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HydroSeev for Enrichment of Hydrogels

HydroSeev

5 mg/ml HydroSeev
Storage: Store HydroSeev at 4^{0}C (refrigerated) after opening.

Intended Use

HydroSeev is a formulation of a biopolymer[1] with mechanical properties similar to spidersilk that is used as an additive for improving the mechanical properties, viability and function of 3D hydrogel models. HydroSeev is supplied as a stock suspension to supplement materials used to create 3D cellular models. It is easy to handle and can be easily incorporated into any bioink with minimal workflow change.

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 printing uniformity and precision of alginate models (basic protocol for partial cross-linking before printing/casting- is based on Freeman et.al.[2]). Any other preferred protocol for alginate gel preparation or different concentration may be used.

Concentrations of gel and HydroSeev suggested below may need further optimization by the researcher depending on the type of cells, matrix used and desired outcome.
This product is intended for research use only.

Alginate-HydroSeev bioink 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 $CaCl_2$. 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 anew 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 minutes 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. End with all of the now cell-laden alginate in the alginate syringe.
9. Couple the syringe with HydroSeev-alginate-cells mix to the syringe with 60mM $CaCl_2$ (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. End 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 $CaCl_2$.
3. Cover the model with culture media.
4. Place the seeded plate in a tissue culture incubator or any other conditions suitable to your work flow.

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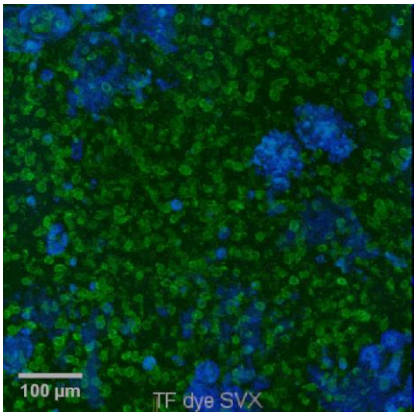

Figure 1 HydroSeev (green) spread in alginate cellular model aggregation of L929 cell line (blue). Evaluated by fluorescent staining by thioflavin-S. Scale bar indicates 100 µm.

1. Stern-Tal D, Ittah S, Sklan E. A new cell-sized support for 3D cell cultures based on recombinant spider silk fibers. DOI: 10.1177/08853282211037781

2. Freeman F E, and Kelly D J. Tuning Alginate Bioink Stiffness and Composition for Controlled Growth Factor Delivery and to Spatially Direct MSC Fate within Bioprinted Tissues. Scientific Reports 7, no. 1 (December 6, 2017): 17042. https://doi.org/10.1038/s41598-017-17286-1.