# DEVELOPMENT OF THERMOSENSITIVE HYDROGELS OF CHITOSAN, SODIUM AND MAGNEISUM GLYCEROPHOSPHATE FOR BONE REGENERATION APPLICATIONS

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## Introduction

Thermosensitive injectable hydrogels based on chitosan neutralized with sodium beta-glycerophosphate (Na-β-GP) have been studied as biomaterials for drug delivery and tissue regeneration [1]. Magnesium (Mg) has been reported to stimulate adhesion and proliferation of bone forming cells [2]. With the aim of improving the suitability of the aforementioned chitosan hydrogels as materials for bone regeneration, Mg was incorporated by partial substitution of Na-β-GP with magnesium glycerophosphate (Mg-GP). Chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels were also loaded with the enzyme alkaline phosphatase (ALP) which induces hydrogel mineralization [3].

## Materials and Methods

4 mL chitosan solution (25 mg/mL in 0.1 M HCl,) 0.4 mL Na-β-GP (1 g/mL Milli-Q water) or Na-β-GP/Mg-GP (0.9 g Na-β-GP and 0.09 g Mg-GP/mL Milli-Q water) solution-suspension, and 0.4 mL ALP solution (25 mg/mL in MilliQ-water) were mixed together to yield 4.4 mL hydrogels. Gelation took place at 37°C overnight.
Hydrogel gelation kinetics was studied by rheometry. Hydrogel mineralization was assessed by incubation in simulated body fluid (SBF) for 14 d, followed by drying and FTIR, TEM and SAED. Hydrogels were characterized biologically by cultivating MG63 osteoblast-like cells on hydrogels and performing a Live/Dead assay. MG63 cells were also cultivated in eluates from hydrogels and growth was assessed using the MTT test.

## Results and Discussion

Substitution of Na-β-GP with Mg-GP did not negatively influence gelation kinetics (FIG. 1). Crystalline deposits were observed in both chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels after incubation in SBF (FIG. 2). Cell biological testing showed that both chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels were cytocompatible towards MG63 osteoblast-like cells (FIG. 3).

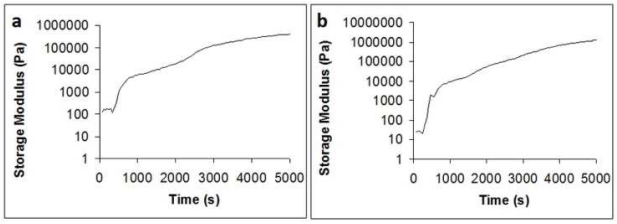


FIG. 1. gelation kinetics of chitosan/Na-β-GP (a) and chitosan/Na-β-GP/Mg-GP (b) hydrogels.

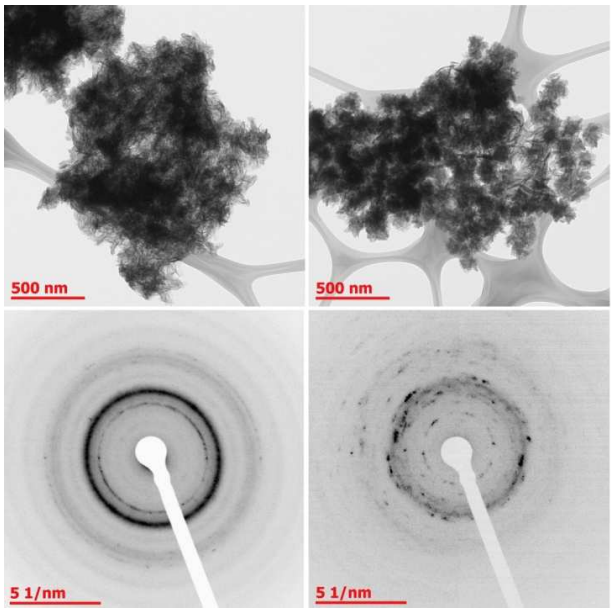


FIG. 2. TEM and SAED of chitosan/Na-β-GP (left) and chitosan/Na-β-GP/Mg-GP (right) hydrogels after incubation in SBF for 14 days.

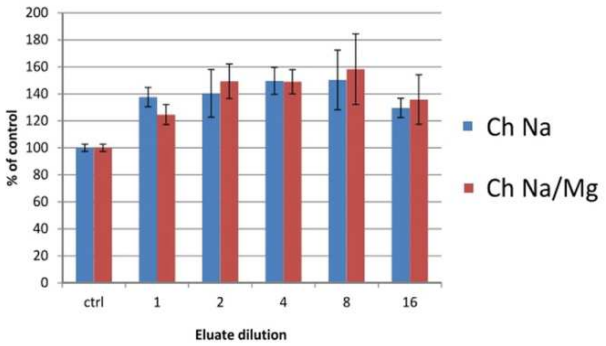


FIG. 3. Growth of MG63 cells in eluate from chitosan/Na-β-GP (Ch Na) hydrogels (blue) and chitosan/Na-β-GP/Mg-GP (Ch Na) hydrogels (red).

## Conclusions

Chitosan/Na-β-GP/Mg-GP hydrogels can be used as an alternative to chitosan/Na-β-GP hydrogels for bone regeneration applications. However the incorporation of Mg in the hydrogels during hydrogel formation did not bring any physicochemical or biological benefit.

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## References

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