The Use of BMP-2 and Novel Polymers for Guided Tissue Regeneration

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Introduction: Bone morphogenetic protein-2 (BMP-2) has been shown to play an integral role in musculoskeletal development by inducing osteoblastic differentiation and is used clinically to promote bone regeneration. Adverse effects of BMP-2 use such as swelling, pain, and difficulty breathing have been documented in patients who underwent spinal fusion due to induced ectopic bone formation. Developing methods to better limit the volume in which BMP-2 acts could increase efficacy and reduce complications. NSAIDs have been proven to delay or prevent bony union in fractures by inhibiting cyclooxygenase-2 (COX-2). It has also been shown that BMP-2 treatment can overcome the negative effects of NSAIDs in bone repair. We have developed a novel material to improve bone healing by incorporating salicylic acid-based poly(anhydride ester) (SAPAE) with polycaprolactone (PCL) for use as a guided bone regeneration membrane. We hypothesized that using the PCL:SAPAE membrane to contain BMP-2 would enable bone regeneration within the defect while reducing bone formation outside the defect when compared to controls. A preliminary dose-response study was conducted to obtain the optimal dose of 12 μg of BMP-2 (data not shown). This optimal dose was then used to test different compositions of the PCL:SAPAE membrane in vivo.

Methods: Polymer Preparation: SAPAEs were synthesized by previously described methods using adipoyl chloride or diethylmalonyl chloride as the linker for fast-degrading (FD) and slow-degrading polymers (SD), respectively. The SAPAEs were dissolved with PCL in dimethylformamide:dichloromethane (1:1) and electrospun to obtain thin, flexible polymer sheets. The PCL:SAPAE ratio was adjusted so that salicylic acid (SA) delivery was maximized without making the material too brittle. The FD and SD polymers allowed for different release kinetics of SA.

Animal Model: The efficacy of these electrospun sheets in containing the BMP-2 to a restricted volume was tested in vivo in a critical sized femoral segmental defect model using male Sprague Dawley rats around 450 g. The electrospun sheets were anchored underneath the fixator plate and wrapped around the femur twice to enclose the defect area (Fig. 1). Each rat received 12 μg BMP-2 via a collagen sponge (15mm x 15mm x 2.75mm) which was wrapped with (i) nothing, (ii) PCL, (iii) FD-SAPAE, or (iv) SD-SAPAE. All animals were euthanized at 4 weeks post-surgery.

Micro-Computed Tomography (microCT): Three dimensional microCT images were used to analyze bone volume and bone volume to total volume ratios using a high resolution microCT system (SkyScan 1172, Bruker, Kontich, Belgium). Analysis was done both within and outside the defect space to determine defect bone regeneration versus bone formation outside the target volume.

Histology: Following microCT, all samples were embedded in poly(methyl methacrylate). Sagittal sections were cut and stained with Stevenel’s blue and Van Gieson’s picrofuchsin for soft and mineralized tissue, respectively (Fig. 2).

Results: The FD-SAPAE showed potential in containing the BMP-2 activity to a defined volume. MicroCT analysis showed no significant difference in defect bone volume between groups. However, there was significantly less bone found outside the defect space using the FD-SAPAE when compared to the unwrapped controls and the PCL (p <0.05) (Fig. 3). Histomorphometric analysis showed the x-ray-dense tissue forming in and around the defect space was indeed new bone. The PCL group showed new bone growth along the edges of the material which suggests that these membranes are not physical barriers to BMP-2 diffusion (Fig. 2).

Discussion: The SA released from the FD-SAPAE appears to limit the amount of ectopic bone being formed, while allowing bone to grow within the defect space, confirming the hypothesis. The SD-SAPAE group produced an unexpected result in that the BMP-2-induced callus formation was not contained within the defect area, but was spread out in a much larger volume when compared to other groups. It is hypothesized that the SD-SAPAE membrane produced an exuberant BMP-2 response by (A) preventing immediate diffusion of the BMP-2 into the surrounding tissue and thus delaying BMP-2 release to a later time in which the host site was more competent to produce bone, (B) the release of SA from the SD-SAPAE membrane which alters the host response to enhance the activity of BMP-2, or (C) a combination of the above processes. This unforeseen response will be further investigated at a cellular level. The FD-SAPAE membrane concentrated bone formation to the defect space. Some ectopic bone formation was noted, but it was significantly less than the unwrapped controls and PCL membrane group (6.6 mm³ vs. 26.7 mm³ and 19.1 mm³, respectively). Further modifications are needed to improve overall efficacy.

Significance: The primary significance in using SAPAE membranes for guided bone regeneration is to provide a safe and cost-effective way to capitalize on the osteoinductive properties of BMP-2 while reducing the adverse effects associated with BMP-2.
These membranes could potentially be used to aid in repairing bony defects, complicated fractures, and non-unions.

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**References:**

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**Figure 1:** Side and front view of plate and polymer fixation to femur

**Figure 2:** Histo pathological and microCT comparison of each polymer membrane wrap using 12 µg BMP-2.

**Figure 3:** Bone volume (A) within and (B) outside the defect space

* = p<0.05