Biocompatibility of Zn-Containing Sol-Gel Derived Glasses in a Murine Wound Model

David Jaroch[1], Lianne Miller[2], Alisha Raehl[2], Sara Gibbs[2], Katherine McKeown[2], Jeremy Goldman[2], Daniel Clupper[1,2]
Departments of [1]Materials Science & Engineering and [2]Biomedical Engineering
Michigan Technological University, Houghton, MI, USA

Statement of Purpose: The goal of this research was to assess the biocompatibility and angiogenic potential of a bioactive zinc containing sol gel system using a murine wound model. Melt derived bioactive glass 45S5 particulate has been shown to induce human endothelial cell proliferation in vitro through the release of vascular endothelial growth factor (VEGF) from glass-exposed fibroblasts[1]. Fibroblast proliferation was, however, decreased in the presence of the 45S5 particulate[1]. In contrast to melt derived bioactive glasses, sol gel glasses can be formulated to release ions such as zinc, which have been shown to induce angiogenesis and reepithelialization[2]. The current work provides insight into the biocompatibility and soft tissue loading tolerance of these controlled release materials.

Methods: Zn-containing sol-gel glass (8 mol% ZnO, 22 CaO, 70 SiO_2 was prepared and heat treated at 650, 750, or 850ºC. Melt derived bioactive glass 45S5 (45 wt % SiO_2, 24.5 Na_2O, 24.5 CaO, 6 P_2O_5) was also prepared.

Female Balb/C mice (6-8 wks old) were anesthetized and a 2 mm long, fully circumferential, region of skin was removed midway up the tail. A protective silicone cuff was placed over the regenerating region and a suspension of glass particulate (<10 μm, 50 mg/ml solution) in a collagen solution (3 mg/ml) was injected under the cuff to replace the excised skin (Fig. 1). Mice (n=5 per group) were euthanized and regenerating regions were collected following 15 days of skin regeneration .

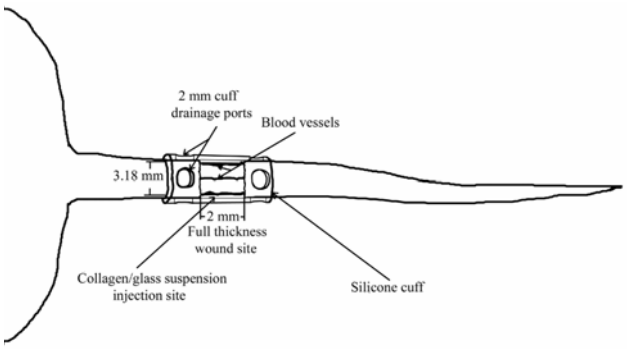

Figure 1. Murine circumferential dermal excisement model of wound healing.

Frozen tail specimens were cryosectioned into 10 μm sections. Tissue sections were co-immunostained for lymphatic endothelial cells (LECs) and blood endothelial cells (BECs). BEC density was determined by counting total BEC cells per mm^2 of regenerating tissue. LEC co-staining was used to exclude LECs from the BEC density measurements. Statistical analysis was performed using Dunnett's comparison test ($p<0.05$).

Results/Discussion: A Dunnett's statistical analysis showed that bioactive glass 45S5 laden samples significantly reduced BEC density (p=0.0002) while the 8Zn850 and 8Zn750 sol gel samples showed no significant change (p=0.799 and 0.663, respectively) relative to normally regenerating control skin (Fig. 2). However, the 8Zn650 sol gel group significantly reduced BEC density relative to controls (p=0.038). Comparison between the 45S5 and 8Zn650 groups demonstrated no significant differences in BEC density (p=0.065).

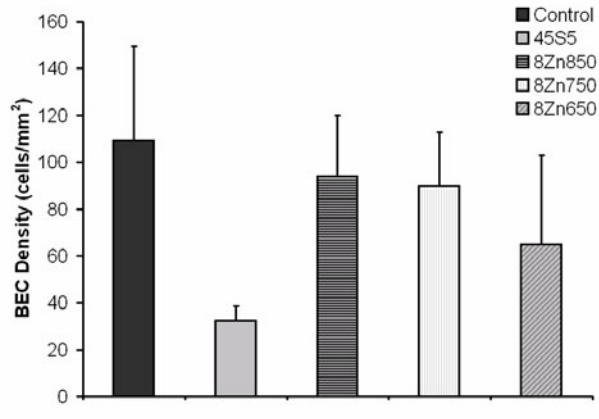

Figure 2. BEC cell counts.

Zn-containing sol gels (8Zn850 and 8Zn750) were well tolerated at high concentrations. Conversely, 45S5 particles, proven to induce VEGF production and stimulate endothelial cell proliferation at lower doses, reduced blood capillary angiogenesis under the current loading conditions as revealed by the significant reductions in BEC density. BEC density decreases by 8Zn650 particles may be attributable to excessive calcium release. Bioactivity testing revealed pronounced Ca release from 8Zn650, 8.9 mM, after 14 d immersion in SBF[3]. Maeno et al. reported that similar concentrations (≥10 mM) produced cytotoxic effects with osteoblasts, cells equipped for high Ca concentrations, in vitro[4].

Conclusions: Zn-containing sol gel derived glasses were well tolerated at concentrations that caused decreased cell densities when melt derived bioactive glass was exposed to regenerating skin. High tissue loading tolerance, coupled with the ability to incorporate a variety of ions proven to enhance regeneration in soft tissues, suggest that sol gel derived silica based bioactive glasses may be ideal vectors for controlled release into a wound site.

References:

1. Day RM. Tissue Engineering. 2005;11:768-777
2. Lansdown ABG. The Lancet. 1990;347:706-707.
3. Jaroch DB. J Biomed Mater Res. Accepted 2006.
4. Maeno S. Biomaterials. 2005;23:4847-4855.