1301 Harbor Bay Parkway
Alameda, CA 94502
T: 510–521–3390, F: 510–521–3389
www.biotimeinc.com

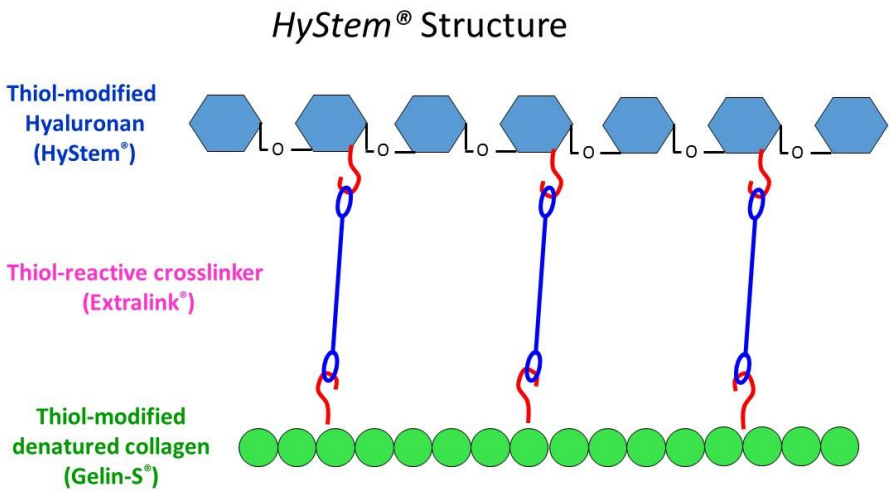
BioTime's HyStem® Technology

BioTime has developed a family of unique hydrogel biomaterials (*HyStem*®) that mimic the natural extracellular matrix (ECM) environment. These materials have applications in 3-D cell culture, stem cell propagation and differentiation, tissue engineering, regenerative medicine, cell based therapies, and as delivery vehicles for bioactive molecules. The *HyStem* hydrogels were designed to recapitulate the minimal composition necessary to obtain a functional ECM.

The technology underlying BioTime's *HyStem* hydrogels was developed at the University of Utah and is based on a unique chemical cross-linking strategy to prepare hyaluronan based hydrogels from chemically modified hyaluronan and other ECM constituents. Building upon this platform, BioTime has developed the *HyStem* family of unique, biocompatible biodegradable hydrogels. Since the first published report in 2002, there have been over 200 scientific publications supporting the biocompatibility of these cross-linked hyaluronan based hydrogels and their applications in cell culture, tissue engineering, and animal models of cell-based therapies.

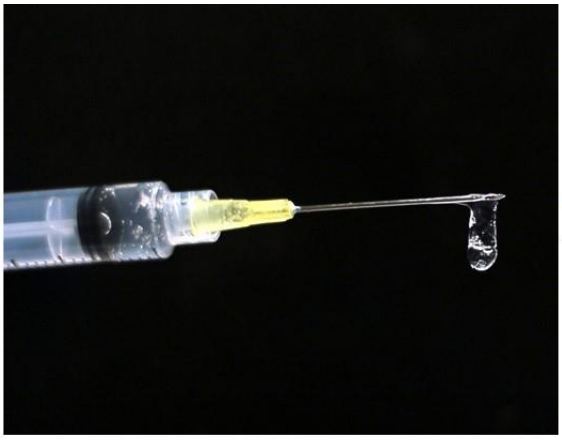
Composition of HyStem® Hydrogels

The building blocks for *HyStem* hydrogels, which include *Renevia*, are hyaluronan and gelatin, each of which has been chemically modified to form a hydrogel in the presence of a cross-linking agent. Specifically, the three components that form the basis of HyStem hydrogels are: Glycosil® (thiol-modified hyaluronan), Gelin™ (thiol-modified gelatin) and the cross-linker Extralink® (polyethylene glycol diacrylate).



For many cell-based tissue engineering applications, creating a three-dimensional space in which implanted cells can attach, proliferate, and differentiate is required. The high failure rate in many applications of cell grafts without such a matrix is high because of difficulties in achieving cell attachment and survival. *HyStem* hydrogels are designed to function as matrices for the stable attachment and survival of cells: Glycosil forms the major backbone of the hydrogel structure while Gelin provides the amino acid sequences necessary for cell attachment and proliferation.

