Synthesis and characterization of elastin-like polypeptide and RGD copolymers and their effect on spheroid formation

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Statement of Purpose: Previously we designed elastin-like polypeptide and polyethyleneimine (ELP-PEI) coatings to develop a 3-D spheroid culture model. Here, the PEI induces the spheroid formation and ELP allows the spheroids to adhere to the substrate.[1] Through various assays and cell adhesion study, it was shown that although, gradual spheroid loss occurs over a longer-term cell culture period, the spheroids mimicked the *in vivo* cellular behavior.[1,2] In the present study, to further improve the spheroid adhesion properties of the ELP-PEI coatings, ELP was fused with RGD residues. We chose the RGD sequence because it is a cell-attachment site present in fibronectin.[3] In this study, we characterized whether the addition of RGD to the C- or the N-terminus of the ELP would affect the spheroid forming ability of the resulting ELP-PEI.

Methods: ELP conjugated with RGD was obtained from genetically modified *Escherichia coli* bacteria modified to express $(VPGVG)_{40}(RGD)_3$ and $(RGD)_3(VPGVG)_{40}$ (where, V = valine, P = proline, G = glycine, R = arginine, D = aspartic acid) via suspension culture. The ELP-RGD conjugates were modified with polyethyleneimine (PEI, MW= 800 Da) as described elsewhere.[2]

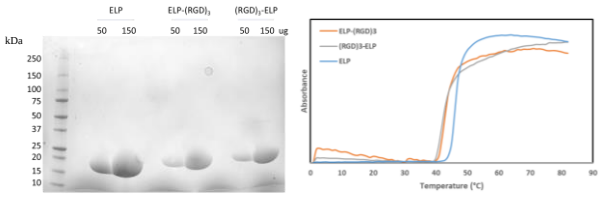
Characterization of conjugates: The ELP-RGDs were characterized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), turbidimetry, and Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). ELP-RGD-PEI conjugates were characterized by o-phthalaldehyde (OPA) assay for evaluating the percentage of conjugation.

Surface modification: 5 mg/mL of 5 mol% ELP-PEI conjugate and 95 mol% ELP was adsorbed onto 48-well tissue culture polystyrene (TCPS) plates by placing 100 µL solution in each well.

Characterization of coating: The coatings were characterized using Atomic Force Microscopy (AFM).

Cell culture: 50,000 cells/mL human adipose derived stem cells (hASCs) were seeded on the coated plates, allowed to form spheroids for 3 days, and then imaged using an optical microscope.

Results: The apparent molecular weight of ELP-$(RGD)_3$ and $(RGD)_3$-ELP on SDS-PAGE were observed to be ~17 kDa, which is similar to ELP **(Figure 1a)**. The ATR-FTIR spectroscopy showed that the ELP-$(RGD)_3$ and $(RGD)_3$-ELP had an equivalent chemical signature as the ELP (data not shown). Turbidimetry data **(Figure 1b)** revealed that, while the phase transition behavior of the ELP was preserved, the transition temperature reduced from 42 °C for neat ELP to ~40 °C when conjugated with $(RGD)_3$. The OPA analysis (**Table 1)** confirmed that the ELP-$(RGD)_3$ and $(RGD)_3$-ELP were successfully conjugated to PEI (more than 5 mol% required for spheroid formation).[4] Therefore, addition of RGD to ELP did not affect the conjugation capacity of ELP to PEI. The coated wells analyzed with AFM revealed that the TCPS wells were uniformly coated with ELP-$(RGD)_3$-PEI and $(RGD)_3$-ELP-PEI (data not shown). Interestingly, the hASCs cultured on the ELP-PEI and $(RGD)_3$-ELP-PEI coatings formed spheroids, while those cultured on the ELP-$(RGD)_3$-PEI spread and did not form spheroids **(Figure 2)**. We hypothesize that negatively charged aspartic acid residues from the $(RGD)_3$ interferes with the positive charges of PEI when the $(RGD)_3$ is placed next to the PEI in the chemical structure of the conjugate and thereby inhibits the PEI's ability to induce spheroid formation.



(a) (b)

Figure 1. (a) SDS-PAGE and **(b)** turbidimetry data for ELP, ELP-$(RGD)_3$, and $(RGD)_3$-ELP.

Table 1. OPA assay showing the percentage conjugation ± SD.

Sample Name	% Conjugation
ELP-$(RGD)_3$-PEI	11.10 ± 0.98
$(RGD)_3$-ELP-PEI	11.92 ± 0.41

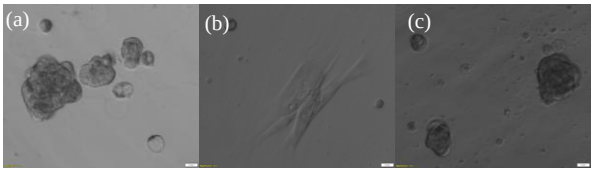


Figure 2. Cell culture images observed on day 3 for TCPS wells coated with **(a)** ELP-PEI, **(b)** ELP-$(RGD)_3$-PEI, **(c)** $(RGD)_3$-ELP-PEI. Scale bars =20 µm.

Conclusions: Our results show that ELP was conjugated with RGD without much alteration in the molecular weight, functional groups present, and transition temperature of ELP. Chemical conjugation ELP-RGD with PEI was successfully performed. However, chemical conjugation of RGD with PEI for ELP-$(RGD)_3$-PEI interfered with the spheroid inducing characteristic of PEI. Therefore, it was concluded that while the site of attachment of RGD, i.e., C or N-terminus of ELP, did not have pronounced effect in the chemical characteristics; the cell spheroid formation was drastically affected.

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