Response of DPSCs to PEG-Melanic Hydrogels

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Statement of Purpose: Found throughout nature, melanins provide numerous functionalities that could benefit biomaterial scientists and tissue engineers. They are secreted to block ionizing radiation[1] and act as antioxidants.[2] Naturally occurring melanin polymers, however, are heterogeneous and difficult to manipulate,[3] highlighting a need for synthetic analogues. We have developed a PEG-melanic hydrogel composed of gallo groups that demonstrates similar properties to natural melanins.[4] Natural melanins are primarily composed of dopaquniones which have demonstrated high cytotoxicity,[5] contrary to our previous work with galloquinone-containing hydrogels.[4] Our synthesis scheme allows us to create melanin-like hydrogels composed of either catechol or gallate groups, thereby allowing direct comparison between the domains. Recent evidence has highlighted that antioxidant-containing materials could improve both longevity and functionality of adult stem cells.[6] The current goal is to examine the response of stem cells from human exfoliated deciduous teeth (SHEDs or DPSCs) seeded onto or encapsulated within melanin-like hydrogels synthesized from both gallate and catechol domains.

Methods: PEG-Gallate (Gal) and catechol (Cat) hydrogels were formulated similar to previous studies.[4] Briefly, 8-arm, 10 or 40 kDa, PEG-NH_2 (JenKem Technology USA Inc.) was substituted with benzyl protected Gal or Cat (TCI America) through carbodiimide chemistry and deportected with a palladium black catalyst (Sigma) under H_2. NMR and FTIR was employed to track phenol substitution and deprotection. Folin-Ciocalteu phenol assay was used to quantify phenol substitution. For 2D studies, individual wells of a 96-well plate were coated with 5 wt% macromer solutions and exposed to an 80 mM $NaIO_4$ solution to initiate crosslinking. GRGDSPC (CycRGD) peptides (American Peptide Company) at different concentrations were allowed to associate with the gels. DPSCs were generously donated by Dr. Maobin Yang's group at Temple University and maintained in α-MEM with L-gluatmine (Gibco) and 20% FBS (Gemini). DPSCs (P7-12) were seeded onto hydrogels at 5000 cells/gel. For 3D encapsulation, DPSCs were rinsed twice with sterile saline before resuspension in PEG macromers with or without DOPA-GRGDS (Amerian Peptide Company). A 7.5 µl droplet was placed onto a PTFE printed glass slide (Electron Microscopy Supplies) and exposed to an equal volume of 15 mM $NaIO_4$ in saline for 2 mins. Cell embedded hydrogels were exposed to excess HBSS to quench remaining periodate. Cell viability was verified with MTS (Promega) and Live/Dead® (Invitrogen) assays following standard protocols. Reverse transcription, qRT-PCR was conducted to assess stem cell phenotype when embedded within PEG-melanic hydrogels.

Results: DPSCs were seeded onto hydrogels formed from 8-arm, 10 or 40 kDa, PEG-Gal and Cat macromers at 5 wt% modified with varying concentrations of CycRGD. DPSCs were cultured up to 17 days on the PEG-melanic hydrogels and cell attachment was assessed with a standard MTS assay corrected with blanks and hydrogels alone. By day 3, DPSCs had fully attached to melanin-like hydrogels synthesized from both 10 and 40 kDa, 8-arm, PEG-Cat without RGD dependency. This was in contrast to hydrogels synthesized from PEG-Gal macromers which demonstrated dependency to RGD inclusion (Figure 1). At day 17, a similar RGD dependency was demonstrated with hydrogels formed from 8-arm, 10 and 40 kDa, PEG-Gal (not shown). Cell attachment and viability was confirmed with Live/Dead® staining (Figure 2). DPSCs demonstrated high viability in all groups except for those hydrogels synthesized from gallate domains without CycRGD addition.

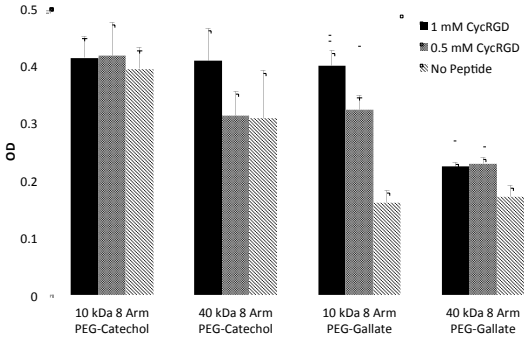

Figure 1. MTS Assays of DPSCs cultured onto hydrogels for 3 days. Bar = $p<0.05$

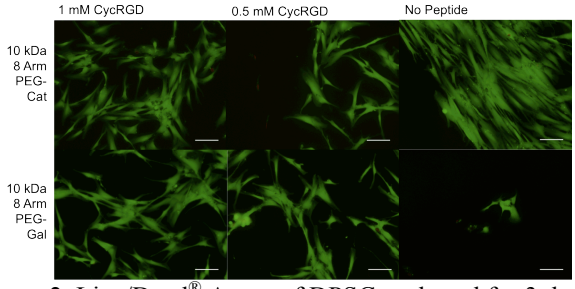

Figure 2. Live/Dead® Assay of DPSCs cultured for 3 days onto hydrogels. Scale bar = 100 µm

Conclusions: Melanin-like hydrogels were synthesized from 8-arm, PEG-Gal and Cat of various molecular weights. Hydrogels were modified with different concentrations of CycRGD to facilitate cell attachment. DPSCs demonstrated a RGD dependency when seeded onto hydrogels synthesized from PEG-Gal macromers but not PEG-Cat macromers. Future work will examine the response of DPSCs when embedded within 3D, PEG-melanic hydrogels.

References: 1. Dadachova, E. Current Opinion in Microbiology 2008;11(6):525-531. 2. Ju, K-Y. Biomacromolecules 2011;12(3):625-632. 3. Nicolaus, RA. Tetrahedron 1964;20(5):1163-1172. 4. Fisher, OZ. Advanced Materials 2012;24(22). 5. Graham, DG. The Journal of investigative dermatology 1978;70(2):113-6. 6. Kasper, G. Stem Cells 2009;27(6):1288-1297.