SYNTHESIS AND CHARACTERIZATION OF A NOVEL BOTTLE-BRUSH MOLECULE INSPIRED BY THE AGGRECAN STRUCTURE

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Introduction

The mechanical properties of articular cartilage result from the ECM network of collagen and the main proteoglycan, aggrecan, which in combination provide the tensile, shear, and compressive stiffness of the tissue [1]. Aggrecan has a linear-shaped protein core with connected brunches made by sugar chains producing a "bottle-brush" like structure that includes ~100 negatively charged chondroitin sulfate glycosaminoglycan (CS-GAG) chains attached covalently to the core protein. Variations in the structure of aggrecan and its GAG constituents are known to exist as a function of tissue age and pathological conditions such as osteoarthritis. The major challenge is to create constructs having biochemical and structural properties that are functionally equivalent to aggrecan. In this regard, a biomimetic aggrecan was designed mimicking the three-dimensional bottlebrush architecture of the natural molecule.

Materials and Methods

The envisioned molecule is composed by a core protein made by a single collagen molecule, and its lysines exposed on its surface connect bristles made by CS chains (Figure 1).

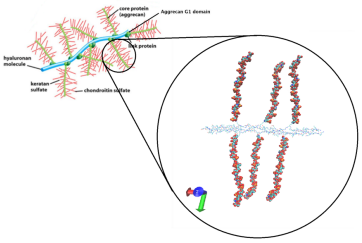

Figure 1 - Representation of the synthesized molecule.

Collagen-free amine content assessment. Ninhydrin is a highly selective indicator for the detection of amino acids with a primary amino group. The free amine residues in collagen molecules react with the 2,2-dihydroxy-1,3-indanedione (ninhydrin assay) to form highly chromogenic derivatives, which can be measured at 570 nm.

Biomimetic aggrecan synthesis. Reductive amination reaction, sketched in Figure 2, is the procedure adopted to obtain the envisioned molecule.

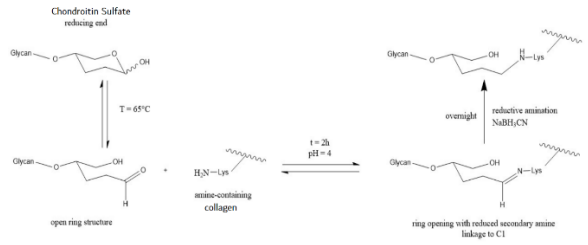

Figure 2 - Scheme of the reductive amination reaction.

Type 1 Collagen (10 mg) and Chondroitin Sulfate type A (600 mg) were mixed in 0.5M Acetic Acid (10 mL), pH = 4. The reaction mixture was stirred at 65°C for 2h, until the imine formation was completed. The imine in AcOH was carefully treated with solid NaBH3CN (3 g) and the reaction was left overnight at 65°C with continuous stirring. In order to purify the product, the precipitated sodium cyanoborohydrate was filtered away using a 100 kDa MWCO Amicon® centrifugal filter and the solvent evaporated at the rotary evaporator and then lyophilized.

Analytical techniques. Lyophilized CS and synthesized molecule samples were reconstituted in deuterium oxide (D2O) and then analyzed with 1H-NMR, HSQC-NMR and DOSY-NMR techniques.

Imaging techniques. Using atomic force microscopy (AFM), it was directly visualized the nanometer scale structure of the synthesized molecule deposited on a mica substrate.

Results and Discussion

Ninhydrin assay on pristine collagen confirms that the 2,7% of the total amino acids present in the collagen are free amine exposed on the surface, available for conjugation [2].

Synthesis. The synthesized molecule has a percentage of functionalization was 48,6%. The success of the reaction was confirmed through a solubility assessment. Pristine collagen is completely insoluble in water, being collagen a largely hydrophobic molecule. After the covalent functionalization of chondroitin sulfate, the obtained product was easily soluble in water.

Analytical techniques. Chemical structures of synthesized molecule samples and unreacted CS were confirmed with 1H NMR. The results suggest that the chemical composition of GAG bristles on the final synthesized molecules was maintained.

Imaging techniques. Atomic force microscopy (AFM) imaging was performed on the molecule (Figure 3).

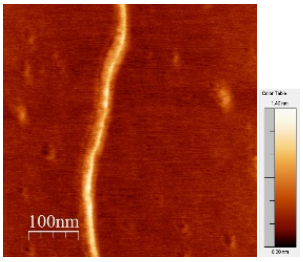

Figure 3 – AFM topography image of the molecule.

References

1. Gautieri et al., Matrix Biol, 34: 89-95. 2014.
2. Chandran and Horkay, Acta Biomat, 8:3-12, 2012.