Cell Colonization on Negatively Charged Polysaccharide Matrices

Annett Ullm*, Jeremy Tillman*, Sundar V. Madihally.
School of Chemical Engineering, Oklahoma State University, Stillwater, OK 74078.

Introduction: The field of tissue engineering offers great potential in rebuilding healthy viable tissues and organs. However, one of the hurdles in this field is incomplete understanding of the influence of 3-dimensional environment on cell colonization. Glycosaminoglycans (GAGs), components of extracellular matrix, influence diverse cellular functions. Despite extensive analysis in various pathophysiological conditions, their effect in three-dimensional environment is not clearly understood. This could be attributed to a) heterogeneity in the type including differences in molecular weight (MW), degree of sulfation, and location, and b) difficulty in synthesizing stable GAG matrices.

This research focused on developing scaffolds using dextran sulfate (DS), a semi-synthetic GAG analogue, using chitosan which provides a positive charge. Influence of DS MW (5kD, 10kD, and 500kD) on cell colonization was also evaluated in 3-D and 2-D scaffolds. Further, to understand the interaction of fibronectin-mediated cellular interaction, fibronectin binding was also characterized.

Materials and Methods Chitosan (>310 kD MW with 85% degree of deacetylation), was used to make structures, combined with negatively charged DS. 2D matrices were formed by air drying chitosan solution and then immobilizing DS. 3D matrices were formed inside 24-well culture plate using 300µL of chitosan solution in each well and frozen at -20°C for at least 4 hours prior to lyophilizing overnight at -86°C. Two methods were used to form the negatively charged scaffolds: i) forming chitosan structures first and then allowing DS to react; ii) mixing DS and chitosan in solution first and then synthesizing scaffolds. Various volumes including 10, 100, and 500 µL of DS were used to determine the optimum combination in both cases. Pore morphology analysis was performed using scanning electron microscopy (SEM). Amount of DS present in the scaffold was measured using Toluidine blue assay.

To test cellular support, 25,000 and 10,000 fibroblasts were seeded on 3-D matrices and 2-D membranes and analyzed for growth by MTT-formazan assay, and spreading by actin staining and confocal microscopy. Histological analysis by H/E staining was also performed to better understand cell colonization. Amount of fibronectin bound to various matrices was evaluated using ELISA.

Results /Discussion: Immobilization of DS after forming chitosan scaffolds produced a scaffold with skinny layer, probably due to surface gelation, blocking cell accessibility to pores. Binding DS before scaffold formation resulted in a matrix with open pores. Analysis of pore structures with the SEM showed increased surface roughness and open pore architecture in-DS-chitosan matrices. Matrices formed with 50 µL of DS solution showed optimum porous properties and structural integrity. Analysis for the quantity and stable immobilization of DS by toluidine blue assay indicated significant presence of DS in the 3D matrices even after seven days of incubation in phosphate buffered saline solution at 37°C. In order to obtain scaffolds with similar number of sulfate group, 4% 5kD, 2% 10kD and 1% 500kD DS solutions were used.

Cell proliferation analysis by MTT-formazan assay showed increasing DS MW-dependent growth kinetics; increased MW showed better support for cell growth on 3D matrices. However, the trend was reverse in 2D matrices. Cytoskeletal organization analyzed by actin staining showed organized actin distribution in 3-D matrices. Histological analysis by H&E staining showed uniform cell distribution inside the scaffold.

When fibronectin binding was analyzed, all DS-containing matrices showed negligible binding, probably due to lack of binding domain and positive charge, unlike chitosan.

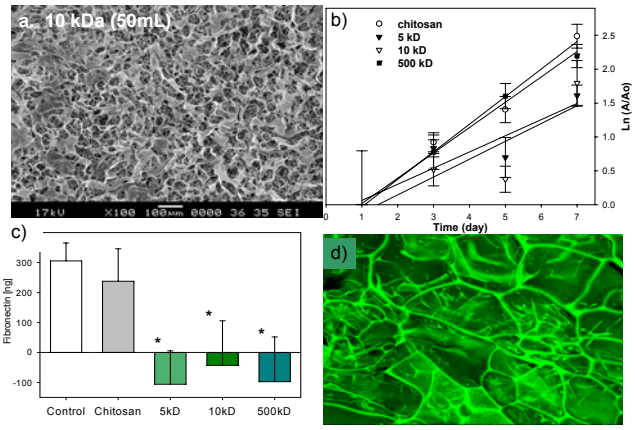

Figure:a) Micrograph of 10kD DS, b) growth kinetics of 3D scaffolds, c)Fibronectin binding on 3D scaffolds and d) confocal micrograph (actin staining) of 3D structure

Conclusions: In summary, results show significant difference in the influence of sulfate charge on cell colonization. There is no influence of fibronectin affecting the cell proliferation in increasing MW of DS-containing scaffolds..

* Both authors contributed equally