Hierarchical Organization of Proteoglycans in Cartilage

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Cartilage is a complex tissue whose extracellular matrix (ECM) mainly consists of charged proteoglycan (PG) assemblies imbedded in a fibrous collagen network. The biochemistry of cartilage ECM has been extensively studied in the last couple of decades. However, the physical-chemical interactions that govern the equilibrium morphology and dynamics of the PG assemblies remain poorly understood.

The major PG of cartilage is aggrecan. Its primary role is to provide the osmotic properties necessary for cartilage to resist compressive loading. The aggrecan monomer exhibits a bottlebrush structure, consisting of an extended protein core to which many chondroitin sulfate and keratan sulfate (linear sulfated polysaccharides) chains are attached. In the presence of hyaluronic acid and a link protein, aggrecan molecules self-assemble into a secondary bottlebrush array with as many as 100 aggrecan monomers condensed on a filament of hyaluronan. This higher-level superstructure yields a hydrated, viscous gel that gives cartilage its resilience and high compression modulus.[1]

Our goal is to quantify microscopic and macroscopic changes resulting from self-assembly and structure formation using a complementary set of physical measurements that ultimately will lead to a comprehensive model of cartilage with quantitative predictability. Achieving this goal entails understanding the static and dynamic properties of ECM's polymer constituents and their assemblies. We recently developed an experimental approach to determine structure at different hierarchal levels by combining scattering techniques, including small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and static light scattering (SLS) with macroscopic methods (osmotic and mechanical measurements).[2]

Figure 1 shows the intensity $I(q)$ measured by SLS, SANS and SAXS for aggrecan solutions at 3 different concentrations. The scattering curves are roughly parallel to each other and the intensity increases with increasing aggrecan concentration. In the lower q-range of the figure, the slope of the $I(q)$ plots is approximately -2. In the q range above 0.01 $Å^{-1}$ another power-law behavior is distinguishable with a slope approximately –2.7. The value of a fractal exponent between 2.5 and 3 is characteristic of a weakly interpenetrating disordered array of branched structures. The upper size of these primary aggregates, calculated from the position of the knee at $q \approx 0.01$ $Å^{-1}$ in the scattering curve, is approximately 500 Å, i.e., the size of a small number of aggregated aggrecan bottlebrushes.

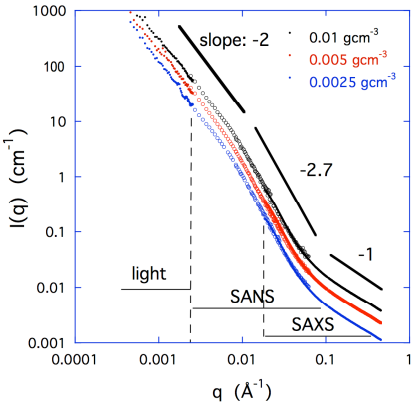

Figure 1. Scattering response of aggrecan at different concentrations in 100 mM NaCl solutions with D_2O.

Osmotic pressure measurements made on aggrecan solutions reveal the self-assembly of the molecules into microgel-like assemblies. In such associating systems, loosely packed microgels several microns in size, coexist with smaller associations, as well as individual aggrecan bottlebrushes. At low aggrecan concentration the formation of the aggrecan-HA complexes reduces the osmotic pressure by roughly 30%.

An important striking finding from the combined scattering and osmotic observations is that the structure of aggrecan and its assemblies are highly insensitive to changes in the ionic environment over the entire range of length-scales probed, i.e., from the nanometer to the micrometer range. These results confirm aggrecan's role to provide and maintain osmotic resilience to compressive loads in cartilage while serving as an ion reservoir mediating Ca metabolism.[3]

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