3-D Cell Encapsulation for Cell Delivery Applications Guide

This user guide describes how to prepare HyStem®-C hydrogels for encapsulation of cancer cells and injection of this suspension into experimental animals for research purposes only. Researchers are responsible for obtaining a valid Institutional Animal Care and Use Committee (IACUC) protocol prior to initiation of any experiments (if applicable). Below pertains to the operational use of the HyStem-C hydrogel in order to assist in preparing an IACUC protocol.

Conducting a bench-top study recommended to confirm the HyStem-C hydrogel characteristics prior to initiating animal experiments in order to gain familiarity with handling and timing of use. The gelation time and final hydrogel properties are highly dependent upon the medium used, the extent of hydrogel dilution, and the final hydrogel pH.

Conducting a pilot animal study to optimize experimental conditions and become familiar with the handling of the hydrogel is recommended prior to doing large-scale animal testing. The pilot study will provide important information on the time course for tumor growth from a given cell line or primary tumor source, optimal injection size, cell concentration, and HyStem-C dilution.

Xenograft Guidelines

This protocol assumes that a suspension of 50 or 100 µl of HyStem-C + cells will be injected into nine research animals.

The cell loading and amount of injected HyStem-C hydrogel used depends upon your application. The amounts discussed in these guidelines are based on published tumor xenograft experiments[1,2] where a cell concentration of 5×10^7 cells/ml was employed. Lower concentrations may also be effective; however, they will require longer tumor-formation times. Please inquire with our Technical Services team, as experiments with additional injection volumes, cell concentrations, and cell lines are ongoing.

A pilot animal study is strongly recommended to determine optimal cell loading for a given cell source.

Key Points

The exact time for the hydrogel to become viscous and gel depends on: medium used, amount of HyStem-C dilution with medium, and hydrogel-solution pH.

The medium used to dilute the HyStem-C and the amount of dilution can profoundly affect the gelation time. The greater the medium dilution, the longer the gelation time.

The pH of HyStem-C is controlled to ~7.4 prior to cell encapsulation and medium dilution. However, the cell-culture medium used can increase or decrease the pH and change the gelation time. For HyStem-C hydrogels, the higher the pH, the faster the gelation time.

If stiffer hydrogels are required, increase the amount of Extralink® used from a 1:4 ratio of Extralink to Glycosil® + Gelin-S® to a 1:3 ratio or decrease the medium dilution.

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HyStem-C forms a hydrogel by reacting thiols in Glycosil and Gelin-S with the thiol-reactive crosslinker, Extralink. Solutions of Glycosil and Gelin-S can form hydrogels in the absence of Extralink via disulfide bond formation; however, this reaction is normally very slow (hours instead of minutes).

Bench-Top Study

Design a pilot animal experiment based on the guidelines below ("Suggested Pilot Study"). Using the same conditions that will be used in the pilot animal study, perform gelation tests with HyStem-C as follows:

1. Prepare cells in medium at the proper cell density/densities.

2. Solubilize the HyStem-C Hydrogel Kit per instructions (see the HyStem-C Hydrogel Kit Product Data Sheet).

Freeze unused solutions at -20 °C with a nitrogen or argon blanket, unless they will be used in less than four hours. Frozen solutions may be usable up to 2 weeks, but slow autocrosslinking can change gel properties. Use freshly reconstituted material when possible.

3. For each condition, add 0.25 mL of Glycosil and 0.25 mL of Gelin-S to a small glass vial. Pipette back and forth to mix.

4. Add the appropriate amount of cells in medium to the Glycosil + Gelin-S mix (0.05 mL for 10% dilution, 0.50 mL for 50% dilution). Pipette to mix.

5. Add 0.125 mL of Extralink to the vial. Record the time. Pipette to mix. The initial solution will be low viscosity (similar to medium).

6. Every few minutes, invert the vial. As the hydrogel forms, the liquid will become more viscous. Record the time at which the hydrogel no longer flows when the vial is inverted.

Note: The gelation time is the difference between the two recorded times. This establishes the maximum length of time you will have to use HyStem-C after the Extralink is added. If you are uncertain about the type of medium or dilution, please determine the gelation time for all of the most likely conditions. If you desire a longer gelation time, increase the amount of medium used to more than 40% dilution.

Suggested Pilot Study

Please use this pilot study to determine the optimal HyStem-C dilution with medium, cell density, hydrogel-injection volume, and coordination of surgical or injection manipulations with hydrogel handling. This protocol is based on nine mice with four subcutaneous injections each (see Table 1 below), where each experimental condition (cell density and injection volume).

Table 1. Injections on each mouse
Inj 1: Control injection: Cells + PBS
Inj 2: 90% HyStem-C + 10% Cells + medium
Inj 3: 70% HyStem-C + 30% Cells + medium
Inj 4: 50% HyStem-C + 50% Cells + medium