Gelatin-based Microcarriers Fabricated as Injectable Bone Scaffolds for Mesenchymal Stem Cell Osteogenesis

Chukwuma Nweke and Jan P. Stegemann
Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.

Statement of Purpose: Complex bone defects frequently disrupt matrix integrity, associated mechanobiological signaling, and the required biological substrate for bone-forming cells, which can leave patients with debilitating bone non-unions. Conventional bulk scaffold implantation therapies address some of these issues, but are invasive and hamper osseointegration. To better avoid these issues, non-cytotoxic mechano-transductive microspheres of gelatin (GmC) have been fabricated and seeded with human bone marrow mesenchymal stromal cells (MSC) for later incorporation within an osteogenic injectable binder. GmC formulations were monitored for suitable control over size, morphology, and mechanics. GmC-cultured MSC were assessed for cell attachment, viability, alkaline phosphatase (ALP) activity, and calcium deposition to establish autonomic cellular matrix deposition. A similar array of tests are being replicated for cell culture on GmC modified through substrate mineralization or binder gel suspension schemes. The overall results of this project will shed further light on the effectiveness of an injectable, mechanically-tuned modular cell substrate at restoring bone cell function and supporting matrix mechanics.

Methods: A water-in-oil batch emulsification process was used to prepare GmC from aqueous 6-14 wt% gelatin solution in liquid polydimethylsiloxane. Specific gelatin formulations were used to modulate substrate stiffness and the associated osteogenic potential of GmC. GmC were subsequently washed in 0.01 v/v% L101-surfactant PBS, collected by centrifugation, and then crosslinked with 1 wt% genipin. Particle size distributions were analyzed in a Malvern Mastersizer 2000S (Malvern Panalytical, Malvern, UK). Materials were mechanically tested by shear rheometry (TA Instruments, New Castle, DE). GmC cytocompatibilities were characterized in terms of MSC seeding efficiency. Long-term cell studies were also conducted through packed-bed culture of MSC (Lonza, Basel, CH) on GmC. Cell viability was examined by live-dead assay. Calcein AM was used for cytosolic staining while ethidium homodimer was used for nuclear staining.

Results: Optical microscopy (**Fig. 1A**) and laser diffraction particle size analysis (**Fig. 1B**) of 6-14 wt% gelatin GmC showed a change in average median diameter from 86.2 ± 14.2 µm to 149.6 ± 6.2 µm. This change is typically observed as a result of increasing solid content in the aqueous phase of emulsions. All GmC were spherical, topographically smooth, and auto-fluorescently red. Seeding efficiency of MSC onto 6, 10, and 14 wt% gelatin GmC was routinely high, with attachment rates of 96.3 ± 1.1%, 95.7 ± 1.7%, and 94.1 ± 4.0%, respectively (**Fig. 1C**). High seeding efficiencies on gelatin-based carriers theoretically promote uniform spatial distribution of bone-forming cells within a filled volume. However, ANOVA did not show statistical significance between formulations of varied gelatin content.

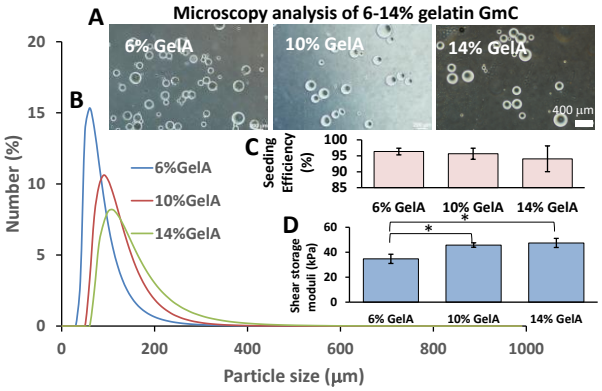

Figure 1. Analysis of 6-14 wt% gelatin GmC

Hydrogel samples of 6-14 wt% gelatin were tested under oscillatory torsion and yielded shear storage moduli of 34.7 ± 3.7 kPa, 45.8 ± 1.8 kPa, and 47.5 ± 3.7 kPa, respectively (**Fig. 1D**). During a three-week culture period in control (CTRL) and osteogenic (OST) media, MSC maintained high viability (>90%) throughout on 6 wt% gelatin GmC (**Fig. 2A**). Correspondingly, assays for ALP activity and calcium deposition on cells seeded at densities of 5,000 (5K/cm^2), 25,000 (25K/cm^2), and 50,000 cells/cm^2 (50K/cm^2) indicated significant osteogenic development. ANOVA showed significant changes in ALP activity from Day 1 to 8 for MSC cultured at 5K/cm^2 in CTRL media and at 25K/cm^2 and 50K/cm^2 in OST media (**Fig. 2B**). All showed significant calcium deposition over the three-week time-span, even without supplements (**Fig. 2C**).

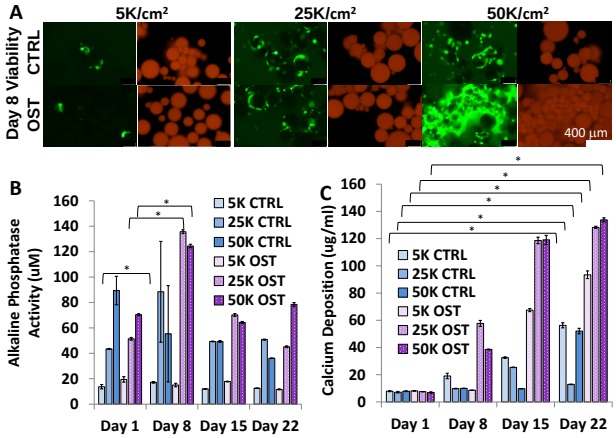

Figure 2. MSC viability, ALP activity, and calcium deposition on GmC

Conclusions: These data demonstrate that genipin-crosslinked gelatin GmC can serve as compositionally and mechanically tunable modules that have advantages over conventional polymeric scaffolds in terms of metabolite exchange, cell distribution, and osteogenic induction. Current experiments focus on syringe delivery of GmC dispersions in an osteogenic, thixotropic binder gel. Such features promise to generate a potent GmC-based, cellular bone filler for regenerating critical-size bone defects.