Hydrogel Microencapslation Permits Critical Size Defect Repair Via Gene Therapy

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Statement of Purpose: We have previously demonstrated that microencapsulated adenovirus-transduced BMP2 expressing fibroblasts are protected from clearance after intramuscular injection, which enables longer gene expression and greater volumes of heterotopic bone formation.[1] In the current study, we extend this system to clinically relevant orthotopic bone formation. Cells were encapsulated within our poly(ethylene) glycol diacrylate (PEGDA) microsphere hydrogels and injected into rat critical size femoral defects in order to elicit repair.

Methods: *Cell culture, transduction, and microencapsulation:* Wistar skin fibroblasts (WSF) were harvested, expanded in DMEM supplemented with fetal bovine serum and gentomicin, penicillin and streptomycin, then trandsuced with replication defective E1-E3 deleted first generation human type 5 adenovirus (Ad5) constructed to contain cDNAs for human BMP2 in the E1 region of the virus as previously described[2,3]. Media was collected and incubated with W20-17 cells to determine alkaline phosphatase (AP) activity. WSFs (1×10^7) were then combined with 0.1 g/ml PEGDA with 1.5% (v/v) triethanolamine/HEPES buffered saline (pH 7.4), 37 mM 1-vinyl-2-pyrrolidinone and 0.1 mM eosin Y to form a hydrogel precursor solution. Sterile mineral oil was combined with 3 µl/ml of 2,2-dimethoxy-2-phenyl acetophenone in 1-vinyl-2-pyrrolidinone (300 mg/ml). The hydrogel precursor solution and mineral oil were emulsified by vortex for 2 s while exposing to white light for an additional 20 s. Microspheres were isolated by two media washes followed by 5 min centrifugation at 1350 rpm, collected injected into rat femoral defects or maintained for 24 hr then stained with ethidium homodimer and calcein AM to assess viability. *Surgical Procedures*: All animal procedures were performed according to the Institutional Animal Care and Use Committee of Baylor College of Medicine. Wistar rats received either autologous or microencapsulated allogeneic WSFs. Critical size defects (~3 mm) were created following exposure and fixation of rat femurs after linear incisions over the lateral aspect of the gluteal region from the palpable region of the greater trochanter of the femur to the knee. The defect was injected with either 1) Ad5BMP2-transduced autologous cells; 2) Ad5BMP2-transduced microencapsulated allogeneic cells; or 3) no treatment. Incisions were closed with surgical clips, antibiotic ointment was applied, and rats received 0.05 mg/kg buprenorphine for pain.

Results: Media from Ad5BMP2-transduced WSFs induced AP activity in W20-17 cells, and at 24 hr 92% of microencapsulated cells were alive (Fig 1). Animals in the autologous group show no healing of the critical size defect at 2 wk (Fig 2A). In contrast, animals receiving microencapsulated Ad5BMP2-transduced allogeneic WSFs show robust bone growth at 2 wk (Fig 2B).

Conclusions: Despite the use of autologous cells to prevent immune clearance of the Ad5BMP2 transduced cells, in these preliminary studies, bone failed to form when these cells were injected. On the contrary, when allogeneic cells were transduced with Ad5BMP2 and microencapsulated within PEGDA microspheres prior to injection within the defect, bone formation crosses the gap within 2 weeks. The data suggest that the PEGDA microspheres provide immunoprotection of the transduced allogeneic cells, permitting them to release sufficient amounts of BMP2 to induce bone formation. Ongoing studies to confirm these results and to additionally compare the outcome when allogeneic cells are directly injected are being conducted.

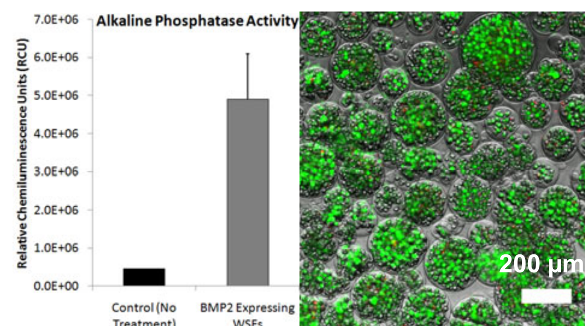

Figure 1. Left: AP activity of Ad5BMP2-transduced WSFs prior to encapsulation. Viability staining of WSFs 24 hr after microencapsulation (green: live; red: dead).

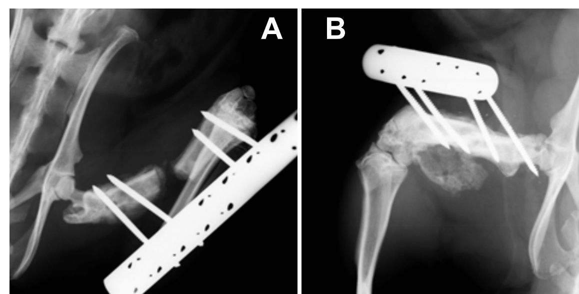

Figure 2. Radiographs at 2 weeks post surgery. **A**: Autologous Ad5BMP2-transduced WSFs. **B**: Micro-encapsulated allogeneic Ad5BMP2-transduced WSFs.

References

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