Engineering a Biomimetic Periosteum for Cortical Bone Allografts: Incorporation of Heparin-Binding Growth Factors into Chitosan-Based Tissue Engineering Scaffolds

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Statement of Purpose: Critical sized defects in long bones formed by traumatic bone loss or osteosarcoma resection are commonly treated with either an autograft or allograft. Bone autografts, currently the gold standard treatment, owe their superior clinical performance over bone allografts to the retention of the periosteum[1]. The periosteum has been shown to be crucial in the bone healing process as it serves as a reservoir for osteoprogenitor cells and osteoinductive factors. Unfortunately, bone autografts suffer from limitations due to graft size availability and the potential for donor site morbidity. While bone allografts do not suffer from these same limitations, the potential for rejection is of concern. Thus bone allografts must go through a rigorous cleansing process which removes the periosteum. Periosteum removal is responsible for the suboptimal clinical performance of allografts where periosteum-mediated healing occurs. Engineering a biomimetic periosteum onto bone allografts that can deliver osteoprogenitor cells and osteoinductive factors should improve the clinical performance of bone allografts. Incorporation of heparin-binding growth factors, such as FGF-2 and TGF-β1, onto our previously developed chitosan-based tissue engineering scaffolds can provide mitogenic and osteoinductive cues to osteoprogenitor cells. The purpose of this work is to characterize the release of heparin-binding growth factors incorporated onto our chitosan-based tissue engineering scaffolds on bone allografts.

Methods: Chitosan (80 kDa, 5% acetylated confirmed through ^{1}H NMR) was acquired from Novamatrix (Sandvika, Norway). Heparin sodium from porcine intestinal mucosa (14.4 kDa, 12.5% sulfur) was purchased from Celsus Laboratories (Cincinnati, OH). Chitosan was methylated to make *N,N,N*-trimethyl chitosan (TMC) following a method previously reported[2]. Murine femurs and humeri allografts (4mm) were harvested from mice sacrificed for another study. The allografts were cleansed and frozen at −80 °C for at least 2 weeks prior to surface modification. Allografts' diaphyseal surfaces were coated with one of three tissue engineering scaffolds—polyelectrolyte multilayers (PEMs), freeze dried chitosan (FD), and electrospun chitosan nanofibers (NF). The FD and NF scaffolds required neutralization with an NH_4OH solution and then subsequently modified with TMC and heparin PEMs using a layer-by-layer deposition procedure. rhFGF-2, rhTGF-β1, hFGF basic Duoset and hTGF-β1 Duoset ELISA kits were purchased from R&D Systems (Minneapolis, MN). Recombinant proteins were reconstituted according to the manufacturer's protocol and were adsorbed under gentle agitation onto PEM-modified scaffolds in triplicate. Growth factor coated scaffolds on allografts were incubated in microtiter wells in PBS at 37 °C and 5% CO_2. At predetermined time points, an aliquot of PBS was removed, frozen down to −20 °C, and fresh PBS was replenished in the microtiter wells. The frozen aliquots were thawed and assayed with ELISA to determine growth factor concentration.

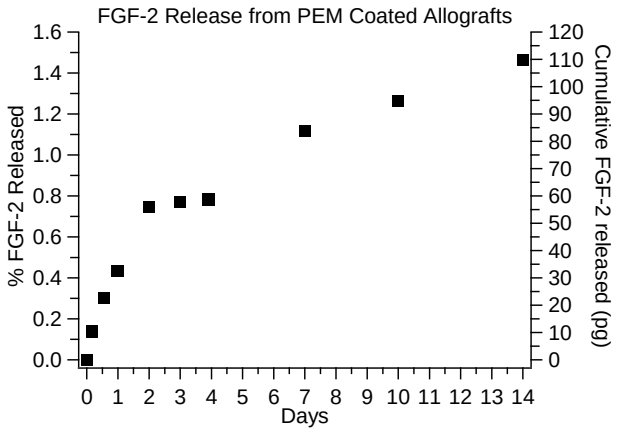

Figure 1. FGF-2 release from cortical bone allografts coated with PEMs is observed out to 14 days

Results: FGF-2 *in vito* release from our PEM-modified allografts was observed for 14 days. PEM-modified allografts coated with 25 ng ml^{-1} of FGF-2 adsorbed an average of 7.5 ng of FGF-2. This resulted in approximately 60% of available FGF-2 adsorbed onto the PEM-modified allografts. FGF-2 cumulative release was 109 pg over the 14 day period. This resulted in 1.5% of the total FGF-2 adsorbed being released. PEM-modified allografts had an average FGF-2 loading density of 350.7 pg mm^{-2}.

Conclusions: PEM-modified allografts demonstrate sustained release of FGF-2 over 14 days *in vitro*. Released FGF-2 can potentially recruit endogenous osteoprogenitor cells. With only 1.5% of total FGF-2 adsorbed released, there is a significant amount of FGF-2 left on our scaffolds which can provide highly localized signaling to osteoprogenitor cells. An average of 7.5 ng of FGF-2 adsorbed can also potentially provide an optimal mitogenic effect for osteoprogenitor cells[3]. PEM-modified tissue engineering scaffolds provide a method for facile incorporation of heparin-binding growth factors. Release profiles of FGF-2 and other heparin-binding growth factors are tunable through the addition of more multilayers or addition of nanoparticles[4]. Further investigation is warranted to assess the bioactivity of the growth factors adsorbed and released.

References:

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