Improved Cellular Recruitment and Mass Transport for Large Bone Defect Regeneration

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Statement of Purpose: Critical defects that result from trauma, complex non-unions, fusions, or resections from pathologies lack the ability to naturally regenerate void-filling bone. Large bone grafts are commonly used in an effort to stimulate bone formation in these types of defects[1,2]. The intrinsic limitation of large bone scaffolds is their inability to promote mass transport and encourage penetration of bone-forming cells and biomolecules into the interior of the graft[3]. Researchers have shown that lack of vasculature leads to necrosis of cells in the interior of large scaffolds, and delayed bone formation in regions furthest from the source of vasculature[4].

To promote sufficient transport of nutrients and bone-forming biomolecules throughout the entirety of a scaffold, as well as provide homogenous cell infiltration and distribution to attain uniform bone formation, we developed and tested a novel synthetic passive transport system. The system leverages wicking phenomena commonly used in the textile industry to move fluids and factors by capillary action. In this work, the passive transport system, consisting of a wicking polylactide (PL) fiber bundle and absorbent component (termed the "modified construct", was characterized.

Methods: The transport properties of modified (with absorbent component) wicking PL fiber constructs, unmodified wicking PL fiber constructs, and round PL fiber constructs were evaluated. The vertical wicking rates of these constructs were determined by analyzing the change in height of the liquid front over time. Bone progenitor cell penetration and retention was assessed in the modified fiber constructs and unmodified fiber constructs by assessing the vertical movment and cell densities of green-labeled D1 mouse mesenchymal progenitor cells (CellTracker green probe; Invitrogen) by fluorescent microscopy and quantifying cell movement along the constructs using Guava EasyCyte™ flow cytometry (Guava Technologies). The modified fibers were incorporated into chronOS strip scaffolds and seeded using custom-made vacuum sealed perfusion packs. Samples were incubated at room temperature for 1 hour. Cell infiltration, distribution, viability, and proliferation were assessed by (1) imaging fluorescent probes DAPI and phalloidin (Invitrogen) in the center and peripheral regions of the scaffold (2) Live/Dead cytotoxicity assay (Invitrogen) and Viacount assay using Guava flow cytometer, (3) PicoGreen Assay (Invitrogen) and biochemistry analyzer (YSI 2700).

Results: The modified wicking fiber constructs had enhanced fluid transport properties. The rate of the fluid front moving vertically was significantly greater in the modified construct than in round or unmodified fiber constructs. The transport and infiltration of progenitor cells into fibers by wicking phenomena was significantly greater in the modified constructs. The modified constructs were successfully incorporated into chronOS strips and infiltrated with cells in a perfusion pack. The total number of cells and number of viable cells was significantly greater in chronOS strip samples with modified constructs. The results showed the central interior region of the modified chronOS strip had enhanced cellular infiltration and distribution as well as significantly more viable cells and greater proliferation.

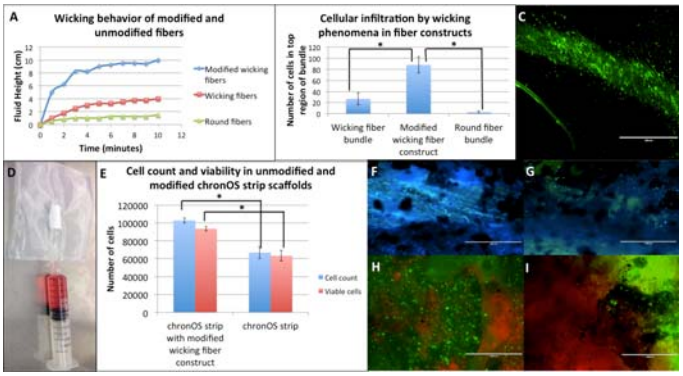

Figure 1. (A) Increased wicking rate of the modified constructs compared to the round and unmodified constructs (B) Significantly greater cellular recruitment into the top region of the modified constructs than into round or unmodified constructs, asterisks indicate significant differences ($p<0.05$) (C) Fluorescent image depicts enhanced cellular penetration into modified construct (D) Perfusion pack method of cell seeding in chronOS strips with and without the modified construct (E) ChronOS strip with modified construct increased both overall cell count and number of viable cells, asterisks indicate significant differences ($p<0.05$) (F) Fluorescent image of central interior region of modified chronOS strip shows greater cell infiltration and distribution than (G) central interior region of unmodified chronOS strip (H) Live/Dead of modified chronOS strip shows significantly more viable cells than in (I) unmodified chronOS strip.

Conclusions: Our preliminary results indicate that our modified, passive transport construct enhances cell distribution, infiltration, proliferation, and viability in a commercially available bone graft. This work suggests the passive transport construct can ultimately improve uniform bone formation.

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References:

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