing magnesium concentration decreased mineral crystallinity. At low magnesium concentrations calcite was formed, while at higher concentrations magnesian calcite was formed. Hydromagnesite formed at high magnesium concentration in the absence of calcium. Amount of mineral formed and compressive strength decreased with increasing magnesium concentration in the mineralization medium. ICP analysis revealed that Ca:Mg elemental ratio in the mineral formed was higher than in the respective mineralization media. Mineralization of hydrogels promoted adhesion and growth of osteoblast-like cells, which were supported best on mineralized hydrogels containing no or little magnesium. Hydrogels mineralized with hydromagnesite displayed higher cytotoxicity.

Discussion: Enzymatic mineralization of GG hydrogels with CaCO₃ in the form of calcite successfully reinforced hydrogels and promoted osteoblast-like cell adhesion and growth, but Mg enrichment had no positive effect. This is in contrast with other studies reporting that incorporation of Mg into GG mineralized with CaP promotes cell adhesion and proliferation[4].

Conclusion: Sample groups UA and UB seem to be the most promising due to the superior amount of mineral formed and cell adhesion and proliferation.

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