Improving Transport Limitations of Tissue Engineered Bone Scaffolds

Suzanne Tabbaa and Karen J.L. Burg
Institute for Biological Interfaces of Engineering and the Department of Bioengineering
Clemson University, Clemson, SC

Statement of Purpose: Over 250,000 spinal fusion surgeries are augmented with bone grafts annually in the United States.[1] Currently, autologous grafts harvested from the patient are considered the gold standard for orthopaedic procedures, including spinal fusions[2,3]. Major limitations using autologous grafts include donor site morbidity and availability. Bone graft alternatives, which include allografts and synthetic bone substitutes, lack vascularity, an essential element of bone formation[2,3]. The development of large volumes of engineered bone is curbed by vascularity and transport limitations[4]. Conventional approaches to improve transport involve incorporation of explanted vascular networks or angiogenic factors, with limited success[5].

We incorporated synthetic engineered fibers of non-circular cross-section (termed "wicking") into a commercially available synthetic bone graft with the goal of improving transport by 1) increasing bulk fluid flow through the bone scaffold, 2) enhancing recruitment of osteoprogenitor cells and facilitating more homogeneous distribution throughout the scaffold, and 3) improving cell viability of osteoprogenitor cells throughout the scaffold.

Methods: Commercially available chronOS™ strips (Synthes) of 1.3×2.0×0.61 cm dimensions were modified with wicking fibers. Three bundles, each containing ten wicking fibers, were securely fit through separately drilled tunnels paralleling the long axis of each chronOS strip. These samples were placed in a 24-well plate and seeded with a cellular solution (10,000 total cells). The fluid transport was characterized over time using a dye solution and digital imaging. Video imaging was used to determine the rate of transport through unmodified and modified scaffolds. Cell distribution was semi-quantitatively analyzed using CellTracker green probe (Invitrogen) and fluorescent microscopy. D1 mouse mesenchymal stem cells (ATCC) were grown in Dulbecco's Modified Eagle's Medium and seeded on scaffolds with and without wicking fibers. Cell proliferation and cell viability were evaluated by determining the amount of DNA on both types of scaffolds using PicoGreen Assay (Invitrogen-Molecular Probes). A Live/Dead viability/cytotoxicity assay (Molecular Probes) was used to assess the cell viability within the cross-section of the scaffolds. Cells were viewed using fluorescence microscopy. The percentage of dead cells was determined from the images for unmodified and modified scaffolds.

Results: Fluid transport tests showed dye solution traveled at a much faster rate in the modified chronOS strip containing the wicking fibers than the chronOS strip without. The distribution of the dye solution within the bone scaffolds containing the fibers was more homogeneous. The DNA content was significantly higher in the chronOS scaffolds containing fibers than in those without, indicating a higher level of cell proliferation. The Live/Dead images of the cross-section illustrate the wicking fibers greatly improve cell viability by reducing the percentage of dead cells by 15%. The wicking fibers in the chronOS scaffold also improved penetration and distribution of the D1 cells. Fluorescent images of scaffolds containing fibers showed a more homogenous distribution of D1 cells with the CellTracker green probe.

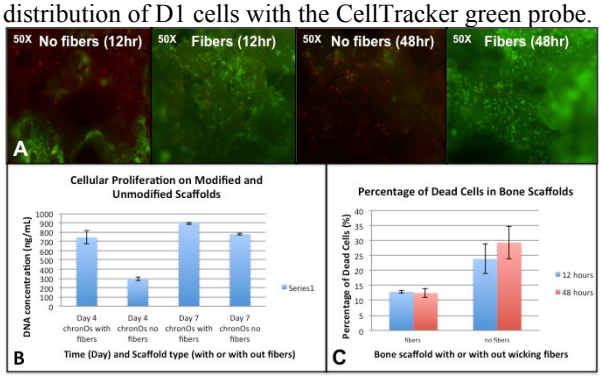

Figure 1. (A) Fluorescent images of D1 cells after Live/Dead. Images show chronOS scaffolds with fibers improve cell viability. (B) Results from Picogreen Assay show increased DNA concentration in wicking-fiber enhanced chronOS. (C) Percentage of dead cells is greater in strip without fibers.

Conclusions: Our preliminary results indicate the wicking fibers improve cell penetration, viability, and proliferation in a large bone scaffold. Transport tests and cell tracker analysis indicate the wicking fibers improve distribution of both fluid and cells within the scaffold. Future work includes longer *in vitro* studies evaluating cell viability and differentiation of mesenchymal stem cells into bone forming cells. *In vivo* studies are also essential to evaluating efficacy of the wicking fibers in the bone scaffold.

Acknowledgements: Synthes and the Clemson University Hunter Endowment

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