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. Calcium carbonate ($CaCO_3$) has been successfully applied as a bone regeneration material, but hydrogel-$CaCO_3$ composites have received less attention. Magnesium (Mg) has been used as a component of calcium phosphate biomaterials to stimulate bone-forming 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 using urease [1] with $CaCO_3$, Mg-enriched $CaCO_3$ and magnesium carbonate to generate composites for bone regeneration.

Materials and Methods

Hydrogels loaded with the enzyme urease (50 mg/ml) were mineralized by incubation in mineralization media denoted as UA, UB, UC, UD and UE (TABLE 1). Mineralized hydrogels were characterized physiochemically by FTIR, XRD, SEM, TGA, ICP-OES and compressive testing, and biologically using MC3T3-E1 osteoblast-like cells.

TABLE 1. Composition of mineralization media UA-UE.

Medium	Concentration		
	$CaCl_2$	$MgCl_2$	urea
	(M)	(M)	(M)
UA	0.27	0	0.17
UB	0.0675	0.2025	0.17
UC	0.135	0.135	0.17
UD	0.2025	0.0675	0.17
UE	0.025	0.27	0.17

Results and Discussion

Increasing Mg concentration decreased mineral crystallinity. At low Mg concentrations calcite (C) was formed, while at higher concentrations magnesian calcite (MC) was formed (FIG. 1). Hydromagnesite ($Mg_5(CO_3)_4(OH)_2{\cdot}4H_2O$) (HM) formed at high magnesium concentration in the absence of calcium (Ca). Amount of mineral formed and compressive strength decreased with increasing Mg concentration in the mineralization medium. Ca:Mg elemental ratio in the mineral formed was higher than in the respective mineralization media. Mineralization of hydrogels with C or MC promoted cell adhesion and growth, while mineralization with HM led to higher cytotoxicity (FIG. 2).

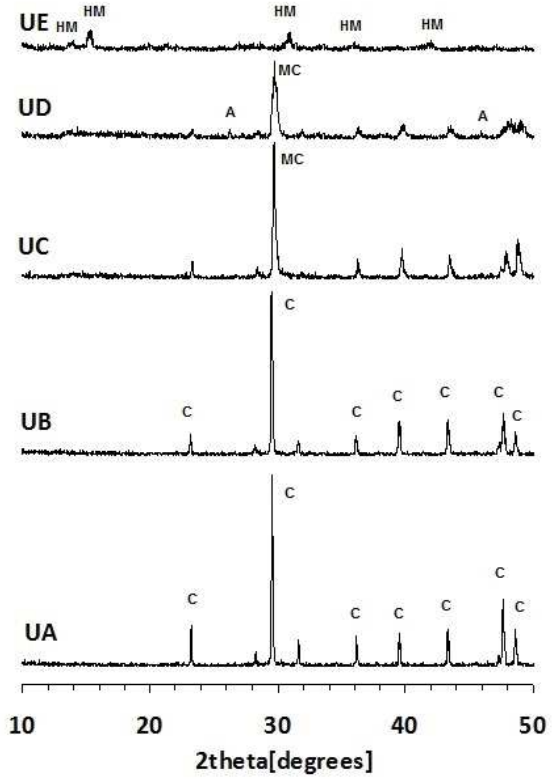

FIG. 1. XRD analysis post-mineralization. A: aragonite; C: calcite; HM: hydromagnesite; MC: magnesian calcite.

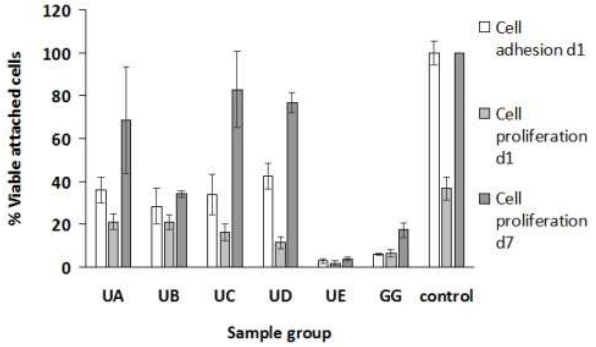

FIG. 2. %viable cells relative to control (tissue culture polystyrene) on day 1 (adhesion) or day 7 (proliferation).

Conclusions

Enzymatic mineralization of GG hydrogels with $CaCO_3$ in the form of calcite successfully reinforced hydrogels and promoted osteoblast-like cell adhesion and growth, but magnesium enrichment had no definite positive effect.

Acknowledgments

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References

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