Immobilization of Chondroitin Sulfate for the Fabrication of Biomimetic Brush Structures

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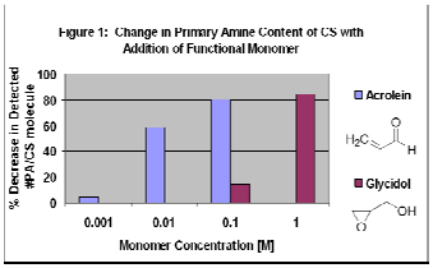
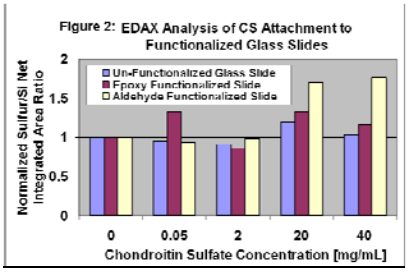
Introduction: Intervertebral disc (IVD) degeneration is accompanied by alterations in the biochemical constituents of the IVD which may be associated with back pain. Among these changes is a loss of proteoglycans (PGs) in the nucleus pulposus (NP), resulting in a loss of the charged glycosaminoglycans (GAGs), primarily chondroitin and keratan sulfates (CSs and KSs), which are covalently attached to the aggrecan core protein.[1] **Statement of Purpose:** The goal of this project is to develop CS brush structures as a biomimetic replacement for the ubiquitous biomacromolecule, aggrecan, for use as a minimally invasive early interventional technique for the treatment and prevention of back pain. We have developed a strategy for the end functionalization of CS and utilized this method for the immobilization of CS onto glass surfaces via their terminal end functionality. End-functionalized CS can be used to fabricate molecular bottle brushes via polymerization strategies such as grafting-to and grafting through, as well as brush surfaces. **Background:** Aggrecan and other similar PGs comprise 15% wet weight of the NP[1]. They work to resist mechanical force in the NP, and provide a hydrostatic tension to the AF via molecular interactions. Aggrecan is composed of a protein core to which GAGs are covalently bound in a very dense array. Charged anionic groups on the GAG chains draw water into the disc and electrostatic repulsions generated between closely packed GAG chains resist deformation thereby allowing the tissue to distribute mechanical forces.[2] **Methods:** *Determination of CS primary amine content.* CS from various vendors (CS-4, Calbiochem, CS-4 and CS-6, Sigma) were investigated for their primary amine (PA) content using the fluorescamine assay (Sigma). Presence of PAs is indicated by an increase in fluorescence. L-serine, with one PA/molecule was used to establish a standard curve. *CS-monomer conjugation.* PA reactive monomers, acrolein (via aldehyde) and glycidol (via epoxide) were reacted with CS in solution (0.1M sodium borate buffer, pH 9.4, room temperature, 4hrs, 10ul/mL cyanoborohydride for acrolein) and subsequently assayed for their PA content. *Surface Modification with CS.* Epoxide and aldehyde functionalized glass slides were purchased from Genetix and incubated in CS solution. Slides were subsequently rinsed to remove any adsorbed CS. Changes in surface hydrophilicity were measured by contact angle and surface sulfur content by ESEM with EDAX chemical analysis software. Experiments were conducted in triplicate. **Results:** *CS PA content.* The presence of PAs in CS from various suppliers was investigated. CS-4 from the supplier Calbiochem contained approximately 7 PAs / CS molecule. CS-4 and CS-6 from Sigma had approximately 3 PAs / CS molecule. The repeat CS disaccharide does not contain PAs however PAs may be available at the terminal end of CS as a result of the cleavage that occurs in isolating CS from the PG core.[3] At least one PA detected is expected to be on the terminal end of CS, and as such, this PA will be most available for conjugation as it is more freely able to probe its environment. The high level of PAs / CS seen in the Calbiochem samples may be attributed to deacetylation of the GalN sugar during processing.. CS-4 in particular, is widely utilized in therapeutic settings therefore CS-4 from Sigma was chosen for use in future studies. *CS-monomer conjugation at the PA site.* When CS was reacted with a functional monomer in solution, the PA signal decreased with increasing monomer concentration, indicating an interaction between the CS PA and the amine reactive functional group in the monomer (Figure 1). This gives evidence that the PA will serve as a reasonable site for attachment to a functionalized surface or polymer backbone. *CS functionalized surfaces.* Utilizing the terminal PA detected in CS, CS chains were attached to functionalized glass surfaces. From EDAX chemical analysis sulfur was detected on CS immobilized glass slides and normalized to Si content. EDAX data indicates an overall increase in surface sulfur content, and thereby surface CS content on aldehyde and epoxide functionalized slides (Figure 2). Attachment of CS to epoxide surfaces was also observed via contact angle where contact angle on epoxide slides incubated in 2mg/mL CS was 50% of that detected on control epoxide slides (0mg/mL CS) indicating an increase in surface hydrophilicity imparted by covalently bound CS chains. **Conclusions:** In this study, we have identified a terminal functional group in commercially available CS that may be used as a handle for the attachment of CS bristles to polymeric backbones or functionalized surfaces. The ability to utilize CS in these structures will allow for the fabrication of a family of biomimetic macromolecules for the replacement of aggrecan in the degenerated NP of the IVD, thereby restoring hydration and mechanical function to the tissue.





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