Bioactive Glass Ionic Dissolution Products Increase Human Osteoblast and hMSC Proliferation and Osteogenic Expression in vitro

Annie Reza, Ph.D., Stephen McIlhenny, Ph.D.
Stryker Orthobiologics, Malvern, PA, USA.

Disclosures: A. Reza: 3A; Stryker Orthobiologics. 4; Stryker. S. McIlhenny: 3A; Stryker Orthobiologics. 4; Stryker.

Introduction: The limited supply of tissue and donor site morbidity associated with bone autograft have continued to motivate investigation into alternative synthetic materials and strategies for bone repair. Bioactive materials, such as 45S5 bioactive glass, remain a focus in orthopaedic regenerative medicine technologies. Upon introduction into a physiological environment, 45S5 bioactive glass releases soluble calcium and phosphorous ions into the localized niche [1]. Reports have demonstrated that these ion dissolution products elicit a cellular response, including increasing cell proliferation [2, 3] and enhancing the osteogenic phenotype [2-4]. The present study evaluated the effect of the ionic dissolution products of Combeite, a specific devitrified modification of 45S5 bioactive glass, on human mesenchymal stem cell (hMSC) and human primary osteoblast cell proliferation and mineralized collagen matrix production in vitro.

Methods: Following a 24-hour preconditioning period, 45S5 Combeite bioactive glass (bimodal distribution of 32-90 and 90-150 µm particle size) was eluted at a 1.1% concentration (w/v) in alpha-MEM with 17% FBS + 1% PenStrep (Gibco) for 24 hours at 37°C, 5% CO₂. The glass particles were removed and the media, containing the ionic dissolution products of the bioactive glass, was collected for use in cell culture supplementation. hMSC (Lonza #PT-2501) and primary normal human osteoblast cells (Lonza #CC-2538) were seeded on fibronectin-coated (2 µg/cm², BD Biosciences) 24-well plates at 6,000 and 9,000 cells/cm², respectively, and allowed 24 hours for cell attachment. Media was then exchanged and cells were cultured for the remainder of the study in glass-free control media or glass-eluted media. Osteoblast control and osteoblast glass-eluted media were further supplemented with 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid 2-phosphate, and 10 nM dexamethasone (Sigma). Media was changed three times per week. Cells were lysed (CelLytic M, Sigma) and proliferation was quantified via DNA content using the PicoGreen assay (Invitrogen). Histology samples were fixed and stained with Picrosirius Red (EMS) to visualize collagen elaboration or Alizarin Red (Ricca) to assess mineralization. Statistical differences in proliferation were measured using a two-sample t-test (p<0.05). Data represent mean ± standard deviation (n=4).

Results: hMSC proliferation was quantified at days 8, 9, and 14, while osteoblast proliferation was quantified at days 3-6 and day 10. Significant increases in DNA content were detected in glass-eluted samples versus control at all time points for both cell types (Figure 1). Proliferation of hMSC glass-eluted samples was 75% greater than control at day 14, while proliferation of osteoblast glass-eluted samples was over 200% greater than control at day 10. Collagen elaboration was visualized at day 14 of culture, with more intense staining exhibited in glass-eluted samples vs. control for both cell types (data not shown). hMSC mineralization (Figure 2, top) was evaluated at day 28 with markedly increased staining detected in glass-eluted samples compared to control. Osteoblast mineralization (Figure 2, bottom) was
assessed at day 14 with a notable increase in staining intensity for glass-eluted samples observed in culture versus control.

**Discussion:** This study examines the effects of the ionic dissolution products of Combeite bioactive glass on both undifferentiated hMSC and primary osteoblasts. These physiologically relevant cell types represent the bone-forming cell populations likely encountered *in vivo* at the site of healing. The data demonstrate a significant increase in DNA content indicative of a significant increase in cell proliferation in response to glass-eluted media compared to controls. This resulted in a larger cell population capable of contributing to the elaboration of a mineralized collagen matrix. Approximately 90% of the organic matrix of native bone is collagen, which, in turn serves as the scaffold upon which mineralized matrix is deposited [5]. The presence of a mineralized matrix is indicative of the mature osteoblast phenotype, thereby providing an important functional marker [6]. Phenotypic calcified matrix production was evaluated via histological stains for collagen and mineralization. Both methods of analysis exhibited an enhancement in the elaboration of matrix components characteristic of the osteogenic niche in response to glass-eluted media. Importantly, undifferentiated hMSC cultures exhibited greater collagen deposition and increased mineralization in response to glass-eluted media in the absence of any other differentiation reagents, indicating the impact of Combeite bioactive glass. This study establishes increased *in vitro* cell proliferation and improved expression of the osteogenic phenotype for hMSC and primary osteoblasts in response to the ionic dissolution products of Combeite bioactive glass.

**Significance:** These studies demonstrate the positive influence of Combeite, a bioactive glass used clinically as a component of synthetic bone void fillers, on primary osteoblast and stem cell proliferation and differentiation in support of the osteogenic phenotype *in vitro.*
**Figure 1.** hMSC and osteoblast cell proliferation in response to culture in glass-eluted media. Significant increases were measured at all time points versus control for both cell types (n=4, p<0.05).
Figure 2. Mineralization of hMSC at day 28 (top) and osteoblasts at day 14 (bottom). Bar = 200 µm