2022

SUPPLEMENTING HYDROGELS WITH HYDROSEEV

Cat # SVX-HY-2
Cat # SVX-HY-10

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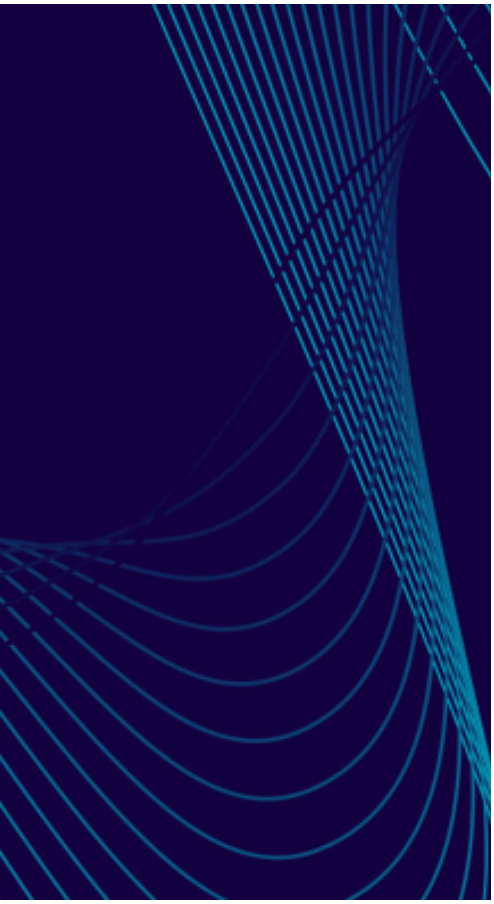
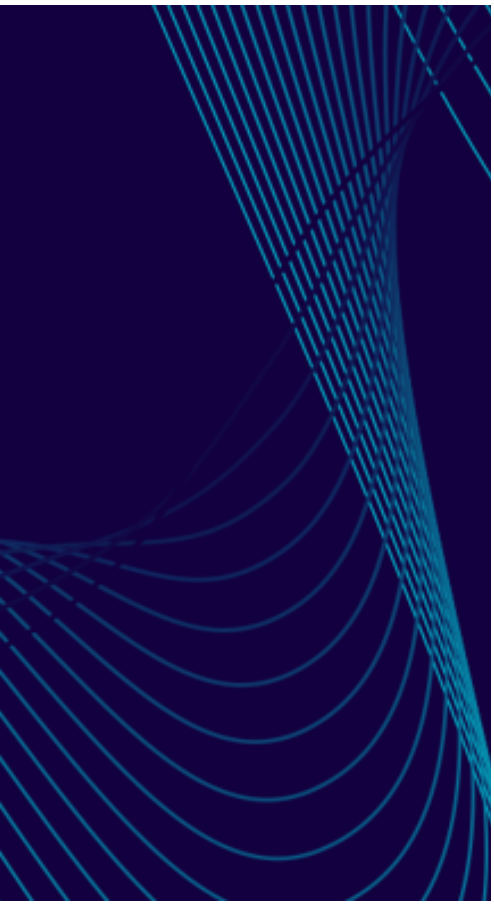




BACKGROUND

There is great appreciation and understanding of the extracellular matrix (ECM) in cellular formation and cellular function. As a result, a growing amount of research is moving away from traditional 2D cell culturing techniques, since cells tend to behave differently when grown on flat, unphysiologically stiff materials such as polystyrene and glass. Hydrogels have presented themselves as more of an organic milieu for cells to proliferate and develop, enabling the delivery of mechanical, structural and compositional cues required for cells for proper (or improper) functioning. The various properties of hydrogels, which mimic the extracellular matrix, provide an environment that is mechanically similar to soft tissues, supporting cell adhesion and protein sequestration [1]. Indeed, hydrogels are useful in cell cultures, revealing important cellular behavioral features and unveiling different responses to drugs as opposed to when cells are grown in a monolayer culture. Hydrogels can be naturally sourced or chemically synthesized. ECM-based hydrogels are commercially available (such as Matrigel®) and have primarily been used to study cell migration, angiogenesis and tumor development. The major drawbacks are the batch-to-batch inconsistencies involving mechanical and biochemical properties of the hydrogel, which are both critical features, and the fact that Matrigel® is derived from a tumorigenic cell line. Alginate is a polysaccharide derived from algae and is notable for its ability to form hydrogels through ionic or covalent crosslinking. Alginate is versatile since it derives from a non-animal origin, thus with low or no risk of animal contamination. It displays high stability at room temperature, is non-toxic and biodegradable, and can easily be degraded by a dissolution buffer in a few minutes, leaving cell aggregates intact for analysis [2]. However, it lacks cell-adhesion properties.

