Enhancing Bone Repair by Tethering Osteoinductive Proteins to Complex 3-Dimensional Degradable Scaffolds

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Synthetic scaffolds were originally proven effective for regeneration of smaller bone defects and have been supplemented with proteins, such as rhBMP-2, rhBMP-7, and rhPDGF-BB, to provide a biological stimulus required to repair larger defects. However, the expense of recombinant proteins together with their ability to diffuse away from the injury site and cause ectopic bone formation are critical weaknesses in the regeneration of large segmental defects. There has been much effort to develop a device with tethered growth factors, yet several key obstacles have prevented translation of tethering technologies, including low tethering efficiency for costly recombinant proteins, slow tethering reaction rates that require hours or days to prepare and inability to tether uniformly in complex 3D scaffolds. In this study, we have developed a tethering system that may contribute to overcome some these obstacles. As a degradable polymer system tyrosine-derived polycarbonates were covalently modified to display biotin moieties at the polymer-liquid interface. As a protein growth factor, rhBMP-2 was biotinylated preferentially targeting the N-terminus rather than the C-terminal receptor binding site as characterized using liquid chromatography after protease digestion followed by mass-spectrometric analysis. Biotinylation of rhBMP-2 did not alter induction of alkaline phosphates activity in osteoblasts after 5 days. We quantitatively compared the tethering of rhBMP-2 through the strong non-covalent biotin-streptavidin interaction1 with the non-specific adsorption of the protein on polymer thin-films using quartz-crystal microbalance with dissipation. Moreover, we were able to translate the tethering approach from a thin-film into a 3-dimensional format, building on a previously developed polymer scaffold technology2. We found uniform tethering of biotinylated rhBMP-2 and Streptavidin throughout the depth of the porous scaffold structure. The tethering efficiency for biotinylated rhBMP-2 in the scaffold was high with 96% as compared to the control with 62% by evaluation of *coomassie*-stained gels. In this comprehensive effort, we currently investigate the effectiveness for bone regeneration *in vivo* using a critical-sized defect model in the rabbit *calvaria*.

1. Stayton, P.S., et al., *Streptavidin-biotin binding energetics.* Biomol Eng, 1999. **16**(1-4): p. 39-44.
2. Magno, M.H., et al., *Synthesis, degradation and biocompatibility of tyrosine-derived polycarbonate scaffolds.* J. Mater. Chem., 2010, **20**, 8885–8893