Promoting Mineralization and Mesenchymal Stem Cell Differentiation in Injectable, Physically and Chemically Gelling Hydrogels for Craniofacial Tissue Engineering

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Statement of Purpose: Full healing of large, complex craniofacial defects is limited by the ability of current treatments, such as autografts and allografts, to provide adequate bony reconstruction and restore facial contours without donor site morbidity, invasive surgeries, and associated complications. Thus, minimally invasive tissue engineering strategies, particularly those involving injectable hydrogels, have been widely investigated to create scaffolding materials that can be injected into large craniofacial defects, be crosslinked *in situ* to fill complex configurations, locally deliver stem cells and biomolecules, and be remodeled into native bone for complete functional and aesthetic craniofacial reconstruction. To this end, we developed injectable, *in situ* forming, and bioresorbable hydrogels capable of physical gelation through copolymerization of poly(*N*-isopropylacrylamide) (PNiPAAm), a thermosensitive polymer, with a hydrolyzable lactone ring and epoxy pendant groups and chemical gelation through crosslinking with diamine polyamidoamine (PAMAM) crosslinkers. We have previously shown that these hydrogels undergo rapid and dual gelation without shrinking at body temperature [1,2], possess tunable physicochemical properties [2], and can bioresorb into soluble degradation products via lower critical solution temperature modulation. The aim of this study was to modulate the mineralization capacity and characterize the ability of the hydrogels to support and direct differentiation of rat mesenchymal stem cells (MSCs) down the osteogenic lineage. We hypothesized that the incorporation of gelatin microparticles (GMPs) as degradable porogens would enhance cell viability, and in combination with the MSCs, would enhance hydrogel mineralization, leading to improved osteogenic differentiation.

Methods: Thermogelling macromers (TGMs) were prepared with PNiPAAm, glycidyl methacrylate, acrylic acid, and the hydrolyzable ring, dimethyl-γ-butyrolactone acrylate, via conventional radical polymerization by adapting the protocol as previously described [1]. Low molecular weight PAMAM crosslinkers were created using a simple polymerization following established protocols [1,2]. 10 mM glutaraldehyde crosslinked 50-100 μm diameter GMPs were prepared as previously described [3]. MSCs were harvested from the long bones of 6 week old Fisher 344 rats in accordance with Rice University IACUC approved animal protocols. MSCs were encapsulated at a density of 15 million cells/mL in 10 or 20 polymer wt % (w/v) hydrogels with and without 20 wt % (w/w) loading of 10 mM GMPs and cultured in dexamethasone-containing media for 0, 7, 14, 21, and 28 days (n=6) following established protocols [4]. At each timepoint, samples and their acellular controls were analyzed via Live/Dead confocal imaging, biochemical assays, and histology.

Results: Several formulations of injectable, physically and chemically gelling hydrogel constructs were successfully fabricated from the mixing of TGMs, PAMAM crosslinkers, GMPs, and MSCs, resulting in highly swollen, non-shrinking hydrogels (Table 1). Live/Dead confocal imaging demonstrated that viable MSCs were encapsulated, and DNA Picogreen assay showed that the presence of GMPs significantly enhanced cell viability over 28 days. Alkaline phosphatase activity increased over time and peaked at 21 days, indicating osteogenic differentiation of the MSCs. Calcium content was significantly enhanced as a function of GMP incorporation and polymer wt % (Figure 1). von Kossa and hematoxylin and eosin staining demonstrated the mineralization of the hydrogels and homogenous distribution of cells, respectively.

Table 1. Hydrogel Formulations Tested

	Polymer wt %	GMP loading %	Encapsulation density (cells/mL)
Levels/factor	2	2	1
Levels	10, 20	0, 20	15 million

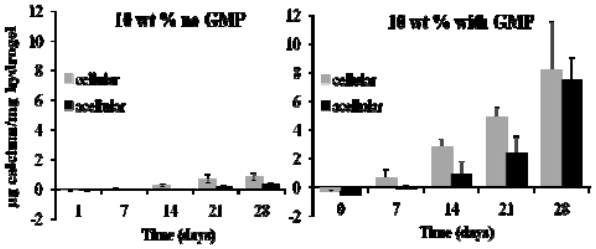

Figure 1. Calcium content of 10 wt % hydrogel constructs with and without GMPs and their acellular controls (n=4)

Conclusions: The results demonstrate that an injectable, dual gelling hydrogel can be used to successfully create cellular composites with MSCs and GMPs. The incorporation of GMPs enhanced MSC viability and hydrogel mineralization. Histological staining and biochemical assays suggested that the hydrogels promoted the osteogenic differentiation of the MSCs. The *in vitro* encapsulation results suggest that these *in situ* forming hydrogels may provide a novel solution for minimally invasive cell delivery for craniofacial bone regeneration.

References:

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