Orbital Floor Regeneration Using Cyclic Acetal Hydrogels

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Statement of Purpose: This work investigates a hydrogel construct for orbital floor regeneration. A novel cyclic acetal based biomaterial, 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD) was investigated. Cyclic acetal biomaterials form diol and carbonyl degradation products, which should not affect the local acidity of the implant. EHD can be mixed with poly(ethylene glycol) diacrylate (PEGDA) and crosslinked to formed a hydrogel. Specifically, a water-soluble radical initiation system ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) was investigated. First, metabolic activity of marrow stromal cells (MSCs) was assessed after continuous exposure to the initiation system to determine if long-term exposure had a significant effect on the MSCs. Second, MSCs were encapsulated in these polymers using the APS-TEMED initiation system to demonstrate that MSCs are able to survive and retain viability within this construct. Lastly, this hydrogel was used to deliver the growth factor bone morphogenetic protein-2 (BMP-2) to a critical size defect in the orbital floor to demonstrate its ability to deliver a growth factor *in vivo*.

Methods: MSCs were isolated from the bone marrow of young Wistar Hannover GALAS male rats. Metabolic activity was assessed after continuous exposure of cells to APS and TEMED at 10, 15, and 20 mM for 30 minutes, 1 hour, and 3 hours. Activity was then analyzed using a dimethylthiazolyldiphenyltetrazolium bromide (MTT) based *in vitro* toxicology kit.

MSCs were then encapsulated in hydrogels. The constructs were prepared using EHD and PEGDA Mn~ 700 at 1:50 molar EHD to PEGDA. Cells were suspended in the gel solution at 2 x 10^6 cells/mL. APS and TEMED were used at 15 mM. Gels were analyzed on days 0 and 7 using the Live/Dead assay.

Hydrogel constructs were then created using EHD-PEGDA and APS-TEMED at 15 mM for the *in vivo* study. Experimental groups included were 0, 10, and 100 ng BMP-2/mL hydrogel and also an empty control with no construct to observe natural healing. New Zealand White Rabbits were used in this study. A defect was created approximately 50% of the orbital floor. At each time point, 7 and 28 days after surgery, the samples and surrounding tissue were retrieved from all groups and prepared for analysis. Histological stains included hemotoxylin and eosin analyzed by light microscopy and standard histomorphometrical analysis. Immunohistochemistry included alkaline phosphatase, osteopontin, and osteocalcin analyzed by quantitative histomorphometrical analysis.

Results/Discussion: First, metabolic activity of MSCs was assessed after continuous exposure to the APS-TEMED initiator system. Results indicate similar levels of activity between the 10 mM, 15 mM and control groups for the 30 min and 1 hr times and decreased activity for the 20 mM group (Figure 1).

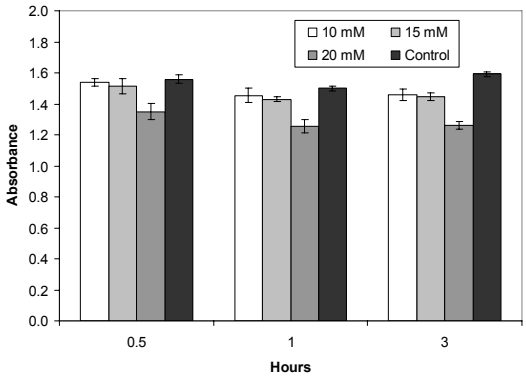


Figure 1. Metabolic activity of MSCs assessed after 0.5, 1, and 3 hours with initiator

Second, MSCs were encapsulated in the EHD-PEGDA gels using the APS-TEMED initiation system. Results show that the majority of MSCs were viable immediately after encapsulation and after 7 days of culture within the gels (Figures 2ab).

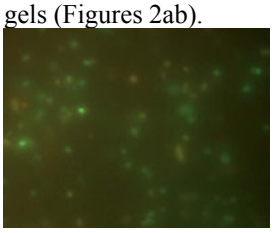
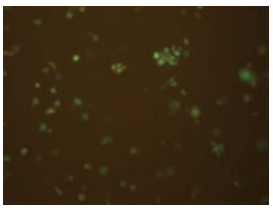

(a)

(b)

Figure 2. Viability of encapsulated cells immediately after encapsulation (a) and 7 days (b) after encapsulation

Third, hydrogel constructs were implanted into orbital floor defects with increasing amounts of BMP-2. Results demonstrate the efficacy of the hydrogel construct to deliver the growth factor *in vivo.*

Conclusions: This study demonstrates the use of the initiators APS-TEMED and EHD-PEGDA as a hydrogel system for tissue engineering of orbital bone. Metabolic activity of MSCs is minimally affected after continuous exposure, viability of MSCs is maintained after encapsulation within the hydrogel construct, and the hydrogels can be used to deliver growth factors *in vivo*. Future studies include augmenting the hydrogels with growth factors while encapsulating MSCs and studying effects on differentiation.