Hyaluronan Microcarriers for MSC Expansion and Cell Delivery

Sarah K Atzet, Te-wei Chu, Chris Paulsworth, Terry Tandeski, Amanda McCollough, Thomas Zarembinski
Glycosan BioSystems, Salt Lake City Utah

Statement of Purpose: The emergence of human mesenchymal stem cell (hMSC)-based therapies requires the growth of a large number of viable, clinical grade hMSC for transplantation and their subsequent delivery into a specific site. Current methods for both large-scale growth on the surface of tissue culture plastic and for subsequent cell harvest using trypsin are both inefficient for cell expansion and transplantation and present opportunities for contamination during passaging[1]. While microcarriers are currently available commercially, they are suboptimal for hMSC expansion since they do not have the specific attachment signals that the hMSC hyaluronic-acid rich, extracellular matrix typically provides. Additionally, some are animal collagen-based, causing regulatory concerns of animal-transmitted disease in subsequent cellular delivery.

HyStem hydrogels, a chemically modifed and crosslinked form of hyaluronan, are used to produce animal-free microcarrier hydrogels for the expansion and seamless tranplantation of hMSC. These microcarriers not only provide the requisite attachment sites for hMSC and thereby increase expansion efficiency but can serve as a delivery vehicle for cellular therapies. HyStem hydrogel have been shown to increase implanted cell engraftment and survivability [2].

Methods: HyStem-C kits are comprised of three components, thiol-modified hyaluronan (HyStem), thiol-modified porcine gelatin (Gelin-S), and polyethylene glycol diacrylate (Extralink). The components are supplied as lyophilized powders that when reconstituted and combined form a soft hydrogel [2]. Prior to gelation the solution can be used to coat plates or in combination with a cell pellet/drug to deliver and localize injections. It is desirable to provide an animal free system and as such, recombinant gelatin has been investigated as a replacement. Recombinant human gelatin of 100 kDa molecular weight will be thiol-modified as previously described (rhGelin-S) [38] and used in the production of HyStem microcarriers. Microcarriers were produced using reverse emulsion techniques. Solutions of HyStem and rhGelin-S were prepared at 10 mg/mL (1 wt%) and Extralink at 50 mg/mL (5 wt%). Pre-gel solutions were prepared by adding the former three solutions in a 2:2:1 volumetric ratio. This solution was then added to vegetable oil stirred by an impeller at 250 rpm and allowed to cure during the 60 minute stirring period. Microcarriers were allowed to settle, collected and the filtered with a cell sieve. Microcarriers were then rinsed exhaustively to remove residual oil. By varying initial concentrations gelation time and mechanical properties the hydrogel can vary over a 100 fold range [3]. Microcarriers were seeded with NIH 3T3 fibroblasts at a concentration of 0.2x10^6 cells per mL. Microcarriers were then cultured in static conditions for 6 days and images were taken at day 1, 4 and 6 (confluent). The diameter and distribution of the microcarriers was analyzed from light microscopy images, as shown in Figure 1. Figure 2 shows the progression of fibroblast growth on HyStem microcarriers. To evaluate the localization and efficacy of microcarriers hMSCs are delivered on HyStem microcarriers in a murine osteochondral defect model [4]. hMSCs seeded on optimized HyStem microcarriers and allowed to proliferate to confluency. Equal numbers of hMSC were injected in one of three forms: hMSC alone, hMSC encapsulated in HyStem, and hMSC attached to HyStem microcarriers. The efficacy and localization of viable hMSC will be evaluated over the course of 10 weeks via histological sampling.

Results

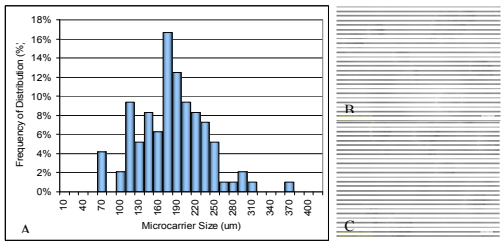

Figure 1. HyStem microcarriers size distribtion (A) and light microscopy images (B, C) scale bar = 100 μm.

Figure 2. NIH 3T3 cells seeded on HyStem microcarriers at day 1 (A), 4 (B), and 6(C).

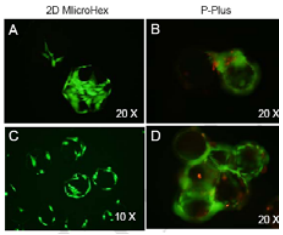

Figure 3. Fluorescent microscopy of hMSC attached to microcarrier for hMSC expansion. Live cells=green, dead

Conclusions: HyStem microcarriers can be produced and tuned by reverse emulsion techniques. These carriers can support cell growth and proliferation and have particular application in cell culture which requires specific attachment site. The HyStem carriers can be used to seamlessly deliver cells for therapeutic applications following expansion.

References:

1)Paul, D., et al, Antioxid Redox Signal, 2009. **11**(8): p1841-55.
2) Prestwich, G.D., J Cell Biochem, 2007. 101(6): p. 1370-83.
3)Vanderhooft, J.L., et al., Macromol Biosci, 2009. **9**(1): p.20-8.
4) Toh, W.S., et al., Biomaterials. **31**(27): p. 6968-80.