HydroSeev

A HYDROGEL SUPPLEMENT

OPTIMAL SCAFFOLD

ECM-LIKE STRUCTURE

NATURAL POROUS STRUCTURE

LARGE SURFACE AREA

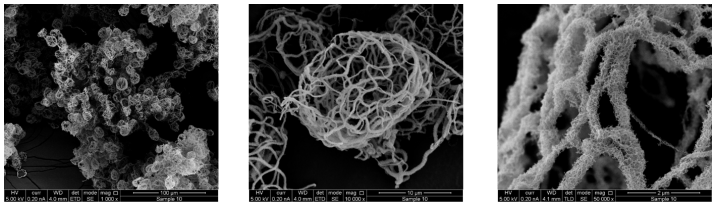


Figure 1. HydroSeev has a spongy structure composed of nano-fibrils with a surface area. Magnification: left 1,000X; middle 10,000X; right 50,000X.

While hydrogels have certainly impacted scientific advances, they have numerous disadvantages involving stability and mechanical properties.

HydroSeev, is a biopolymer providing mechanical support and an ECM-like environment to cells in a consistent and reproducible manner [3].

HydroSeev fibers' nano and micro dimensions are similar to a naturally occurring ECM, providing a tissue-like environment for cells. Its fibers have high porosity, enabling better access to oxygen and nutrients, thereby mimicking the function of vascularized tissue. Finally, HydroSeev fibers have extra strength and elasticity, providing mechanical cushioning and protection to individual cells within the 3D culture (Figure 1).

USING HYDRROSEEV FOR SUPPORTING HYDROGELS

0.02% HYDROSEEV IS ALL YOU NEED

Adding as low as 0.02% HydroSeev to cells cultured in hydrogels improves viscosity, promotes better porosity, improves mechanical properties and stability of the hydrogel, and improves cellular distribution within the hydrogel (Figure 2).

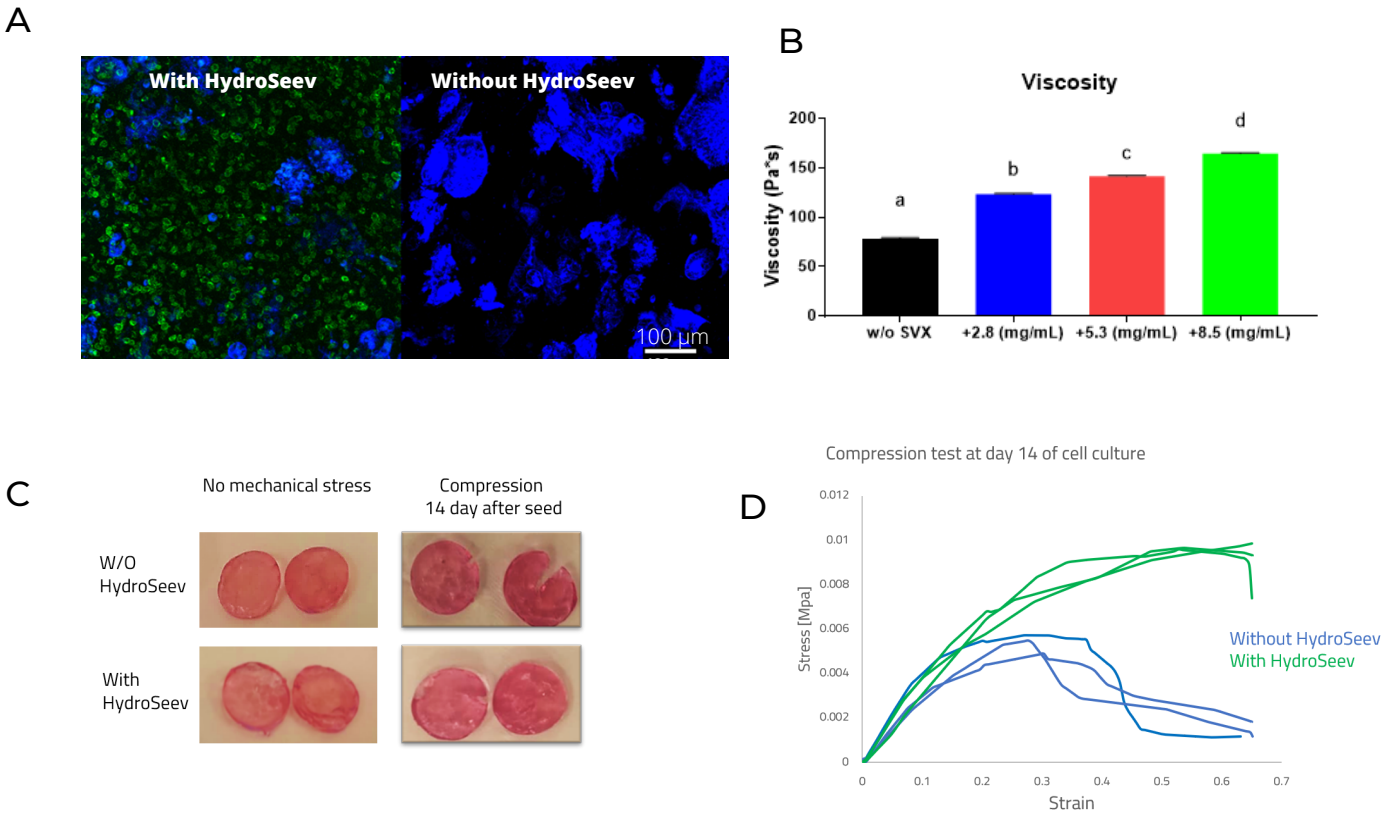
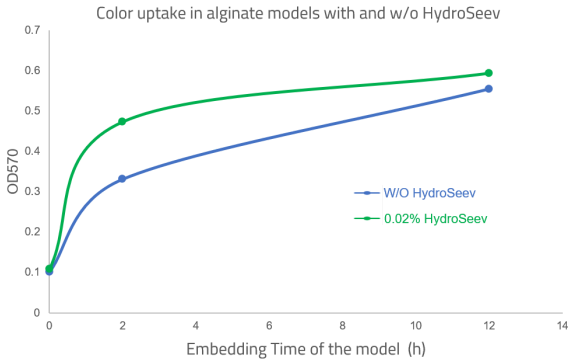


