IN VITRO EVALUATION OF OSTEOGENIC ACTIVITY OF HUMAN ADIPOCYTE DERIVED STEM CELLS ON VARIED SCAFFOLD FORMULATIONS

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Statement of Purpose: To control the loss of alveolar bone that may result in periodontitis and regenerate the lost bone, guided bone regeneration (GBR) is indicated. The currently used collagen scaffolds for GBR suffer from rapid degradation by collagenases[1] and lack of rigidity.[2] Our studies have shown elastin like polypeptide (ELP)–collagen composite hydrogels were biocompatible and more rigid than the collagen hydrogels.[3,4] Human adipocyte derived stem cells (hASCs) are multipotent cells that can differentiate along osteogenic lineage and have potential to be used in GBR. We hypothesized that hASCs would differentiate along the osteoblastic lineage in more rigid ELP-Collagen composite hydrogels. Hence, we investigated the efficacy of composite hydrogels made with varying concentrations of ELP and collagen for hASCs culture to produce viable osteoblast-like cells.

Methods: Hydrogel Preparation. ELP was prepared as described elsewhere.[4] Hydrogels were formed by mixing ELP and collagen (rat tail collagen type I, Corning) at varying concentrations (Table 1) and incubating at 37ºC.

Table 1: Composition of Hydrogels

Hydrogel	ELP: Collagen Ratio	Concentration (mg/mL)	
		ELP	Collagen
H1	0	0	2
H2	3:1	6	2
H3	0	0	6
H4	3:1	18	6

Cell Culture. hASCs were obtained from discarded whole adipose tissue provided during elective procedures from patients in accordance with the Univ. of Mississippi Medical Center Institutional Review Board protocol #2012-0004. hASCs (50,000 cells/hydrogel) were incorporated during gelation for 3 days of acclimation in DMEM with 10% FBS. Subsequently, the cells were supplemented with osteogenic medium cocktail composed of DMEM, 50 µM L-ascorbic acid, 10 mM β-glycerophosphate, 0.05 nM dexamethasone, 10% FBS, and 1% penicillin/streptomycin for 3 weeks.

Biochemical Characterization. Viability was assessed by Live/Dead assay (Invitrogen). Metabolic activity and osteogenic differentiation were assessed by total protein assay (Thermo Sci) and alkaline phosphatase assay (ALP; BioAssay Sys). Osteogenic maturation was assessed by osteocalcin assay (OCN; Invitrogen). Mineral deposition was visualized and quantified by Alizarin red assay (Millipore). Manufacturers' protocols were followed. All assays were performed on days 1, 8, 15, and 22.

Statistical Analysis. ANOVA and Games-Howell post hoc test for unequal variances was performed. Values with $p \leq 0.05$ were deemed significantly different.

Results: The Live/Dead assay showed high number of live cells attesting the biocompatibility of all hydrogels (Fig 1). The increasing total protein content for all hydrogels indicated that cells were metabollically active (data not shown). The normalized ALP activity (~10-30 nM/min/mg protein) and osteocalcin (~0.5 pg/mg protein) were similar on all days for the H1, H2, and H3 hydrogels (Fig 2a,b). However, the H4 hydrogel showed maximum ALP activity (44 nM/min/mg protein) and osteocalcin (1.6 pg/mg protein) on day 22 indicating continual high differentiation and maturation activity along the osteoblastic lineage (Fig 2a,b). Alizarin red staining on day 23 showed very minimal amount of mineralization in the H1, H2, and H3 hydrogels, while the H4 hydrogel had greater Alizarin red staining indicating mineralization (Fig 2c). Scanning electron microscopy (SEM) paired with energy dispersive spectroscopy (EDS) revealed high amount of calcium and phosphorous deposits on the H4 hydrogel indicating mineralization (Fig. 3).

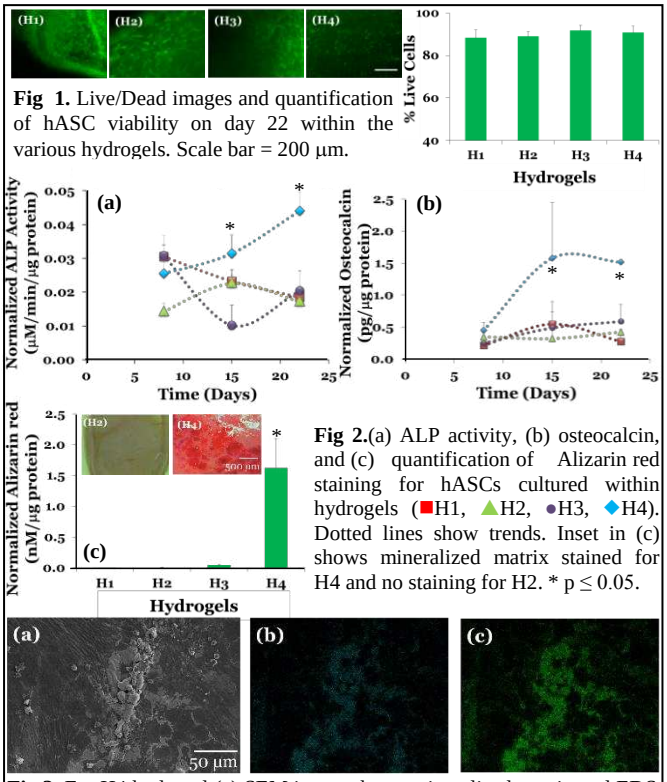

Fig 1. Live/Dead images and quantification of hASC viability on day 22 within the various hydrogels. Scale bar = 200 µm.

Fig 2.(a) ALP activity, (b) osteocalcin, and (c) quantification of Alizarin red staining for hASCs cultured within hydrogels (■H1, ▲H2, ●H3, ◆H4). Dotted lines show trends. Inset in (c) shows mineralized matrix stained for H4 and no staining for H2. * $p \leq 0.05$.

Fig 3. For H4 hydrogel (a) SEM image shows mineralized matrix and EDS image shows (b) calcium and (c) phosphorous ions. Scale bar = 20 µm.

Conclusions: Our results show that a composite hydrogel containing higher concentrations of ELP and collagen to be a suitable scaffold for long-term, 3-dimensional culture and subsequent osteogenic differentiation of hASCs resulting in superior mineralization. This study has laid a foundation for the future of ELP-collagen composite hydrogels in guided bone regeneration with further studies focusing on the evaluation of their efficacy in vivo.

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