Mechanical Stiffness Testing of HyStem® Hyaluronic Acid Products

Introduction: There is a need for biomaterials that represent the full range of tissues ranging from nerve to cartilage and are well suited for tissue engineering and general cell culture. Hyaluronic Acid is likely the most plentiful glycosaminoglycan (GAG) found in many tissues. Hyaluronic Acid hydrogels have emerged as promising biomaterials due the fact that they have a high-water content, and they allow cells to be encapsulated within the material in a 3D environment.

Mechanical stiffness is a critical parameter to consider when designing a cell culture system. In the context of 3D cell culture, matrix stiffness can impact a wide variety of pathways including stem cell differentiation[1], cancer metastasis[2], and cardiac tissue function[3]. Furthermore, depending on the application, the structural integrity of hyaluronic acid constructs may be of importance. Thus, as culture systems become more tissue specific, the need to quantify the mechanical stiffness of biomaterials becomes more important and relevant.

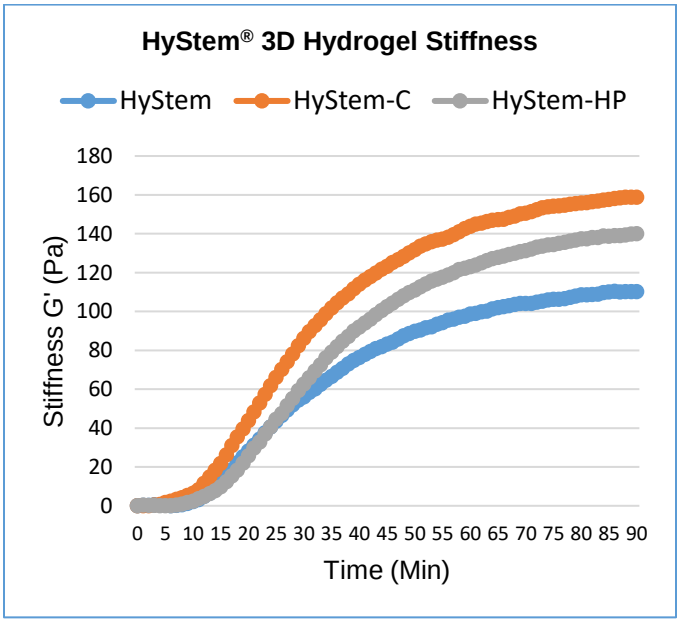
HyStem® Kits consist of thiol-modified hyaluronic acid called Glycosil®. Glycosil® hyaluronic acid is the primary component that provides the mechanical properties of the HyStem® kits. The table below lists the components in each kit.
Table 1:

Product Name	Catalog Nos.	Pkg Size	Kiit Components
HyStem®	GS310 GS311 GS1004	2.5 ml 7.5 ml 12.5 ml	-Glycosil® -Extralink®-Lite -DG Water
HyStem®-C	GS312 GS313 GS1005	2.5 ml 7.5 ml 12.5 ml	-Glycosil® -Gelin-S -Extralink® -DG Water
HyStem®-HP	GS314 GS315 GS1006	2.5 ml 7.5 ml 12.5 ml	-Heprasil® (2) -Gelin-S -Extralink® -DG Water

(1) Extralink®-Lite is ½ the concentration of Extralink®
(2) Heprasil® is Glycosil® containing thiolated Heparin

Methods: Typical methods used to evaluate the mechanical properties of hydrogels include atomic force microscopy (AFM) and dynamic shear rheometry. Another method for evaluating the mechanical properties of hydrogels is a contactless method for assessing material stiffness. Contactless measurements were carried out on an ElastoSens® Bio2 (Rheolutions Instruments). Product was loaded into a sample holder and incubated at 37°C within the ElastoSens® instrument and continuous measurements were taken. Moduli were measured throughout the polymerization time period until full polymerization was achieved and a plateau region realized.

Results: Hydrogel stiffness results were measured as storage modulus G' and are plotted and shown in figure below.
Figure 1:



The results provided above represent the average of a minimum of three tests per product with differences between tests being minimal. Although not shown here, HyStem® products can be manipulated to produce tunable stiffness results within the soft hydrogel range. (4)

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Discussion: Mechanical moduli are dependent on the stress conditions under which the material test was carried out. The contactless measurements using the ElastoSens® provide moduli in terms of storage modulus (G'). Table 2 provides plateau results for each product as measured in G'.

Table 2:

Product	Plateau (Pa)
Product	G'
HyStem®	110
HyStem®-C	159
HyStem®-HP	140

The mechanical stiffness (G') of HyStem® hydrogels ranges between of 100Pa to 160Pa which covers many soft material applications and will guarantee a solid hydrogel. However, for stiffness in excess of 200Pa, using other hyaluronic acid materials may be required. Advanced BioMatrix offers PhotoHA® (methacrylated hyaluronic acid) which is a tunable hydrogel that can be manipulated to achieve mechanical stiffnesses (G') approaching 8000Pa depending on hyaluronic acid concentration, photoinitiator used and light exposure type and time. The Table 3 provides a list of methacrylated hyaluronic acid accompanied with a variety of photo initiators available from Advanced BioMatrix.

Taken together, HyStem or PhotoHA products provide a basis for selecting a hyaluronic acid product that abides by the mechanical necessities of your cell culture system.

Table 3:

Product Name	Catalog No.
PhotoHA® Methacrylated hyaluronic acid only	5212
PhotoHA®-IRG Methacrylated hyaluronic acid with Irgacure 2959	5220
PhotoHA®-LAP Methacrylated hyaluronic acid with LAP	5274
PhotoHA®-RUT methacrylated hyaluronic acid with Ruthenium	5275

References:

1) Engler, Adam J., et al. "Matrix elasticity directs stem cell lineage specification." Cell 126.4 (2006): 677-689.

2. Paszek, Matthew J., et al. "Tensional homeostasis and the malignant phenotype." Cancer cell 8.3 (2005): 241-254.

3) Qiu, Yiling, et al. "A role for matrix stiffness in the regulation of cardiac side population cell function." American Journal of Physiology-Heart and Circulatory Physiology 308.9 (2015): H990-H997.

4) Vanderhooft, J. L., et al. "Rheological properties of cross-linked hyaluronan-gelatin hydrogels for tissue engineering." Micromol. Biosci. (2009): 9, 20-28.