Chitosan-Glycerophosphate hydrogel imparts injectability and thermosensitivity to calcium phosphate composite for bone repair

Abdellatif Chenite, Matthew Shive and Claire Jarry
BioSyntech Inc., 475 Armand-Frappier Blvd, Laval, H7V 4B3, QC, Canada

Introduction

The development of an injectable composite comprised of a thermosensitive chitosan solution and granules of biphasic calcium phosphate (BCP) is expected to greatly impact the field of minimally invasive orthopedic and reconstructive surgery. BCP is a solid mixture of synthetic hydroxapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA), a bone mineral component, and tricalcium phosphate $Ca_3(PO_4)_2$ (TCP), a potent bioresorbable hydroxyapatite precursor, in an approximate proportion of 60% wt. HA and 40% wt. TCP[1]. This biomaterial has been well-recognized as osteoconductive and biodegradable via chemical and cellular process, but despite clinical use[2], BCP is only available as granules or blocks, limiting its value particularly in cases where the defects or injured bones are not easily accessible. Here, the thermosensitive solution described previously[3] consists of a chitosan solution neutralized with β-glycerophosphate that is liquid at room temperature but gels when heated to body temperature. The osteogenic bioactivity recognized for chitosan[4] makes this solution of particular interest for this composite, since it not only delivers mineral particles but also contributes actively to osteoconduction whereby it may accelerate new bone formation. The present work describes the preparation and the characterization of fully injectable chitosan-GP/BCP composite.

Methods

BCP granules with diameter sizes ranging from 125 to 250 μm were purchased from Biomatlante (France). The preparation method for the chitosan solution was based on that described previously[3], but modified. In order to enable incorporation of mineral granules at RT, the composite was designed as two-component system intended to be reconstituted at the time of use.

The composite was characterized by rheology using a CVO rheometer (Bohlin Instruments Inc., USA) equipped with concentric cylinders. Composite hydrated morphology was examined with an ESEM-Quanta 200 FEG (Czech Republic) and injectability through a syringe and needle size 16G was evaluated using a Universal Mechanical Tester, Mach-1 (Biosyntech, Canada).

Results and Discussion

The addition of chitosan-βGP to BCP to form a thermosensitive injectable composite brings intrinsic handling and biological advantages which are particularly amenable to minimally invasive orthopedic and reconstructive surgery compared with other bone graft materials. This BCP-loaded chitosan-βGP solution was characterized rheologically, where the gelation process for the composite at 37°C was monitored as a function of the time dependence of the elastic modulus (G') and viscous modulus (G"), (Figure 1). The composite gelled within 10 minutes and after 30 minutes obtained sufficient mechanical consistency believed to be critical for clinical applications.

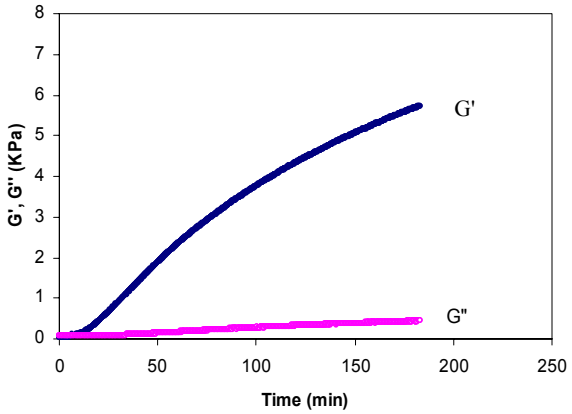

Fig. 1: *Time evolution of G' and G" of chitosan-βGP/BCP*

Morphological investigation of hydrated specimens by environmental scanning electron microscopy (ESEM) revealed a 3-D dispersion of BCP granules embedded within the chitosan-GP hydrogel matrix. This suggests that the gelled composite possesses suitable physico-mechanical properties for maintaining a well-dispersed mineral phase. This is advantageous for clinical use in the prevention of granule migration into the tissue surrounding to the injection site. The compressive force needed for the injection of chitosan-GP/BCP through a relevant needle size before gelation was determined to be approximately 6.6 N, only 6 times that required for water, and much lower than the average force exerted by the majority of adults.

Further discussion will highlight the characteristics that chitosan-βGP imparts to the composite in relation to the unique biological activity and biodegradation.

Conclusions

The chitosan-GP/BCP composite fulfills the required characteristics for an injectable bone graft, while adding several highly attractive unique characteristics. Its proposed osteogenic bioactivity is a consequence of the synergy between both components, and will likely favor new bone growth. In addition, the suitable rheological properties and *in situ* gellation of the composite offers an appropriate environment for an improved interaction between local cells and the implanted composite.

References

1. Gauthier O. et al. *Biomaterials*, **19**, 133-139, (1998).
2. Delecrin J et al. *Spine*, **25**, 563-569, (2000).
3. Chenite A et al. *Biomaterials*, **21**, 2155-2161, (2000).
4. Klokkevold PR et al. *J. Periodontol.*, **67**, 1170-1175, (1996).