Incorporation of Gelatin into Self-Assembled Copper-Capillary Alginate Gel Scaffolds Enables Stem Cell Adhesion

B.J. Willenberg, F-W. Meng, T. Zheng, M.D. Weiss, D.A. Steindler, C. Batich and N. Terada.
University of Florida.

Statement of Purpose: Derivation of biomaterials from capillary alginate gels is a recent and promising tissue scaffold research focus. Due to the area's nascence however, there exists a paucity of studies detailing the synthesis, characterization and use of different derivative materials; hence, ideal capillary alginate gel-derived scaffolds or applications remain to be identified. To help address this dearth of scaffold variety, we developed gelatinized copper-capillary alginate gel (GCCAG) scaffolds specifically targeted at facilitating cell adhesion. We tested the scaffolds with NIH-3T3 fibroblasts as a model differentiated cell line and multipotent astrocytic stem cells (MASC) as a model stem cell line.

Methods: CCAG was made as previously described (Willenberg, BJ. J Biomed Mater Res A. 2006;79A:440-450). Briefly, a 2% (w/v) alginate (Keltone® LV CR, ISP Alginates, San Diego, CA) solution was poured into an alginate-smeared petri dish and submerged in a bath of 0.5M $CuSO_4$ solution for two days. The resulting gel was then removed, washed in water and sectioned into blocks (5×5×3 mm). The process was the same for GCCAG except the gelling solution was 2% (w/v) alginate/2% (w/v) gelatin type A (Sigma-Aldrich, St. Louis, MO) and the gel was grown in an oven at 40°C. Sectioned CCAG blocks were then crosslinked with 2% (w/v) chitosan oligosaccharide (oligochitosan) lactate (Sigma-Aldrich) as described previously (Willenberg, BJ. J Biomed Mater Res A. 2006;79A:440–450) to produce OCCAG scaffolds; GCCAG was crosslinked by first soaking blocks in a phosphate buffered saline (PBS) solution with *N*-hydroxysulfosuccinimide (Pierce, Rockford, IL, 0.14 g Sulfo-NHS/g hydrated GCCAG). *N*-Ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma-Aldrich) was then added (0.13 g EDC/g hydrated GCCAG) and the mixture gently shaken at room temperature for 20-24 h; resultant GCCAG scaffolds were then removed and washed with water. All scaffolds were sterilized by soaking in 70:30 ethanol solutions prior to cell experiments.

For NIH-3T3 experiments, cell suspensions were pipetted on top of thin OCCAG or GCCAG sheets (cut from scaffold blocks parallel to the capillary long-axis), and cultured Dulbecco's® Modified Eagle Media (DMEM, GIBCO®, Invitrogen, Grand Island, NY) containing 10% fetal bovine serum (FBS, Atlanta Biologicals, Norcross, GA), 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 25 mM HEPES (GIBCO®) for 3 days. For MASC experiments, green fluorescent MASCs, prepared as previous described (Zheng T. Neuroscience. 2006;142:175-185), were seeded into GCCAG scaffold blocks via centrifugation of cell suspensions atop capillary openings, and cultured in DMEM with F12 and N2 supplements (GIBCO®), 5% FBS, 20 ng/mL epidermal growth factor (Sigma-Aldrich), and 10 ng/mL basic fibroblast growth factor (Sigma-Aldrich) for 10 days. Cell-scaffold combos were then fixed in 4% paraformaldehyde in PBS, vibratome sectioned, mounted, stained with DAPI nuclear stain and immunolabeled with antibodies against either glial fibrillary acidic protein (GFAP, 1:10, Shandon, Pittsburgh, PA) or ß-III tubulin (1:1000, Promega, Madison WI) as previously described (Chen, KA. Exp Neurol. 2006;198:129-135).

Results/Discussion: Figure 1 shows the typical microstructure of CCAG-derived scaffolds (Fig. 1a and b), and the results of the cell culture experiments (Fig. 1c-f). Fibroblasts showed a well-spread spindle morphology on GCCAG (Fig. 1d) indicating good cell adhesion. In contrast, fibroblasts were rounded and granular on OCCAG (Fig. 1c), a typical morphology of dead or dying cells. MASCs cultured within GCCAG blocks were positive for either ß-III tubulin (Fig. 1e), a pan neuronal marker, or GFAP, an astrocyte marker; cell processes of both types were stretched in the direction of GCCAG capillaries indicating cells were well adhered.

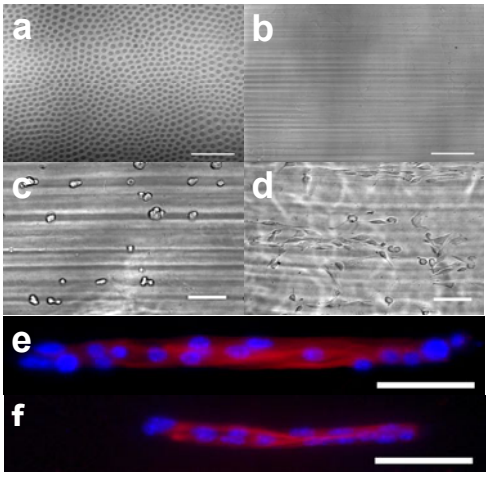

Figure 1. a) CCAG perpendicular and **b)** parallel to capillary long-axis. **c)** NIH-3T3s on OCCAG, and **d)** GCCAG scaffolds. **e)** Fluorescent micrographs of ß-III tubulin$^+$ (red), and **f)** GFAP$^+$ (red) MASCs cultured within GCCAG scaffolds. Scale bars = 250 µm for **a** and **b**, 100 µm for **c** and **d**, and 50 µm for **e** and **f**. Capillary long-axis is horizontal for **b**-**f**.

Conclusions: GCCAG scaffolds crosslinked with EDC chemistry are adhesive to different cell types including stem cells. These novel scaffolds are easily produced using relative mild processes, and should prove advantageous over previously reported capillary alginate gel-derived scaffolds (Dittrich R. Adv Sci and Technol. 2006;49:159-164. Prang P. Biomaterials. 2006;19:3560-9).Comparative studies using multiple stem cell lines to investigate stem cell-scaffold interactions in our CCAG-derived scaffolds are currently underway.