

Injectable Chitosan Sponge Delivers Active BMP-7 for Potential Use with Critical Size Defects

Lamees Nayef^{1*}, Mina Mekhail^{2*}, Reggie Hamdy³, Maryam Tabrizian⁴.

1*, 2* McGill University, Montreal, Qc, Canada 3. McGill University, Shriners Hospital for Children, Montreal, Qc, Canada 4. Department of

Biomedical Engineering McGill University, Montreal, Qc, Canada.

*Authors have Equal Contribution

Statement of Purpose: Critical Size Defects (CSDs) are fractures that exceed the maximum size that can be healed spontaneously by the body and require external intervention to aid bone regeneration¹. Bone Morphogenic Protein 7 (BMP-7) is a potent growth factor that is known to accelerate bone growth². The injection of BMP-7 to CSDs is not effective because the growth factor's size is small and results in its rapid diffusion away from target³. An injectable chitosan sponge is investigated as a scaffold and drug delivery system (DDS) for BMP-7 to accelerate bone regeneration in CSDs. In this study we investigate the release kinetics profile of this new DDS and confirm the activity of the BMP-7 it delivers.

Methods: BMP-7 was mixed with chitosan solution at pH 6 and then crosslinked with Guanosine Diphosphate (GDP). Excess BMP-7 that was not entrapped was separated and tested with ELISA to work out the encapsulation efficiency of the fast gelling sponge. The sponges loaded with BMP-7 were then incubated at 37°C in PBS buffer and allowed to release BMP-7. Aliquots of PBS buffer with the released BMP-7 were taken at constant intervals for 30 days and replaced with a fresh buffer solution. The BMP-7 concentration in the aliquots was checked using ELISA and used to work out the release kinetics of this new DDS. Chitosan sponges were also loaded with 1µg of BMP-7 and allowed to deliver to cultured MC3T3 preosteoblast cells. The activity of the released BMP-7 was checked by measuring the alkaline phosphatase activity (ALP, marker of bone growth) of the cells using a colorimetric assay for ALP, and the picogreen single strand DNA assay to obtain ALP activity per mass of DNA. Cell growing under an unloaded sponge and without any sponge were tested and used as controls for comparison.

Results and Discussion:

The encapsulation efficiency for the sponge was 80%. The release profile is shown in Figure 1. Only 30% of BMP-7 was released by day 7 showing the system does not have burst release. Approximately 80% was released by day 30, showing prolonged controlled release.

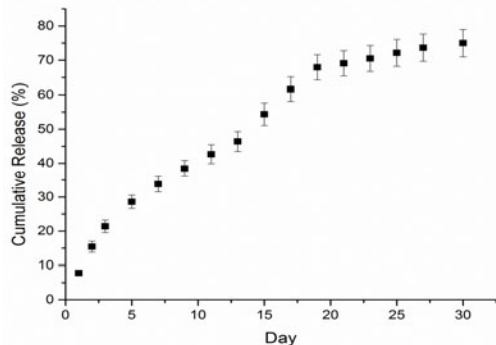


Figure 1: In vitro release kinetics profile of BMP-7 from Chitosan Sponge

The release profile was fitted into different kinetic models of release. The Higuchi model showed the best fit (Figure 2), showing that release from the sponge mainly occurs by diffusion.

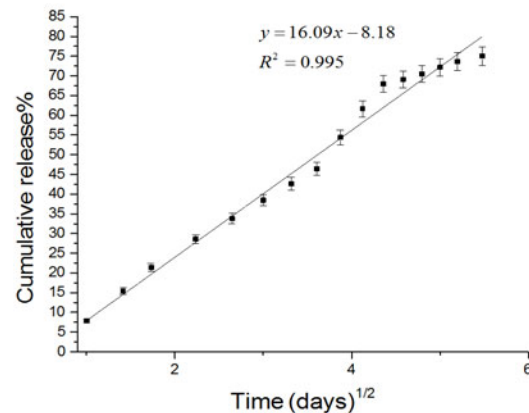


Figure 2: Release kinetics data fitted into the Higuchi Model Delivery of BMP-7 from the sponge increased ALP activity indicating that the released BMP-7 is active and capable of accelerating mineralization in vitro. The activity is higher than that of cells growing with an unloaded sponge and those growing without any sponge.

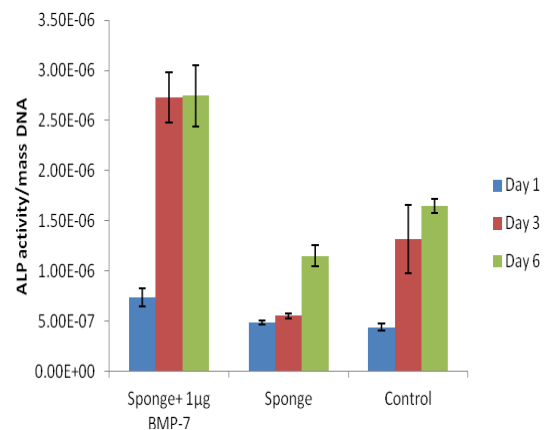


Figure 3: ALP activity per mass DNA of cells that are supplied with BMP-7 released from the chitosan sponge compared with controls

Conclusions:

In vitro studies show that the release kinetics of the chitosan sponge are favorable and that the BMP-7 delivered from the sponge is active. The results so far make the sponge a good candidate for a DDS for acceleration bone growth in critical size defects.

References:

1. Cooper G.M. et al. *Plast Reconstr Surg.* 2010; 125(6):1685-1692.
2. White A.P. et al. *Int Orthop.* 2007; 31(6):735-741.
3. Lissenberg-Thunnissen, S.N., et al. *Int Orthop.* 2011; 35(9): 1271-1280.