Controlling Immobilization of BMP on PLGA scaffolds via Heparin

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Introduction

It is hypothesized that delivery of osteotropic growth factors directly to the bone-implant interface can promote bone formation. However, it is not enough to have growth factors randomly bound to the implant surface. If the orientation of the growth factors can be controlled such that the receptor binding site has maximum exposure for cells then bone growth may be further enhanced.

Poly(D,L-lactic-co-glycolic acid) (PLGA) has been frequently used as a vehicle for drug delivery and to make tissue engineering scaffolds. Other studies have investigated immobilization of proteins and peptides on PLGA, either directly or through spacer molecules.

Heparin has binding affinity for a number of growth factors, including bone morphogenetic proteins (BMPs). Because of this property, heparin has been attached to various implant surfaces in efforts to enhance the immobilization of growth factors, thereby improving host tissue growth. More specifically, the N-terminal region of BMP-2 binds with heparin and this interaction modifies protein bioactivity (Eur J Biochem 237:295, 1996).

With this information in mind, heparin was immobilized on porous, dihydrazide-derivatized PLGA scaffolds to facilitate the oriented binding of BMP.

Materials and Methods

PLGA (12 kDa, carboxyl-terminated) microspheres were prepared using a single emulsion process. Scaffolds were prepared by adding 25mg of microspheres (50-150 µm diameter) to a mold approximately 4.2mm in diameter and then heating at 43ºC for one day.

Adipic dihydrazide (AAD) was attached to the scaffolds by immersion for two hours in a solution containing 43.6 mg/ml AAD, 12.95 mg/ml EDAC, and 3.13 mg/ml NHS in 0.1 M MES, pH 6.0. Control scaffolds were treated with only MES.

Heparin was then attached to scaffolds. In addition to covalent attachment to AAD-derivatized PLGA using EDAC/NHS, heparin (20 µg) was adsorbed to scaffolds. For comparison, heparin was also bound directly to EDAC/NHS-activated PLGA.

The amounts of heparin attached to scaffolds were measured using a toluidine blue binding assay. After rinsing samples of excess heparin and other reactants, 0.005% toluidine blue in 0.2% NaCl and 0.01M HCl was added to each sample and allowed to react for 2.5 hours. Following centrifugation at 3000 rpm for 10 min, an aliquot of the supernatant was diluted 1:10 with ethanol and the absorbance read at 650 nm. A standard curve was prepared using serial dilution of heparin.

To enable quantification of BMP, the protein was fluorescently labeled with Alexa 488. Five µg of BMP-2 in PBS, pH 7.4, were allowed to bind for 3 hours. The samples were then rinsed and dissolved in 0.5 ml of acetone before reading the fluorescence.

Results and Discussion

Heparin binding

Figure 1 shows negligible binding of heparin directly to PLGA. Because of the short half life of the activated intermediate, carboxylate groups were rapidly regenerated, possibly resulting in electrostatic repulsion of the negatively charged heparin molecules. Approximately 1.1 µg of heparin was adsorbed to each hydrazide-derivatized scaffold. Almost twice as much heparin (2.3 µg) was immobilized on derivatized PLGA.

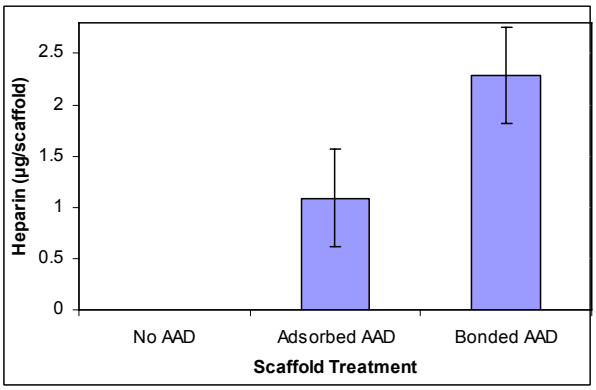

Figure 1. Amount of heparin adsorbed or covalently attached to PLGA scaffolds.

BMP-2 binding

Figure 2 shows that all samples bound BMP-2. However, covalent bonding of heparin to PLGA with dihydrazide-derivatized spacer molecules resulted in attachment of about 30% more BMP-2 relative to the other surface treatments. Interaction of BMP's N-terminus with heparin orients the protein on the PLGA surface.

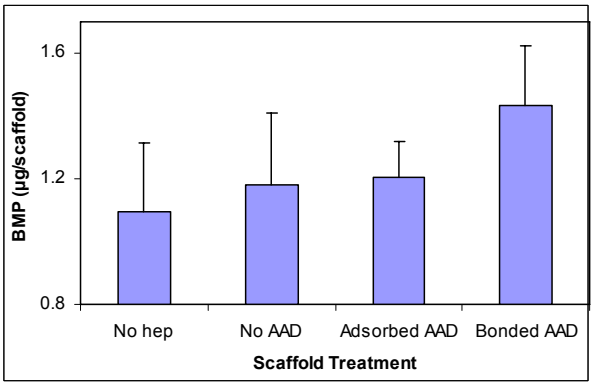

Figure 2. Amount of BMP-2 adsorbed or covalently attached to PLGA scaffolds.

Conclusion

BMP immobilization on heparin-loaded PLGA scaffolds was achieved. Ongoing research is directed at probing orientation and bioactivity of immobilized BMP.

Acknowledgement

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