Variance of Extracel Hydrogel Compositions and the Osteogenic Effects on GFP-reporter Preosteoblasts

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Statement of Purpose: The Extracel™ hydrogel is a thiolated hyaluronan based, self-crosslinkable hydrogel. Gelation can take place spontaneously in cell-friendly conditions which makes it potentially suitable for cell encapsulation for 3D cultures and *in vivo* cell delivery. Each component of Extracel™ is chemically defined. Variation of the hydrogel compositions may change the physical and chemical properties and subsequently may create the most suitable microenvironment for specific cells. In the present study, the composition was manipulated in terms of relative ratio of the three components, i.e., Glycosil™, Gelin-S™ and PEGDA. The effect of the gels on the osteogenic differentiation of GFP-lineage-reporter mouse calvarial pre-osteoblasts [1] were studied in 2D culture and correlated to the compositional variations.

Methods: Extracel™ Hydrogel was a kind gift of Glycosan Biosystems (Salt Lake city, UT). To make hydrogels with varied compositions, the ratio of Glycosil™ to Gelin-S™ was varied from 75:25, 50:50 and 25:75 (vol:vol). PEGDA was added to the pre-mixed Glycosil™ and Gelin-S™ with a final concentration of 0.1, 0.2, 0.4 and 0.8% (vol). Coatings were made in the 12-well tissue culture treated plates (TCPS; Falcon, BD). Cells were harvested from calvaria of 6 d old pOBCol2.3GFP transgenic mice according to an established protocol [1]. Proliferation medium contained DMEM, 10% FBS, 1% penicillin/ streptomycin, 1% mM non-essential amino acids (Gibco, USA). Cells were seeded at a density of 15K/cm^2. Upon 7 d, medium was changed to osteogenic medium which contained alpha-MEM, 10% FBS, 1% penicillin/streptomycin, 50 µg/ml ascorbic acid and 4 mM β-glycerophosphate. Medium was changed every 2-3 days. Xylenol orange (XO) dye (20 uM) was added to the culture medium for 12 hrs prior to imaging to observe mineral deposits non-destructively. Fluorescence images were taken using a computerized inverted microscope Zeiss Axiovert 200 (Carl Zeiss, USA). A series of 6x6 adjacent pictures (15% overlap) at 5x objective were acquired and concatenated into a single image. The intensity of GFP and XO fluorescence was quantified based on these fluorescent pictures using ImageJ (NIH).

Results: Cells were confluent by 7 d in all cultures. Mature osteoblasts as evidenced by GFP expression was not observed until 14 d. The GFP expression was higher on all hydrogels than on TCPS controls. On the 0.1% and 0.4% PEGDA crosslinked hydrogels, the GFP expression was highest on 50:50 (vol:vol) (Glycosil: Gelin-S), whereas the other hydrogels showed a minimum GFP expression at the same Glycosil: Gelin-S ratio. Note that the hydrogel containing 0.2% PEGDA exhibited the lowest promoting effect on GFP expression for 75:25 and 50:50 gels. Upon 21 d, the GFP expression increased in all the cultures. The GFP expression was still higher on the hydrogels than on TCPS (Fig. 1, Fig. 2) and trends with composition remained the same as that of the 14 d cultures. XO staining level was consistent with the GFP expression levels as expected because Col2.3-GFP expression has been designed to be expressed only by mature bone matrix-depositing cells (Fig.1).

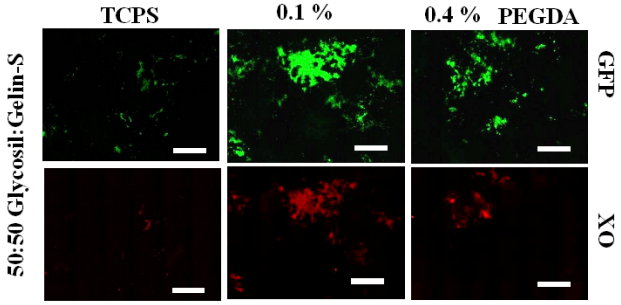

Fig. 1 Images of GFP and XO staining after 21d.

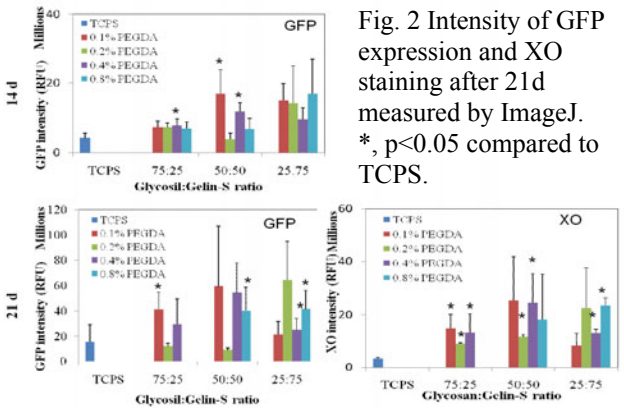

Fig. 2 Intensity of GFP expression and XO staining after 21d measured by ImageJ. *, $p<0.05$ compared to TCPS.

Conclusion: Extracel hydrogel displayed significant promoting effects on osteogenic differentiation of mouse calvarial preosteoblasts. The potency to promote osteogenesis varied in accordance with the composition of the hydrogel. Generally, a maximum promoting effect was obtained with Glycosil: Gelin-S at a 50:50 (vol:vol) ratio and the PEGDA concentration at 0.1% or 0.4% (vol). If the PEGDA concentration was 0.2% and 0.8% (vol), then the Glycosil: Gelin-S ratio of 25:75 (vol:vol) gave the maximum promoting effects. The low 0.1% cross-linker amount resulted in a gel that rapidly degraded before 21d as compared to the 0.8% which remained stable throughout the study. The use of the lineage reporter mice allowed us to rapidly screen 12 different formulations and identify optimal hydrogel formulations to support and promote osteogenesis of preosteoblasts.

Acknowledgment: Extracel™ hydrogel was a kind gift from Glycosan Biosystems (Salt Lake City, UT).

References: [1] Kalajzic I, et al., *J Bone Miner Res*. 2002;17:15-25.