Surface Modified Chitosan Tissue Engineering Scaffolds for Biomimetic Periosteum on Cortical Bone Allografts

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Statement of Purpose: Bone grafts are commonly used to heal large bone defects due to traumatic injuries and diseases such as cancer. Of the treatments available, autografts are the gold standard treatment as they provide superior healing due to the presence of an osteoconductive scaffold, osteoinductive growth factors, and osteogenic cells. However, due to the limited donor site availability of bone autografts, bone allografts present another viable treatment option. In order to mitigate the host-allograft immune response, the periosteum is removed from the allografts. The periosteum is known to contain bone tissue's regenerative capacity, and its removal severely inhibits the healing of bone allografts.[1] Glycoaminoglycans (GAGs) are important components of skeletal tissues with biochemical and biophysical functions. Sulfated GAGs, like heparin, can bind and stabilize growth factors. Therefore we propose that GAGs-based surface coatings on bone allografts can aid in recovering the regenerative potential of the periosteum by creating a biomimetic periosteum. This work demonstrates that GAGs-based nanostructured surface coatings can uniformly coat cortical bone allograft surfaces.

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 C3H mice (Age 7-9 weeks) sacrificed for another study. The allografts were cleansed and frozen 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 were neutralized with an ammonium hydroxide solution and also modified with PEMs of alternating TMC and heparin layers using a procedure described elsewhere[2]. The FD scaffolds were applied using a custom mold. The NF were directly electrospun onto the diaphyseal surface of allografts using a custom collection apparatus. Scaffold morphology was evaluated by Scanning Electron Microscopy (SEM). Surface chemistry modifications were analyzed by X-ray Photoelectron Spectroscopy (XPS).

Results: Murine femur and humeri allografts' diaphyseal surfaces were successfully modified with TMC and heparin PEMs (A, Fig. 1) and scaffolds composed of chitosan. We report a porous freeze dried chitosan coating on bone diaphyseal surface with pore sizes on the order of 100 μm in B, Fig. 1 (data not shown). We also report for the first time the successful electrospinning of chitosan NF directly onto cortical bone confirmed by SEM micrographs in C, Fig. 1. Both FD and NF chitosan scaffolds were attached to the diaphyseal bone surface and were able to endure further aqueous modification (without significant morphological change) with PEMs of TMC and heparin as evidenced by the high resolution XPS spectra of these surfaces in Fig. 1.

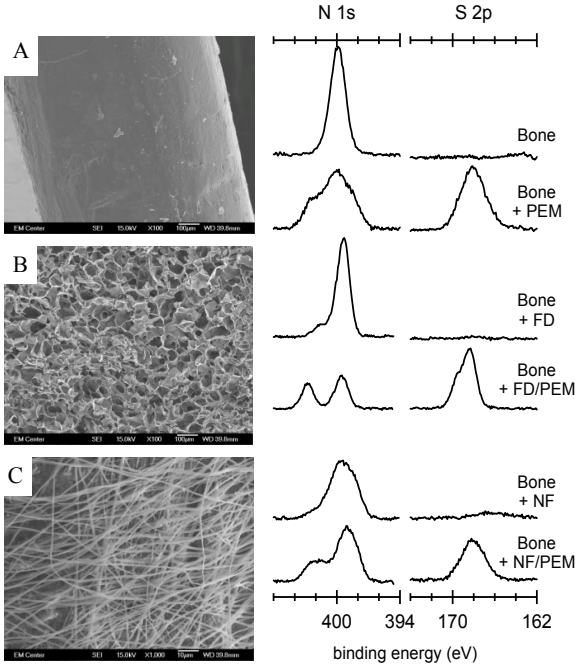

Figure 1. (Left) SEM micrographs of (A) PEM-, (B) FD-, and (C) NF-modified bone. (Right) XPS spectra of N1s and S2p envelopes confirm modification.

Conclusions: We report new approaches to developing biomimetic periosteum. Chitosan has previously been shown to be biocompatible for a variety of skeletal tissue engineering applications. The scaffold morphologies on allografts shown in Fig.1 have feature sizes suitable for bone tissue engineering. Chitosan NF were directly electrospun onto the diaphyseal surface of allografts thus enabling a thin tissue engineered scaffold to be produced on the cortical bone surface. Further PEMs deposition of TMC and heparin are confirmed by XPS analysis (appearance of sulfate at 168 eV and ammonium at 402 eV after addition of TMC-heparin PEMs). Our group has also previously shown TMC and heparin PEMs possess the ability to bind and stabilize heparin binding growth factors.[3] This allows our tissue engineered scaffolds to bind important growth factors such as FGF-2, which may play a part in healing large segmental defects in cortical bone.

References:

1) Zhang, XP. J Bone Miner Res. 2005;20:2124-37
2) Almodovar, J. Macromol. Biosci. 2011;11:72-76
3) Volpatio, FZ. Acta Biomater. 2012;8:1551-1559