The individual components of a *HyStem* hydrogel are supplied in vials as pre-measured, sterile, lyophilized solids that, when reconstituted and mixed together, form a clear, transparent, viscoelastic hydrogel in approximately 10-60 minutes at room temperature (*see photos below*). The rate of gelation and hydrogel stiffness can be controlled by varying the amount of cross-linker. An important attribute of *HyStem* hydrogels is their large water content, >98%. As a result, the hydrogel is highly permeable to oxygen, nutrients and other water-soluble metabolites [1].



Hystem hydrogel extruded through a 20-gauge needle

~20 min

Fully formed *Hystem* hydrogel (5 mL)

The ability to mix cells with HyStem hydrogels during the pre-gel phase, deliver the cell/hydrogel mixture through a cannula, and have the subsequent gelation occur in situ without compromising the cells or the recipient tissue differentiates HyStem from other matrices or scaffolds.

***HyStem* Research Reagents:**

BioTime manufactures and distributes a variety of *HyStem* hydrogels for pre-clinical research use through its division – ESI Bio (www.esibio.com). *HyStem* hydrogels have been shown to support attachment and proliferation of a wide variety of cell types. These biomaterials exhibit a high degree of biocompatibility when implanted *in vivo*, without evidence of inflammation or an immune response arising from the implanted hydrogel. When implanted in *HyStem* hydrogels, cells remain attached and localized within the hydrogel and slowly degrade the implanted matrix and replace it with their natural ECM.

Animal studies using *HyStem* hydrogels have demonstrated the importance of a biocompatible matrix for the implantation of cells for certain therapeutic applications. Investigations with neural progenitor cells in animal models of stroke have demonstrated statistically significant enhanced survival of the cells implanted in *HyStem* hydrogels relative to cells implanted without a protective matrix [2]. Additionally, the hydrogel group showed less glial scarring at the stroke site and significant recovery of stroke-impaired limb function. *HyStem* hydrogels have also been used successfully in animal models of cell-based therapies for cancer [3], coronary infarct repair [4, 5], and cancer immunotherapy [6].

***HyStem* Hydrogels for Therapeutic Applications:**

BioTime has developed a family of unique hydrogel formulations, designed as implantable matrices for a broad range of cell-based therapies in humans. *HyStem* hydrogels intended for clinical use are manufactured to pharmaceutical standards in cGMP compliant facilities and meet the strict quality requirements of the FDA and other regulatory bodies for a Class III, permanently implantable, medical device. Data from animal studies along with ISO 10993 biocompatibility testing have shown that *HyStem* hydrogels are biocompatible and provide an excellent protective matrix within which implanted cells can proliferate and generate new tissue.

***Renevia*™** is one of the *HyStem* hydrogel formulations, designed as an implantable matrix for the delivery of autologous adipose tissue-derived cells to treat the facial lipoatrophy associated with HIV. *Renevia* provides a uniform, biocompatible, resorbable delivery matrix on which the cells can attach, proliferate, and differentiate into adipose tissue. As the implanted cells proliferate within the *Renevia* filled space, they resorb the hydrogel through enzymatic hydrolysis and replace it with their own native extracellular matrix. *Renevia* is currently being investigated in a pivotal efficacy clinical trial in HIV-positive subjects with facial lipoatrophy.

References

1. Shu, X.Z., et al., Synthesis and evaluation of injectable, in situ crosslinkable synthetic extracellular matrices for tissue engineering. *J Biomed Mater Res A*, 2006. **79**(4): p. 902-12.
2. Zhong, J., et al., Hydrogel matrix to support stem cell survival after brain transplantation in stroke. *Neurorehabil Neural Repair*, 2010. **24**(7): p. 636-44.
3. Shah, K., Encapsulated stem cells for cancer therapy. *Biomatter*, 2013. **3**(1).
4. Cheng, K., et al., Functional performance of human cardiosphere-derived cells delivered in an in situ polymerizable hyaluronan-gelatin hydrogel. *Biomaterials*, 2012. **33**(21): p. 5317-24.
5. Smith, R.R., E. Marban, and L. Marban, Enhancing retention and efficacy of cardiosphere-derived cells administered after myocardial infarction using a hyaluronan-gelatin hydrogel. *Biomatter*, 2013. **3**(1).
6. Compte, M., et al., Tumor immunotherapy using gene-modified human mesenchymal stem cells loaded into synthetic extracellular matrix scaffolds. *Stem Cells*, 2009. **27**(3): p. 753-60.