Application of Colloidal Gas Aphron Foaming Techniques to Alginate: Creation of a Novel Tissue Scaffold

Elizabeth E Johnson[a,b], Norman Meznarich[a,c] and Buddy D. Ratner[a,b,c].
[a]University of Washington Engineered Biomaterials Research Center, Seattle, WA 98195 USA
[b]Department of Materials Science and Engineering, [c]Department of Bioengineering
University of Washington, Seattle, WA 98195 USA

Statement of Purpose:

We previously reported use of colloidal gas aphrons (CGAs) for the generation of tissue engineered scaffolds based on poly(vinyl alcohol)-amino acid (PVA-AA) hydrogels. CGAs eliminate the need for solvents, crosslinkers or salt templates and are applicable for many hydrogel systems. Here we adapt the CGA method for development of a biodegradable scaffold based on the natural polymer, alginic acid. Alginate has been shown to induce neovascularization, attenuate left ventricular dilation and prevent heart failure *in vivo* making it a promising material for use in cardiovascular engineering [19,139].

Methods:

Glycine, alginate, pH 7.4 phosphate buffered saline (PBS) tablets, calcium chloride, L-α-phosphatidyl choline from fresh egg yolk, dicyclohexyl carbodiimide (DCC), dichloromethane and acetone were purchased from Sigma-Aldrich. Sodium dodecyl sulfate (SDS) was obtained from BioRad. F68-NF Prill, F98 Prill, F87 and P85 PLURONIC® surfactants were obtained as gifts from BASF Corp. Nanopure water was used in all work. To obtain a usable alginate CGA a surfactant was needed. We investigated three surfactant types: (1) charged, synthetic (SDS), (2) uncharged, synthetic (Pluronic®, BASF Corp.) and (3) a natural surfactant (phosphotidyl choline). Pluronics® were determined to be the safest effective choice. Four different Pluronics® were evaluated to test the effects of surfactant strength, gelation ability and molecular weight on CGA production. Alginate foam was generated using a 5 wt% aqueous alginate solution mixed with glycine monomer (10 wt% overall) and frothed for 2 minutes at 2000 rpm. 10 mL of pluronic solution (3, 5, 7 or 10 wt% in water) was added and the mixture was frothed for an additional 10 min. Samples were frozen at -80 ºC and lyophilized (VirTis, benchtop 6.6). The scaffolds were non-covalently crosslinked in 4 wt% $CaCl_2$ solution, rinsed in acetone and dried overnight at room temp. Half of the samples were covalently crosslinked by reaction with dicyclohexyl carbodiimide (DCC) in dichloromethane (0.4 wt%). Residual DCC was removed by dichloromethane and water washes over a 24 hour period. Samples were dried by lyophilization and sterilized by ethylene oxide gas. Changes to processing parameters and scaffold chemistry were explored through systematic evaluation of chemical and physical variables. Characterization of scaffold pore structure was achieved using scanning electron microscopy (SEM) (SEI Siron XL 30, 5kV beam) and Digital Volumetric Imaging (DVI). The tensile properties of PBS swollen scaffolds (15mm x 1.5mm x 5mm) were measured on an Instron™ 5500R by straining at 10mm/min until failure. The volumetric swelling ratio (Q) of the scaffolds was examined by measuring the weight of swollen samples (m_s) after soaking in water for 1 week and the final dry polymer weights (m_{fd}) following lyophilization.

Cytotoxicity testing was conducted using a modification of International Standards Organization 10993 recommendations. Cell adhesion and viability experiments were conducted on 8mm scaffold disks rehydrated in sterile PBS. 100,000 cells (C2C12 mouse myoblasts) were seeded on each scaffold under static conditions and in the presence of fetal bovine serum (20%v/v). Cell adhesion was monitored at 4 and 8 days in culture using a LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes). Cells were imaged using epi-fluorescent microscopy.

Results / Discussion:

The CGA technique was successfully used to produce solid, microporous alginate materials using Pluronic® F87 (10 wt%) coupled with a mild calcium chloride crosslinking step. DVI and SEM imaging show a highly porous, open network on the scale of a few hundred micrometers. *In vitro* cell culture studies found the scaffolds to be non-cytotoxic and to induce cell adhesion and spreading.

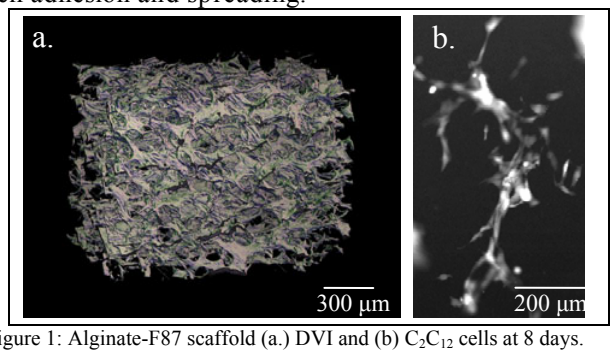

Figure 1: Alginate-F87 scaffold (a.) DVI and (b) C_2C_{12} cells at 8 days.

Alginate-F87 scaffolds were found to have a breaking strain of 30 ± 9, tensile strength of 25 ± 10 kPa and an elastic modulus 121 ± 50 kPa.

Conclusions and Future Work:

We have utilized CGA foaming to develop a versatile new technique for the generation of tissue engineering scaffolds. Here we adapted the technique to a degradable hydrogel system using alginate. These alginate scaffolds have tensile properties with great promise for use in soft tissue applications. In addition *in vitro* studies show both cell adhesion and spreading without further modification. In vitro degradation studies are underway and evaluation these scaffolds *in vivo* is planned.

Acknowledgements:
American Heart Association fellowship 0415312Z, NIH grant R24HL64387 and NSF grant EEC-9529161.