Figure 2. Addition of 0.02% HydroSeev to cell cultures improves: **A.** Cell distribution in alginate (3.5%) hydrogel (green - HydroSeev, blue- cell clusters). **B.** Hydrogel viscosity is improved upon addition of HydroSeev. **C.** Mechanical properties of cell-seeded alginate hydrogel are significantly improved when HydroSeev is added (left). Stress-strain curve of compressed cell-seeded alginate hydrogel (right).

USING HYDRROSEEV FOR SUPPORTING HYDROGELS

The addition of HydroSeev increases the permeability of hydrogels, allowing for better oxygenation, nutrient delivery, and diffusion of signaling molecules and small molecules to cells, thereby optimizing screening processes. HydroSeev increases cell viability and stability; cells are homogenously spread as discrete individual cells or small clusters in the gel (Figure 3).

A



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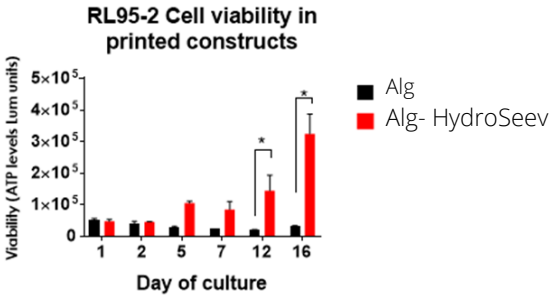

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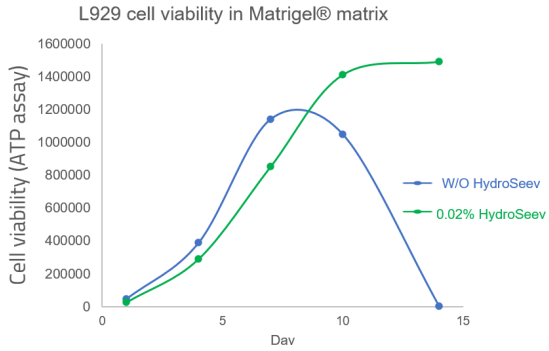

