# Evaluation of the *In Vivo* Residence and *In Vitro* Degradation Characteristics of a Unique Divinyl Sulfone Modified Hyaluronan

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**Introduction**: Hyaluronan (HA) and its derivatives are utilized in various medical applications including the treatment of pain due to osteoarthritis, prevention of post-surgical adhesions, drug delivery and cosmetic applications. Unmodified HA has a very short residence time once implanted into the body. HA can be chemically modified to increase its residence time and therapeutic properties[1]. A unique formulation of HA modified with divinyl sulfone (DVS) and processed to produce a soft and elastic gel has been characterized by its rheological properties, *in vivo* residence time, and *in vitro* degradation rate.

**Methods**: Bacterially fermented sodium hyaluronate was modified with 0.035 Meq and 0.070 Meq DVS, respectively, and acid washed to form two soft and elastic, modified HA gels. These test materials were denoted as hylastan gel A and hylastan gel B, respectively. The two hylastan gels were characterized for rheology and degree of modification (Table 1). The viscoelastic properties were determined by an oscillation test in the frequency range of 0.04 Hz to 7 Hz on a Controlled Stress Rheometer (Bohlin) at 25°C. The viscosity at shear rate 1 $sec^{-1}$ ($\eta(1)$) was also determined via the stress viscometry test. The percent modification of gels was determined by enzymatic digestion with *streptomyces*-derived hyaluronidase followed with an HPLC separation and chromatographic profile analysis. For the *in vitro* degradation study, samples were treated with hyaluronidase (from sheep testes) at a final hyaluronidase/HA ratio equal to 7.4 units/mg. After the gel degraded to soluble fluid, the HA molecular weight (MW) was measured every half hour up to five hours by SEC/MALLS (Size Exclusion Chromatograph /Multi-Angle Laser Light Scattering). For the *in vivo* residence time study, Guinea pigs were randomized into two treatment groups of 6 animals and a control group of 4 animals. Each hylastan test material or non-modified HA control material was injected into the intraarticular space of the femoropatellar joint of the assigned animals. On days 2 and 29 following injection, animals were sacrificed and their fat pads removed and fixed in 10% neutral buffered formalin. Tissue was embedded in paraffin and serially sectioned (5µm thick) onto consecutive slides. Sections were stained with H&E and evaluated for the presence of residual HA-based material.

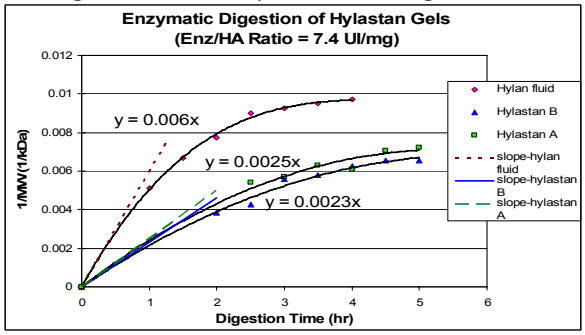
**Results**: The hylastan gels had lower phase angles than the high MW HA due to crosslinking although they were very soft gels, as indicated by their low modulus values (Table 1). From the enzymatic degradation study, the reciprocal of MW was plotted against digestion time, then the initial degradation rate of each sample was calculated (Figure 1). There were no significant differences in the degradation rate between the two hylastan gels, but they degraded at a much slower rate than hylan fluid[1].

*Table 1: % Modification and Rheology of Test Articles*

| Sample ID | FPC (mg/ml) | % Modif. | G'(5) (Pa) | G"(5) (Pa) | δ(5) (°) | η(1) (Pas) |
|---|---|---|---|---|---|---|
| Hylastan Gel A | 7.5 | 3.5% | 56 | 12 | 12 | 71 |
| Hylastan Gel B | 7.5 | 5.5% | 46 | 7 | 9 | 53 |
| Hylan Fluid | 10 | NA | 88 | 59 | 34 | 21 |

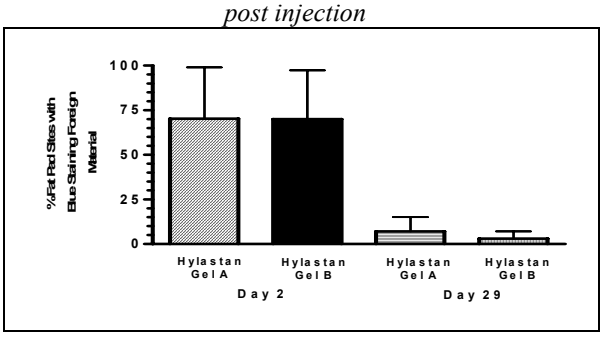
FPC - Final Polymer Concentration

*Figure 1: MW vs. hyaluronidase digestion time*




Histological analysis of the fat pad tissues taken 2 days following injection showed approximately 70% of the sites contained residual HA-based material. At day 29, only 4% to 7% of the sites contained HA-based material (Figure 2). None of the animals treated with non-modified HA showed any residual material at either 2 or 29 days post injection.

*Figure 2: Residual hylastan found in fat pads at 2 & 29 days post injection*




**Discussion**: The hylastan gels showed a significantly slower degradation rate than hylan fluid *in vitro* which correlated to the *in vivo* histological results. DVS modified HA gave soft and elastic gel properties and longer *in vivo* residence time than non-modified HA.

**Reference:**

[1]Balazs EA. J Rheumatol. 1993;20 (S39):3-9.