Rheological properties of cross-linked Hyaluronic Acid for Dermal Fillers

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ABSTRACT

In this work sodium hyaluronate produced by fermentation of a novel, superior and safe strain, namely *Bacillus subtilis*, has been cross-linked with divinyl sulfone to produce hydrogels. The visco-elastic properties of the hydrogels after sterilization and injection through needles typically used for dermal fillers applications have been tested against benchmark products. The results show that the cross-linked material can be tailored and formulated to target the desired properties of ideal dermal fillers.

INTRODUCTION

Hyaluronic acid (also referred to as HA or hyaluronan) is a natural linear and unbranched polysaccharide belonging to the class of non-sulphated glycosaminoglycans. HA is composed of beta-1,3-*N*-acetyl glucosamine and beta-1,4-glucuronic acid repeating disaccharide units (Fig. 1).

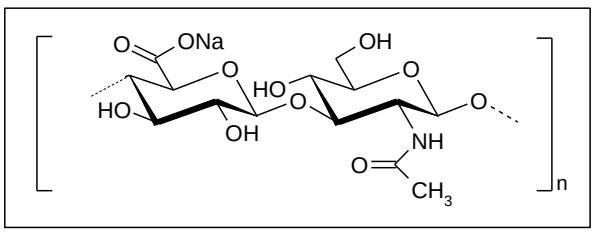

Figure 1. Chemical structure of HA disaccharide repeating unit.

HA is ubiquitous in human and animal tissues, where it exhibits significant structural, rheological, physiological, and biological functions. HA is now recognized as a high-value biopolymer with numerous proven and marketed applications within the cosmetic, biomedical and pharmaceutical fields.

A wide range of modification methodologies, including cross-linking and attachment of hydrophobic pendant groups, have been investigated to produce new and robust HA-based materials with enhanced biostability and improved rheological characteristics, with potential to be used as visco-augmentation products[1,2].

In this work sodium hyaluronate produced by fermentation of *Bacillus subtilis* strain[3], has been cross-linked to produce hydrogels[4] (Fig. 2). The visco-elastic properties of the hydrogels after sterilization and injection through needles typically used for dermal fillers applications have been tested against benchmark products.

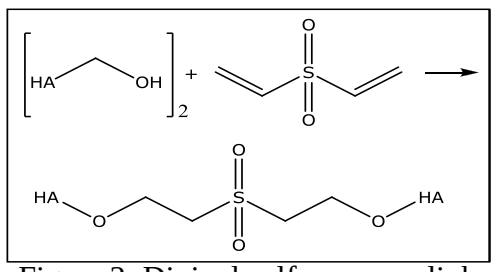

Figure 2: Divinyl sulfone cross-linked HA.

MATERIALS AND METHODS

The sodium hyaluronate is produced by Novozymes Biopharma DK A/S by fermentation of *Bacillus subtilis* strain. The weight average molecular weight of the starting material was 0.85 MDa.

Formulation of cross-linked gels

The hydrogel was produced according to the method described in the patent application using divinyl sulfone (DVS) as the cross-linking agent[4].

Hydrogel was prepared with HA/ DVS weight ratio of 5:1 with a final HA concentration of 1.7 w/w%.

The cross-linked gel was mechanically degraded by extrusion through first a 22G x 1", then a 27G x ½" and finally a 30G x ½" needle. A HA-solution (20 mg/mL) was mixed into the gel. The visco-elastic properties as well as extrusion force were measured on starting material, intermediate samples and the final product.

Analytical methods

The visco-elastic properties were evaluated on a rotational rheometer (Physica MCR301, AntonPaar, Austria) using a parallel plate geometry (PP25 or PP50). The tests were carried out at the controlled temperature of 25 °C, by using a Peltier heating system. The hydrogel samples were subjected to periodic oscillation in a dynamic experiment (small amplitude frequency sweep tests) to evaluate the dependence of the elastic (G') and viscous (G'') moduli. The frequency range investigated was 0.1 to 10 Hz. In order to indentify the linear visco-elastic response range, preliminary strain sweep tests were performed at the oscillation frequency of 1 Hz. The tests were repeated at least twice on each sample.

The extrusion force was evaluated with a texture analyzer (TA.XT plus, Stable Micro Systems, UK) applying syringes containing HA-samples to an extrusion force of variable strength, thereby measuring the force (N = Newton or kg) needed to inject the material.

RESULTS AND DISCUSSION

Visco-elastic properties as function of Gel/fluid ratio

The elastic modulus (G') of the hydrogels was always higher than the viscous one (G'') and both moduli did not vary significantly with the frequency: this visco-elastic behaviour was typical of gel materials. Figure 3 shows how visco-elastic properties increase as gel/HA-solution ratio changes (Gel/fluid ratio "Square": 90:10, through "triangle" 80:20 to circles 70:30).

The elastic and viscous moduli increased with increasing HA-solution concentration (Table 1).

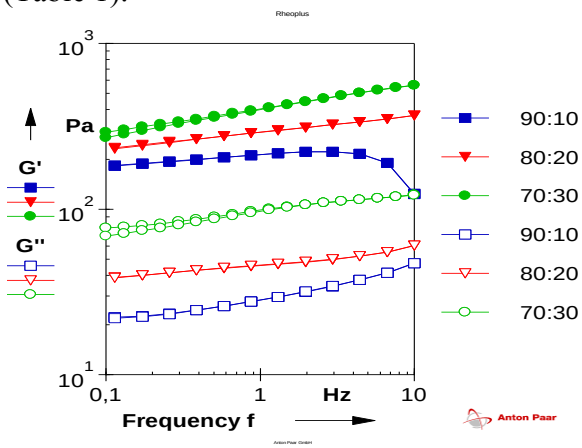

Figure 3: G' and G'' of various DVS-CL-HA-gel/HA-solution ratios

Visco-elastic properties for dermal filler applications

Tailor made cross-linked HA material was prepared by variation of the gel/HA-solution ratio to target dermal filler applications. A comparable evaluation of the materials obtained to benchmark dermal fillers (BM1 and BM2) was carried out and results are shown in Figure 4 and Table 1.

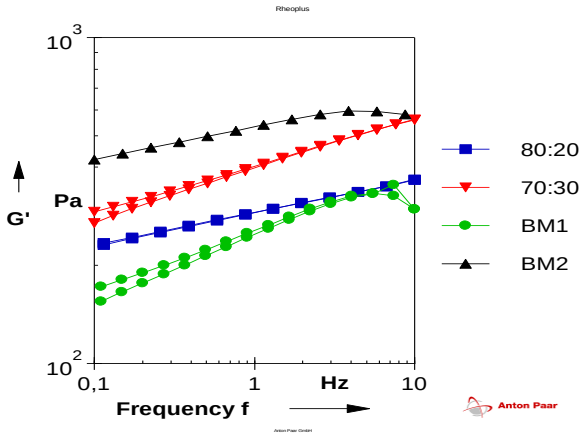

Figure 4: G' and G'' of various CL-HA gel/HA-solution ratios

Table 1. Storage moduli of benchmark products vs. gel/HA-solution ratios.

Ratio	G' (1Hz)
Benchmark 1 (BM1)	243 Pa
Gel/fluid ratio70:30	286 Pa
Gel/fluid ratio 80:20	390 Pa
Benchmark 2 (BM2)	540 Pa

Extrusion force

It was demonstrated that the formulation of cross-linked gel and HA-solution results in materials with similar extrusion force properties compared to benchmark products.

REFERENCES

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