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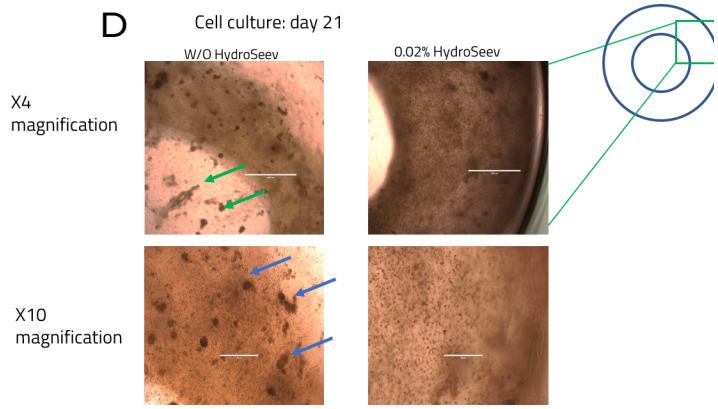


Figure 3. Addition of 0.02% HydroSeev to cell cultures improves: **A.** Permeability of cells grown in alginate (3.5%) hydrogel. **B.** RL-95 cell viability significantly improved upon addition of HydroSeev to alginate matrix. **C.** L929 cell viability is prolonged upon addition of HydroSeev to Matrigel matrix. **D.** Cells are better distributed HydroSeev is added to alginate (blue arrows- large cell clusters in the gel, green arrows- large clusters that disintegrated from the gel).

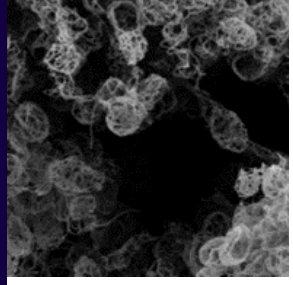
References

1- Tibbitt, M.W. and Anseth, K.S. (2009) *Biotech. Bioengineer.* **103,** 655.

2- Habanjar, O. *et al.* (2021) *Int. J. Mol. Sci.* **22,** 12200.

3- Stern-Tal, D. *et al.* (2021) *J. Biomater. Appl.* 8853282211037781.

PROTOCOL FOR ALGINATE GEL ENRICHMENT



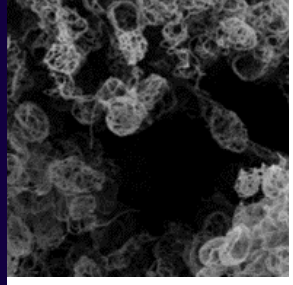
The following protocol is suggested for HydroSeev enrichment of alginate gel prepared with partial cross-linking before printing/casting to improve the printing precision of alginate and model uniformity. Any alternative preferred protocol for alginate gel preparation or different concentration may be used..

Alginate-HydroSeev biolink preparation:

1- Dissolve 3.5% (W/V) alginate (0.35gr/10 ml) in ultra-pure water. Filter using a 0.2µm filter.
2- Prepare a 60 mM solution of CaCl2. Filter using a 0.2µm filter.
3- Add **0.02%** (W/V) **HydroSeev*** (0.2mg/1 ml) **to the volume of alginate required for the experiment**. Mix well by slowly pipetting up and down several times.
*Optional- To avoid dilution of alginate, the amount of HydroSeev to be used in the experiment can be transferred to a new tube and centrifuged at 7000g for 3 minutes. Then, as much liquid as possible can be carefully aspirated, and the required amount of 3.5% alginate can be added to the HydroSeev-containing tube.

4- Remove cells of interest from the culture vessel by trypsinization.
5- Calculate the desired number of cells for seeding in the gel. Cells can be concentrated by centrifugation at 100-500 g for 5 min and medium removal.
6- Load three sterile Luer-lock syringes with the following materials (total volumes may be adjusted according to the application but a constant ratio between ingredients in the different syringes should be kept):
Syringe 1- cells suspended in 100 µl culture medium.
Syringe 2- 3 ml of 3.5% alginate solution supplemented with 0.02% HydroSeev.
Syringe 3- 1 ml 60mM CaCl2.
7- Attach a sterile Luer-lock coupler on the end of the syringe containing the cell suspension (Syringe 1). Couple the syringe with cells (Syringe 2) to the syringe with the HydroSeev-alginate (Syringe 1). Ensure that there are no air bubbles in the system.
8- Slowly push plungers back and forth ~40 times to ensure thorough mixing. Finish with all of the now cell-laden alginate in the alginate syringe.

