Effect of Proteoglycans on the Surface Modulus of Agarose Gels : Indentation Testing

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Statement of Purpose: The mechanical properties of tissues are dependent on the composition and structure of the extracellular matrix (ECM). Proteoglycans (PGs) are a major constituent of mammalian ECM and play a significant role in the mechanical properties of various tissues, in both the normal and pathological states. For example, in osteoarthritis, decreases in PG content of cartilage correlates with a decrease in tissue stiffness.[1] Chondroitin Sulfate PGs (CSPGs) are upregulated in Central Nervous system (CNS) injuries, creating a biochemical barrier to nerve regeneration.[2] We hypothesize that this increase in CSPG concentration in the glial scar also creates a mechanical barrier to regeneration. We have developed a simple mesoindentation system with an indenter size comparable to the diameter of a mammalian cell, in order to study the mechanical properties of soft, hydrated materials and tissues. The goal of this work was to determine the effect of CSPG addition on the modulus of model agarose gels, using this mesoindentation system.

Materials: Agarose gels (Seaprep, Cambrex Bio Science Rockland, Inc) with concentrations of 0.6, 1, 1.5, 2 and 5% (w/v) were fabricated according to Balgude et al.[3] Embryonic chick brain CSPGs (Chemicon International, Temecula CA) were added, separately, to the surface and the bulk of agarose gels (1, 1.5 and 2%) at a concentration of 50 µg/ml. Surface modified gels were prepared by adding CSPG solution to agarose gels and incubating at 37°C, 5 % CO_2 for 4 hours. For fabrication of bulk modified gels, CSPGs were added to non-gelled agarose at 40°C and then the samples were gelled. The effect of enzymatic digestion on gel modulus was studied by adding chondroitinase ABC (cABC) in varying concentrations (50 µl of 0.2, 0.5, 1 and 2 U/ml) to 1% CSPG-modified agarose gels.

Mechanical testing and analysis: Experiments were performed using a custom built, mesoindentation system with a 14µm diameter, flat cylindrical indenting tip. Samples were preloaded to 0.05 mN to ensure complete contact with the sample surface. Once the preload was achieved, the load and displacement values were zeroed. Blank and CSPG-modified gels were cyclically indented by means of a piezoelectric actuator, using a triangular waveform with a peak piezo displacement of 60 µm and a frequency of 1 Hz, for a period of 10 s. cABC treated gels were tested in a similar manner, but with for a period of 5 min after enzyme application. A modified version of Sneddon's equation was used to calculate modulus from the load-displacement data.[4] SPSS software was used for statistical analysis and Tukey HSD post hoc tests were performed where significant differences ($p<0.05$) were indicated by ANOVA.

Results and Discussion: As shown in Figure 1, the modulus of agarose gels increased significantly with concentration, except between the 0.6% and 1% agarose gels. Upon addition of CSPG, there were significant increases in modulus in the 1 and 1.5 % agarose gels. For these two agarose concentrations, there was a significant increase in modulus between the modified (bulk and surface) and unmodified gels and also between the bulk CSPG modified and the surface CSPG modified gels. CSPG modification did not significantly affect the modulus of the 2 % agarose gels. The mechanical contribution of CSPGs is likely masked by the higher stiffness of the blank 2% gel.

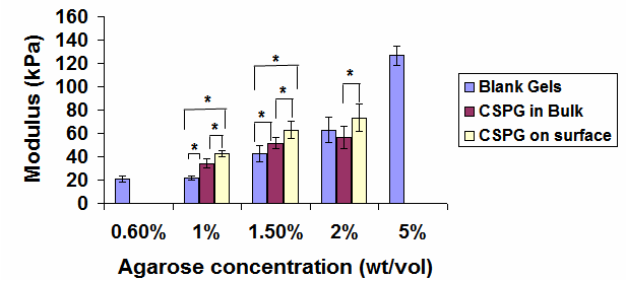

Figure 1. Modulus as a function of gel composition. Asterisks (*) imply significant differences between groups ($p< 0.05$).

Figure 2 shows the effect of cABC treatment on the modulus of CSPG surface modified, 1% agarose gels. Treatment with 2 U/ml cABC resulted in a rapid decrease in modulus of the gels, with values reaching those of blank 1% agarose gels within 5 min. The addition of 0.2 U/ml cABC had little effect on the modulus over this time frame.

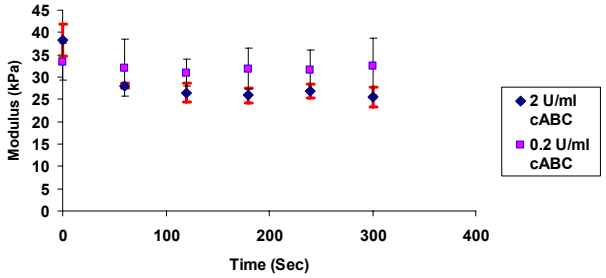

Figure 2: Effect of cABC addition to 1% agarose gels with CSPG surface modification. Addition of 2 U/ml cABC results in a rapid decrease in modulus.

Conclusions: We have a system capable of measuring the mechanical properties of soft materials and tissues on a cellularly relevant scale. This is important because substrate stiffness governs cellular response.[5] Using the mesoindenter we have shown that CSPG content and distribution affects the modulus of soft gels significantly. This increase is reversible by enzymatic digestion of CSPG and can be monitored using the mesoindenter.

References:

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