Hydrogel microspheres increase cell survival and increase new bone volume in a gene therapy bone formation model.

Ronke Olabisi[1], Zawaunyka Lazard[2], Mary Hall[3], Eva Sevick[3], Alan R Davis[2], Elizabeth A Olmsted-Davis[2], Jennifer L West[1]
[1]Department of Bioengineering, Rice University, Houston, TX, USA
[2]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA
[3]Center for Molecular Imaging, The University of Texas Health Science Center, Houston, TX, USA

Statement of Purpose: We have previously described a system in which adenovirus-transduced BMP-2 expressing fibroblasts were encapsulated within PEG-DA hydrogels, which allowed the diffusion of sufficient amounts of BMP-2 protein from the cell-seeded hydrogels to induce bone formation *in vivo* in a murine model.[1] These poly(ethylene) glycol diacrylate (PEG-DA) hydrogels were 25 µl droplets and more transduced cells were required when encapsulated than when directly. In order to improve diffusion of nutrients and BMP-2, these hydrogels were formed as microspheres, enabling placement through injection.

Methods: Hydrogel precursor solution was created by combining 0.1 g/ml PEG-GGGMGPSGPWGGK-PEG with 1.5% (v/v) triethanolamine/HEPES buffered saline (HBS, pH 7.4), 37 mM 1-vinyl-2-pyrrolidinone, 1.0 mM eosin Y and transuduced MRC-5 cells for a final concentration of $6x10^4$ cells/µl. Acetophenone was combined in 1-vinyl-2-pyrrolidinone at a concentration of 300 mg/ml. The acetephenone solution was then added to mineral oil at 3 µl/ml. Microspheres were created through vortex induced emulsion in the oil. MRC-5 cells were transduced with Ad5dsRED, Ad5F35BMP2 or the empty virus HM4 and analyzed *in vitro* and *in vivo* to determine viability and BMP-2 expression. For in vitro assays, microspheres were incubated with media and 2 µM calcein acetoxymethyl ester and imaged under a fluorescent microscope. In order to evaluate BMP-2 expression, transduced cells were microencapsulated or plated directly at $1x10^5$ cells/well in 0.4 µm pore polycarbonate membrane transwell inserts and W20-17 cells were cultured in the wells of 6 well plates. After 72 hours W20-17 cells were assayed for AP activity using a chemiluminescence procedure[2]. For *in vivo* assays, microspheres or direct cells were injected into the right lateral hind limb of the mouse. Mice injected with dsRED cells or microspheres were monitored with live animal imaging, while mice injected with BMP-2 cells or microspheres were x-rayed at 2 weeks. All animal procedures were according to the protocols established by the Institutional Animal Care and Use Committee (IACUC) of both Baylor College of Medicine and the University of Texas.

Results: Transduced cells showed good survival of the microencapsulation process (Fig. 1) and BMP-2 release was comparable to that of the monolayer (Fig. 2, left). Animals injected with BMP-2 cells showed greater bone formation with microspheres than direct cell injections. (Fig. 2, middle and right). Similarly, animals injected with dsRED cells (Fig. 3) showed greater fluorescence in the microspheres than in the cells directly injected (Fig. 4).

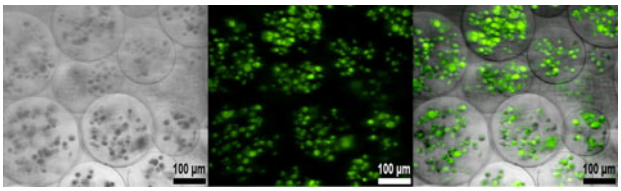

Figure 1. Viability of microspheres.

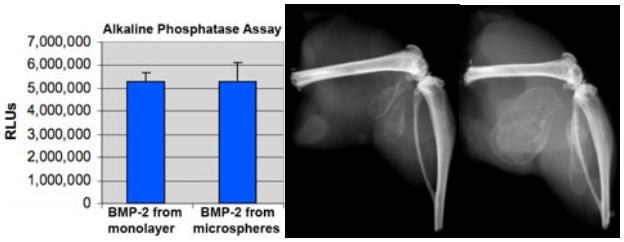

Figure 2. Left: AP activity *in vitro*. Bone formation following direct injection (Middle) and microspheres (Right).

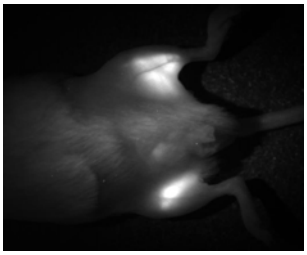

Figure 3. *In vivo* dsRED fluorescence in a mouse.

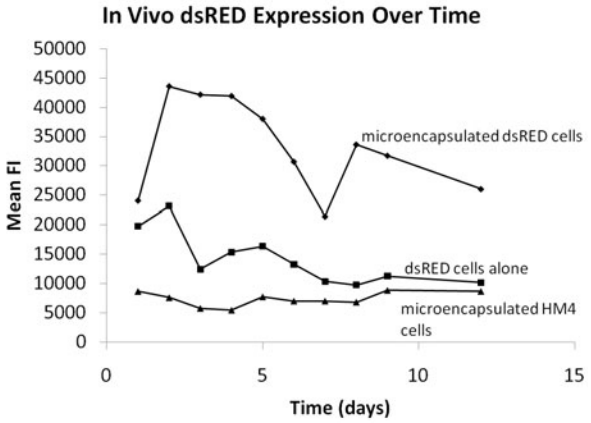

Figure 4. *In vivo* dsRED fluorescence over time.

Conclusions: The results indicate that hydrogel microencapsulation provides protection of the transduced cells from clearance, permitting more effective use of *ex vivo* gene therapy treatments.

References

1. Bikram M. et al. Annals of Biomedical Engineering. 2007;35: 796-807
2. Olmsted, E. et al. Journal of Cellular Biochemistry 82, 11-21 (2001).