Biophysical Effects of Matrices on Cell Colonization

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Introduction: Much of tissue engineering research has been conducted on two dimensional (2D) membranes and three dimensional (3D) porous scaffolds generated from various natural and synthetic polymers. Using injectable hydrogels as a less invasive alternative is also an option. Although temperature sensitive and pH sensitive hydrogels have been developed, there has been no systematic comparison of cell colonization in these different forms. The objective of this study was comparing the cell colonization characteristics on 2D membranes, 3D porous structures and hydrogels made from same materials. Chitosan was selected as the base material as it does not have any specific cell binding domain and it can be easily processed into various forms without altering the functional groups. In addition, these forms are known to have a wide range of elastic moduli (2 mP to 2 P). Further, gelatin can be blended without any chemical reaction to study the influence of cell binding domain in various matrices [1].

Materials and Methods: Chitosan (200-300 kDa molecular weight, Mw, 85% DO), Gelatin type – A (300 Bloom) and 2-glycerolphosphate (2-GP) were obtained from Sigma Aldrich Chemical (St. Louis, MO). 0.5% w/v chitosan solution was prepared in 1N HCl and neutralized with 0.56 g/mL of 2-GP. To obtain chitosan-gelatin matrices, equal amount of gelatin was added to chitosan solution. 400 µL of the solutions were frozen in 24-well plates overnight at -20°C and lyophilized for 12 h to obtain porous structures. 2D membranes were prepared by air drying the same solution on Teflon and then transferring these to a 24 well plate, precoated with 100 µL of the same solution. To prepare hydrogel samples, 400 µL of the solution was incubated overnight at 37°C in a 24 well plate.

To understand the cellular interactions, human foreskin fibroblasts were cultured in serum-free medium. Cells were prestained with non-toxic cytoplasmic stain (CFDA) prior to seeding on to the structures. Viability was assessed by measuring the amount of fluorescence in the medium and by flow cytometry. To assess cell morphologies, cells were stained after four days for cytoskeletal actin using Alexa Fluor 546 phalloidin and DAPI for nuclei after fixing the structures in Paraformaldehyde and permeabilizing with ethanol. Cell proliferation was analyzed using flow cytometer studies. Further, total protein content in the media supernatant was assessed by BCA. The amount of secreted collagen in the spent medium was assessed using the Sircol™ Assay. A negative control (only samples, without exposure to cells) was incubated for four days, and the growth medium collected was used to quantify total collagen secreted. Matrix metalloprotease 2 (MMP-2) and matrix metalloprotease 9 (MMP-9) fluorogenic peptide (DNP-Pro-Leu-Gly-Met-Trp-Ser-Srg-OH) was used to assess gelatinase activity.

Results: Based on the amount of fluorescence leached into the growth media, chitosan structures showed less viability relative to chitosan-gelatin structures. Cell proliferation analysis using flow cytometer showed that there were more cells on Chitosan-gelatin 3D than in other structures (**Figure 1**). However, the growth rate was significantly less as the CFDA intensity did not change. Analysis of cell spreading results showed spindle shaped cells on chitosan-gelatin porous structures. There was minimal spreading on all other structures. Total amount of protein in chitosan hydrogels was 25% lesser than 3D and 2D structures. However, the total protein content remained the same for all three forms of chitosan-gelatin mixture. For both chitosan and chitosan-gelatin, collagen content was less on 2D matrices (**Figure 1**).

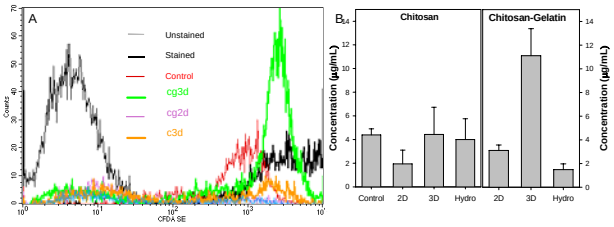

Figure 1. Cellular Activity on matrices after four days (a) CFDA expression. (b) secreted collagen content.

When MMP-2/MMP9 activity was measured, growth media on 3D chitosan porous structures had an increased activity. However, MMP activity was higher on 2D and hydrogel structures of chitosan-gelatin.

Conclusions: Results show that hydrogels support less cell spreading relative to 3D matrices. Presence of gelatin increased cell spreading on porous structures, however, there was no biological advantage in hydrogels. From the collagen assay, gelatin containing 3D structure supported cell colonization better than other components. More tests such as histology and matrix-bound extracellular matrix analysis would provide more information on cell colonization.

References:

[1] Huang Y, Onyeri S, Siewe M, Moshfeghian A, Madihally SV. In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. Biomaterials. 26(36):7616-27, 2005.