Composite Chitosan-Calcium Phosphate Scaffolds for Local BMP-2 Delivery and Enhanced Bone Regeneration

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Statement of Purpose: The clinical success of recombinant human bone morphogenetic protein-2 (rhBMP-2) in musculoskeletal applications has been well documented. Medtronic's popular INFUSE® system consists of an absorbable collagen sponge (ACS) which is loaded with a 1.5 mg/mL solution of rhBMP-2 and costs several thousand dollars[1]. Although efficacious, this delivery system is not ideal. Much of the BMP-2 is released from the ACS within the first few hours of implantation and has little effect *in vivo*. For this reason, large amounts of costly rhBMP-2 must be loaded initially to be successful. A delivery system which releases a majority of the loaded growth factor in an extended fashion without displaying a large burst effect is desirable and would greatly decrease the costs of receiving a needed but expensive therapeutic agent like BMP-2. Our lab has developed composite chitosan-calcium phosphate scaffolds bone scaffolds for this purpose[2]. The ability of these scaffolds to deliver BMP-2 and promote osteogenesis was tested in rat muscle pouch model.

Methods: Composite microspheres were fabricated by dropping a 3.5% chitosan (92.3% DDA) solution containing $NaHPO_4$ and $CaCl_2$ in 2% acetic acid into a basic precipitating solution. After neutralization in deionized water, scaffolds were formed by washing the composite microspheres in 1% acetic acid and packing into 15 mL centrifuge tubes. Some of the scaffolds were pre-frozen at -20°C and lyophilized in a Labconco freeze-dryer for 48 hours. Composite scaffolds were cut into dimensions of 5mm dia. x 5mm height using a scalpel. Scaffolds were sterilized using 25kGy gamma irradiation.

To test the osteoinductivity of composite scaffolds *in vivo*, the following groups of scaffolds (n=5) were randomly implanted into bilateral muscle pouches in the *latissimi dorsi* of Wistar rats: Group A- Lyophilized scaffolds without rhBMP-2, Group B- Lyophilized scaffolds with rhBMP-2, Group C- Non-lyophilized scaffolds with rhBMP-2, Group D- Absorbable Collagen Sponge with rhBMP-2 (Control). Groups B, C, and D were loaded with 4 mL of a 9.0 µg/mL solution of rhBMP-2 for 48 hours. The animals were sacrificed after one month. The implants and surrounding tissue were excised and stored in 10% formalin. Decalcified histological processing was performed, and three slices of each scaffold were stained with hematoxylin and eosin. Slides were analyzed for the amount of residual implant material, new bone, and osteoid.

Results: In a previous experiment, composite microspheres demonstrated an extended release of BMP-2 for one month[2]. In the current study, all of the composite scaffolds were found to be osteoinductive *in vivo*. Osteoid was deposited around the edges of the scaffolds; furthermore, in localized areas where the scaffolds fragmented, significant amounts of osteoid and some new bone tissue were observed (Figure 1). However, very little degradation of the scaffolds occurred after one month. For this reason, the percentage of osteoid and new bone development was normalized to the amount of actual open space (Table 1). Although only a small percentage of the total implant area was filled in with new bone or osteoid, approximately 30% of the unoccupied implant area contained bone-like tissue.

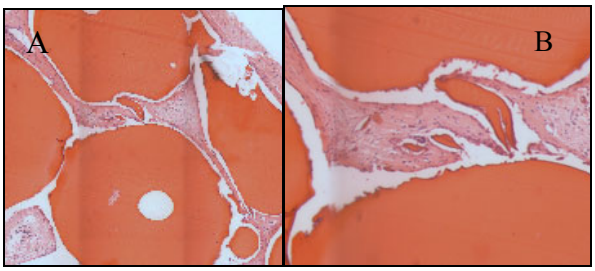

Figure 1: Osteoinductivity of Composite Scaffolds. A- Osteoid surrounding the implant (Group B) and tissue ingrowth into the scaffold is seen, B- Osteoid and new bone in the pore spaces of the composite scaffold

Table 1: Histological Analysis of Composite Scaffolds

Group	Implant (%)	Osteoid (%)	New Bone (%)	Normalized (%)
A	65.2 ± 3.7	8.8 ± 2.6	1.77 ± 0.75	30.6 ± 8.5
B	59.2 ± 6.1	10.4 ± 1.2	1.18± 0.29	28.8 ± 5.4
C	71.8 ± 3.0	7.7 ± 2.2	1.01 ± 0.70	30.5 ± 5.7
D	N/A	N/A	94.0 ± 4.4	N/A

Group A- Lyophilized, Group B- Lyophilized w/ BMP-2, Group C- Non-lyophilized w/ BMP-2, Group D- Absorbable Collagen Sponge w/ BMP-2 (Control); Normalized is (osteoid + bone)/(100 - implant).

Unexpectedly, the scaffolds without BMP-2 (Group A) displayed osteoinductivity; in addition, the osteoinductivity of the composite scaffolds was not enhanced by the addition of BMP-2. The rapidly degrading collagen sponge with BMP-2 was the most osteoinductive group in this model.

Conclusions: The composite scaffolds displayed the ability to promote and support new bone development. In Group A (no BMP-2), it is possible that BMP-2 diffused to the implant area from the other experimental implant. This observation means that these scaffolds exhibited significant osteoinductivity even at very low levels of BMP-2. The slow degradation rate may have attenuated the advantages of local BMP-2 delivery by preventing cell ingrowth. A faster degradation rate can be achieved (and is being pursued) using chitosan with a lower DDA or other acid solvents. With an improved degradation profile, the extended drug delivery of BMP-2 using composite scaffolds may provide osteoinductivity levels similar to or better than an ACS.

References:

1. Sandhu H. Spinal fusion using bone morphogenetic proteins. Orthopedics 2004;27(7):717-8.
2. Reves B et al. 2008 SFB Translational Research Meeting.