# Plastically Compressed Bioglass Incorporated Collagen Matrices for Orthopedic Applications

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**Statement of Purpose:** Bone defects are a major healthcare problem worldwide. In the United States alone, around 1 million bone grafting procedures are performed each year costing an estimated $2.5 billion[1]. Autologous bone grafts are currently the gold standard for the repair of bone defects; however, donor site morbidity and the need for multiple surgeries are major limitations. Cadaver-derived allografts are often associated with risks of immune related complications and disease transmission. Bone tissue engineering has been pursued as an alternative treatment methodology; albeit with little success. Therefore, there is an unmet need for the development of functional biomaterials to augment bone repair and regeneration. Bioglass 45S5 developed by Hench et al. is an osteoinductive ceramic that has been shown to expedite bone formation and improve osteointegration[2]. In this study, bioglass particles were incorporated into collagen matrices using plastic compression method and the bioactivity of these matrices was assessed by culturing SaOS-2 cells for 14 days.

**Methods:** The protocol for the synthesis of plastically compressed bioglass incorporated collagen matrices was modified from previously published literature[3-4]. Briefly, type I collagen with or without bioglass particles (45S5; 10 μm; obtained from Dr. Larry Hench) was mixed with 10x PBS and 0.1N NaOH and gelled in circular silicone molds (radius=5mm) by incubation at 37 ºC for 1 hour. Following gelation, the gels were sandwiched between a set of polyethylene terephthalate (PET) mesh and metal mesh using a custom set up (Fig. 1A) and plastically compressed by applying a weight of 10 g for 5 min. The resultant matrices were stained with Alizarin red S to confirm the incorporation of bioglass particles. Scanning electron microscopy was performed to assess the distribution of bioglass particles within the plastically compressed collagen matrices. To test the bioactivity of the bioglass incorporated collagen matrices, SaOS-2 cells (ATCC) were seeded on the matrices (60% by weight of bioglass) at a density of 30,000 cells/cm$^2$ and cultured for 14 days. Collagen only matrices were used as control. Culture medium composed of RPMI 1640 with 10% FBS. At days 7 and 14, samples were fixed, processed and imaged under SEM to check for mineralized nodule formation. Further, the chemical composition of the mineralized nodules was confirmed using EDAX.

**Results:** Alizarin red S staining confirmed the incorporation of bioglass within plastically compressed collagen matrices (Fig. 1B). Further, scanning electron microscopy revealed that bioglass is uniformly distributed throughout the volume of the collagen matrices (Fig. 1C). SEM images showed SaOS-2 cells mediated deposition of mineralized nodules on bioglass incorporated collagen matrices as early as day 7 (indicated by arrows in Fig. 2). Mineralization was not observed on collagen only matrices at day 7 (Fig. 2). By day 14, mineralized nodules were also observed on collagen only matrices. However, a significantly higher amount of mineralization was observed on collagen:bioglass matrices compared to collagen only (Fig.2). Further, EDAX confirmed that the Ca to P atom ratio in these nodules was comparable to that of native bone (1.4-1.6). Together, these results demonstrate that incorporation of bioglass improves the bioactivity and expedites mineralization on plastically compressed collagen matrices.

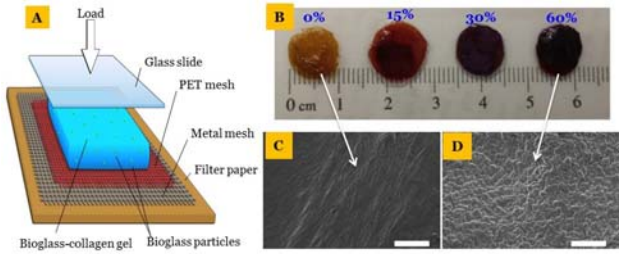


**Fig. 1.** (A) Plastic compression process for the synthesis of bioglass incorporated collagen matrices. (B) Alizarin red staining confirms incorporation of bioglass and SEM shows uniform distribution of bioglass within plastically compressed collagen matrices (C&D). Scale bar: 100 μm.

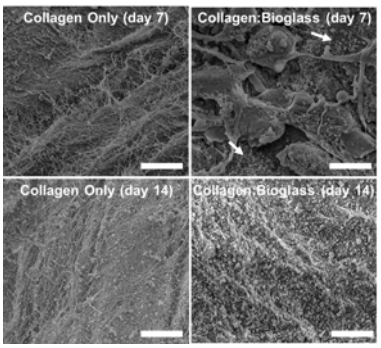


**Fig. 2:** SEM micrographs of plastically compressed collagen and collagen:bioglass matrices at days 7 and 14. Collagen:bioglass matrices induce rapid mineralization. Scale bar: 10 μm

**Conclusions:** In this study using SaOS-2 cells, we have shown that plastically compressed collagen-bioglass matrices induce rapid mineralization that is significantly faster than that observed in collagen only matrices. These results are in agreement with previous studies that have demonstrated the mineralization potential of plastically compressed collagen:bioglass scaffolds using simulated body fluid[3] and MC3T3-E1 cells induced alkaline phosphate expression[4]. While evidence of mineralization using preosteoblast cell lines is promising, studies on the assessment of osteogenic differentiation of mesenchymal stem cells on these matrices are imminent. Successful realization of such differentiation in the absence of external cues will demonstrate the true functionality of the material and be an important step towards its clinical translation. Overall, plastically compressed bioglass incorporated collagen matrices have the significant potential to be used in orthopedic applications.

**References:** [1] Desai BM: Osteobiologics. Am J Orthop 2007;36:8-11; [2] Xynos ID: Calcified Tissue International 2000;67(4):321-29. [3] Marelli B: Biomacromolecules 2010;11:1470-79. [4] Marelli B: Biomaterials 2011;32:8915-26.