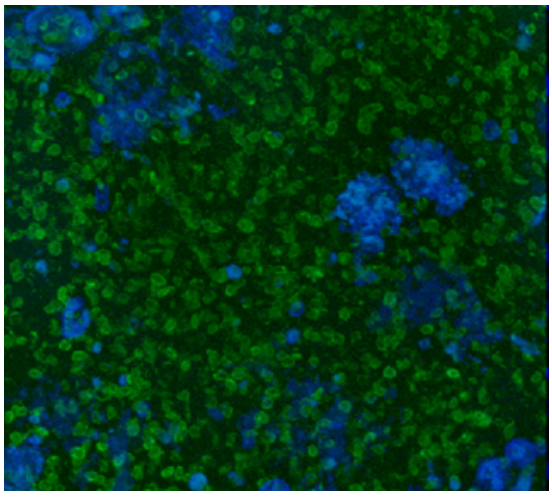
PROTOCOL FOR ALGINATE GEL ENRICHMENT



9- Couple the syringe with HydroSeev-alginate-cells mix to the syringe with 60mM CaCl2 (Syringe 3). Ensure that there are no air bubbles in the system.
10- Slowly push plungers back and forth ~40 times to ensure thorough mixing. Finish with all of the material in one syringe. This will start the gelation process.
11- Place the matrix in a cell culture incubator for 40 minutes before printing or casting the models.

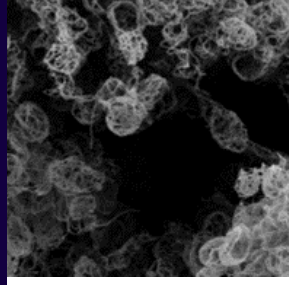
Gel printing or casting:

1- Print, manually dispense into a mold or a tissue culture well/plate or use any method to create your 3D model.
2- Optional: It is possible to increase the stiffness and stability of the gel by performing an additional cross-linking. For higher cross-linking immerse the gel for 30-60 seconds in 60 mM CaCl2.
3- Cover the model with culture media. Place the seeded plate in a tissue culture incubator or any other conditions suitable to your workflow.



HydroSeev (green) spread in alginate cellular model aggregation of L929 cell line (blue). Evaluated by fluorescent staining by thioflavin-S. Scale bar indicates 100 µm.

PROTOCOL FOR MATRIGEL® (CORNING) ENRICHMENT

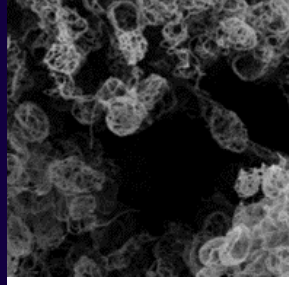


The following protocol is suggested for HydroSeev enrichment of Matrigel® for improvement of gel stability and viability. Any alternative preferred protocol for Matrgel® matrix preparation or different concentrations may be used.

Matrgel®-HydroSeev biolink preparation:

1- Thaw Matrigel® (Corning) overnight at 4oC (for details, see protocol for Matrigel® thawing and use on Corning's website).
2- For dome application method, preincubate the cultureware in a humidified 37˚C incubator overnight. For embedded application method, pre-chill cultureware at 2-80C.
3- Pre-chill all consumables (tips, plates, tubes and more) that you will need during the experiment and keep Matrigel® matrix on ice during the entire process (See Corning's Matrigel® detailed protocol).
4- Remove cells/aggregates of interest from the culture vessel.
5- Calculate the desired number of cells for seeding in the gel. Cells can be concentrated by centrifugation at 100-500 g for 5 min and medium removal.
6- Depending on cell/organoid type, concentration of Matrigel® and purpose, use a concentration of 50% or more Matrigel® in the final seeded mixture.
7-Add 0.02%-0.04% (W/V) HydroSeev (0.2-0.4mg/1 ml) and the required amount of cells to a tube containing the volume of medium required for the experiment (cells-HydroSeev-medium mixture should be 50% of the final volume of the matrix or less). Mix well by slowly pipetting up and down several times. Keep the mixture on ice.
8- Add 1:1 or more Matrigel® to the tube containing the cells-HydroSeev-medium mixture to generate a ready to use mixture containing at least 50% Matrigel®. Keep the mixture on ice.

PROTOCOL FOR MATRIGEL® (CORNING) ENRICHMENT



9-For dome formation - carefully dispense 5 to 50 µl of the mixture on a tissue culture plate pre-heated to 37oC. If possible, working on a heated platform is recommended to maintain 37oC. Transfer plate to a 37oC incubator.

10-For embedded application - to a pre-chilled plate carefully add 150 to 200 µL mixture/cm2. Transfer plate to a 370C incubator.

11- Once dome solidifies (typically 10 minutes for dome application and at least 30 minutes for embedded application), gently add room temperature culture medium along the well wall to avoid disruption of the matrix. Transfer plate to a 370C incubator.