AMINATED ELASTIN-LIKE POLYPEPTIDE COPOLYMER COATINGS FOR LIVER CELL CULTURE

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Statement of Purpose: Researchers use in vitro hepatocyte culture models to study liver disease and screen substances for toxicity. Existing models typically feature a hepatocyte monolayer, which dedifferentiates during a short culture period. Induction of spheroidal aggregates of hepatocytes atop thin films has been shown to increase hepatic differentiation over longer periods compared to monolayer.[1] This research systematically probes the effects of charge type and content on spheroid formation by comparing the chemical makeup and cell culture performance of two aminated copolymers based on elastin-like polypeptide (ELP), a genetically engineered variant of mammalian elastin. To this end, we formulated ELP-polyethyleneimine (ELP-PEI; protonated by synthetic imine groups) and ELP-polylysine (ELP-PL; protonated by amino acid lysine groups) coatings to have different levels of primary amine content. We then evaluated the performance of primary rat hepatocyte spheroids cultured atop these coatings over a 3-week period.

Methods: Copolymer Synthesis: ELP (MW = 17,000 Da) produced from genetically engineered E. coli[1] was reacted to PEI (Sigma, MW = 1,200 Da) and PL (Sigma, MW = 1,000 Da) in 0.1 M MES using carbodiimide chemistry.[1,2]

O-phthlaldehyde (OPA) Assay: Amine content of bulk copolymers was indicated by reacting to OPA (Thermo Sci) and was used to prepare formulations with varying levels of amine contents by mixing unreacted ELP (neutral charged) with the ELP copolymer (highly charged) (Fig 1).

X-ray Photoelectron Spectroscopy (XPS): A PHI1600 XPS Surface Analysis System (Physical Electronics) was used to scan 3 areas on surfaces prepared by solvent coating of 1 mg materials per TCPS well. CasaXPS software was used to analyze survey scans and detailed scans of the nitrogen 1s binding energy region (385−415 eV).

Cell Culture: Primary rat hepatocytes (75,000/well) were cultured in Williams-E medium within 24-well plates atop the coatings in triplicate over a 3-week culture period. Cells were maintained in a 37°C, 5% CO_2 static environment. Media was changed every 48 hours. Albumin secretion in media was assessed by ELISA (Bethel Labs) on days 2, 6, 10, 14, and 20. Total protein content (Thermo Sci) of cell lysate was used to normalize albumin production.

Results: Fluorescence of OPA-treated solutions of ELP-PEI and ELP-PL reaction products indicated primary amine content of ~2.5 times, respectively, of that of neat ELP (Fig 1). This directed how we diluted the reaction product with uncharged ELP to render the coatings with varying charge contents (Fig 1). Based on our previous research, which showed 1-20 mol% of charged conjugate was required to form stable spheroids,[2] we chose to investigate two charge grades for this research, with a fluorescence reading 1.1 and 1.7 times that of neat ELP (representing ~ 5 and ~ 20 mol%).

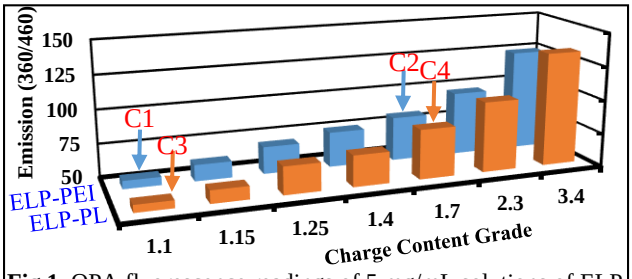

Fig 1. OPA fluorescence readings of 5 mg/mL solutions of ELP copolymers. Error bars showing a 95% confidence interval were less than 5 % of each measured value.

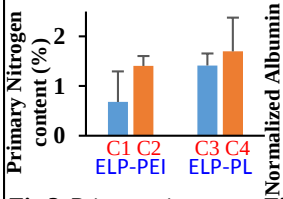

Fig 2. Primary nitrogen content of the various coatings by XPS.

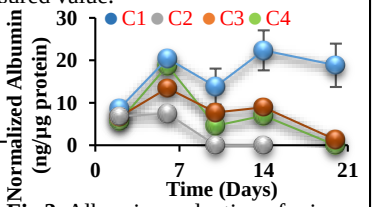

Fig 3. Albumin production of primary rat hepatocyte spheroids cultured atop various coatings.

XPS analysis indicated that all coating surfaces had similar overall nitrogen content for both ELP-PEI and ELP-PL coatings (data not shown). A slight increase in protonated amine content (charge) was shown by XPS (Fig. 2) for ELP-PEI (C1 vs. C2), but not ELP-PL (C3 vs. C4). Only 3 data points were collected per coating for XPS with additional data needed to establish statistical significance.

The initial albumin production by the primary rat hepatocyte spheroids was ~ 6 ng/µg protein on all coatings on day 2. The ELP-PEI coating with the higher level of amination (C2) did not support long term cell differentiaton, with essentially no albumin production by day 10. The ELP-PL coatings with both levels of amination (C3 and C4) showed equivalent cell differentiaton, with highest albumin production by day 6, which however, steadily declined during subsequent culture period. In contrast, the ELP-PEI coating with the lower level of amination (C1) supported long term cell differentiaton, with a sustained increased albumin production over the 3-week culture period (Fig 3).

Conclusions: We have developed a method using the OPA flourescence assay to facilitate the creation of ELP copolymer coatings with varying levels of amination. Our results suggested that both the type of the charge group (PEI or PL) and the level of surface charge affected hepatocyte differentiation. This result provides a starting point for extensive mechanistic studies in how charge type and content affect long term cell culture performance.

References: 1. Janorkar et al., Biomaterials 2008;29:625.
2. Turner et al., J Biomed Mater Res 2014;102A:852.

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