### **GMHA Hyaluronan Hydrogels**

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* This chemistry is a two-step process for making cross-linked hyaluronan (HA) hydrogels. The first step is the modification of HA with glycidyl methacrylate (GM) to yield GMHA conjugates; the second step is photocrosslinking at physiological conditions. The entire process takes about 2½ - 4 days.
* See also similar published chemistries using GM and methacrylic anhydride (references below).

# Preparation

Hyaluronan Collaborative Labs

Glycidyl methacrylate\* Aldrich 151238

Triethylamine\* Aldrich 471283

Tetrabutyl ammonium bromide Fluka 86860

Acetone

Phosphate buffered saline (PBS)

Irgacure 2959 Ciba Specialty Chemicals

Vinyl pyrrolidone\* Aldrich V340-9

# \* Hazardous component—be sure to read MSDS before use.

# 5% methacrylated GMHA

1. Dissolve 0.5g HA in 50 ml dI on a stir plate in a chemical fume hood. Thorough dissolution can take several hours to overnight.
2. Add the following components separately and in the following order: 1.0 ml triethylamine, 1.0 ml glycidyl methacrylate, 1.0 g tetrabutyl ammonium bromide. Thoroughly mix before adding the next component.
3. Mix at room temperature in a sealed flask in a chemical fume hood overnight.
4. Incubate the reaction at 50-60°C for 1 h (open the flask seal slightly) and cool to room temperature.
5. Precipitate in acetone, using one part HA solution to 20 parts acetone.
* Pour acetone into a large beaker and while stirring with a glass rod or pipet, slowly add the HA solution.
* The GMHA will precipitate as a cottony white solid.
* If the acetone starts getting cloudy and precipitation stops, start again with fresh acetone.
1. Rinse the precipitate in fresh acetone.
2. Thoroughly dissolve the precipitate in about 30 ml dI.
3. Repeat steps 5-7 to remove all residual excess reactants.
4. Lyophilize frozen solution 24-48 hours and store desiccated at –20 or 4°C.
5. To verify the extent of methacrylation: make a 0.5% solution of GMHA with D2O and submit for 1H-NMR. Compare the peak heights per proton of the HA methyl peaks (at 1.82 or 1.93) and methacrylate peaks (at 5.55 and 5.24).

# Crosslinking UV Lamp: Blak-ray B-100A (from UVP)

* Vinyl pyrrolidinone (VP) acts as a reaction accelerant and co-monomer.
* While concentrations of VP up to 3% produce more extensively cross-linked gels with shorter UV exposures, concentrations above 0.5% have been shown to be cytotoxic. Similarly, concentrations of 2959 above 0.1% have been shown to be cytotoxic to photo-encapsulated chondrocytes (Bryant, Nuttelman, Anseth *J Biomater Sci Polym Ed* 2000, 11(5): 439-57)
* The first procedure below outlines 1-3% VP gels, which may have interesting material properties; the second procedure outlines a less cytotoxic procedure with 0.3% VP and PBS as the 2959 solvent.
* Although initiation requires intense UV light, minimize the exposure of 2959 solutions to ambient light.

*1-3% VP Gels*

1. Make a 0.5-1.5% solution of GMHA in PBS and allow to dissolve thoroughly overnight.
2. Make a 250 mg/ml solution of IRGACURE 2959 in vinyl pyrrolidone. Store at room temp. in the dark.
3. Add 2959 solution\*\* to the GM-HA solution in a chemical fume hood and mix well.
4. Expose to long-wave 365 nm UV light 0.5-4 minutes\*\* to produce a solid gel.

*0.3% VP Gels*

* + 1. Make a 1% 2959 solution in PBS. To dissolve, sonicate the solution and heat to 50°C until the 2959 is completely dissolved. Add 0.3% VP in a chemical fume hood. Store at room temperature in the dark.
		2. Make a 0.5-1.5% GMHA solution in 2959/PBS/VP solution\*\*; allow to dissolve thoroughly in the dark.
		3. Expose to long-wave 365 nm UV light 0.5-4 minutes\*\* to produce a solid gel.

\*\* The amount of 2959, VP and UV exposure needed is determined experimentally and depends on the degree of methacrylation of the particular GMHA batch.

##### Variables to Achieve Varying Degrees of Crosslinking

*Modification*

 Molar excess GM, triethylamine, tetrabutyl ammonium bromide in reaction

 Reaction temperature

 Length of reaction

*Crosslinking*

 Concentration of 2959, VP, GMHA

 Intensity or time of UV exposure

 Addition of other components (e.g., acrylated PEG)

Additional references on similar chemistries

*Glycidyl Methacrylate*

Jin, Yamanaka, Sato, et al. *J Control Release* 2001, 73: 173-81.

Trudel, Massia. *Biomaterials* 2002, 23: 3299-3307.

*Methacrylic Anhydride*

Smeds, Pfister-Serres, Hatchell, Grinstaff. *J.M.S.—Pure Appl. Chem.* A36(7&8): 981-9 (1999).

Smeds, Grinstaff. *JBMR* 54:115-21 (2001).

