3D Cell Instructive Scaffolds to Direct Stem Cell Fate

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ABSTRACT

Here we present the fabrication of novel three dimensional scaffolds using emulsion templating. The surfaces of these scaffolds present defined domains of adhesive (poly(acrylic acid)) and inert (poly(ethylene glycol)) patches. This was achieved by exploiting amphiphilic block copolymers (poly(styrene)-b-poly(acrylic acid) and poly(styrene)-b-poly(ethylene glycol)) phase separation at the oil-water interface.

Keywords: 3D scaffolds, emulsion templating, amphiphilic block copolymers, domains, chemical force spectroscopy.

1 INTRODUCTION

With increasing evidence in the literature that physical properties such as elasticity of a matrix dictates stem cell lineage commitment [1] as well as micro and nanotopographies [2] and surface chemistry [3], there is an increasing necessity in the field of three dimensional scaffold design and synthesis to incorporate such physicochemical properties to obtain the desired cellular response. Ideally, synthetic scaffolds must be able to mimic the extracellular matrix which provides both physical and chemical cues for cellular function. This includes spatiotemporal presentation of cell inert and binding sites that control cell shape and morphology; an important indicator in mesenchymal stem cell lineage commitment. A strategy for scaffold fabrication method to incorporate such complexity in surface chemistry is water-in-oil emulsion templating [4]. While this is a commonly used technique, here, we used amphiphilic block copolymers as w/o surfactants. Subsequent polymerization of the emulsions produce porous scaffolds resulting in surface functionalization by the hydrophilic blocks of the copolymer. The de-mixing or phase separation of two block copolymers in solution of various ratios have shown to form discrete domains or patches on the nano scale in bilayer systems [5]. Thus similar phase separation is expected at the oil-water interface allowing for the fabrication of 3D matrices with hierarchical surface structure and pattern. It is hoped that by controlling cell shape through adhesion, the lineage commitment of mesenchymal stem cells maybe controlled.

2 MATERIALS AND METHODS

The scaffolds were synthesized by using a water-in-oil HIPE. The aqueous phase consisting of de-ionized water and the radical initiator, potassium persulfate (0.1 wt/v %) was slowly added to the oil (organic) phase consisting of divinylbenzene and the block copolymers polystyrene-b-poly(ethylene oxide) (PS-PEO), poly(acrylic acid) (PS-PAA). The hydrophilic blocks; PEO and PAA were particularly chosen for their affects of cell adhesion. The surfactant used was either one block copolymer on its own, e.g. PS-PEO only or or PS-PAA only or as mixtures of PS-PEO:PS-PAA in varying molar ratios of 75:25, 50:50 and 25:75. Once the emulsions were formed, they were cured at 60 ºC for 24 hours. The resulting foams were then extracted in a soxhlet apparatus for 24 hours using propan-2-ol and then dried under vacuum. Scaffolds made with block copolymer were compared to those made with a low molecular weight surfactant (sorbitan monooleate). Scaffold morphologies were assessed by scanning electron microscopy. Scaffold surface chemistry, particularly the carboxylic acid moiety of the hydrophilic blocks were characterized by X-ray photoelectron spectroscopy and contact angle measurements. In order to elucidate the formation of PEO and PAA domains, chemical force spectroscopy mapping (CFSM) was carried out. A standard gold tip with a nominal spring constant of 20nN/nm was functionalized with Poly(L-Lysine) as described before [6]. All samples were measured in dH2O at pH7.

3 RESULTS AND DISCUSSION

3.1 Scaffold Structure and Morphology

Figure 1. shows high and low magnification of scaffold morphology using PS-PEO and PS-PAA block copolymer mixtures. All foams display a porous structure in the order of 100 µm while the surface of the wall of the voids show an apparent surface roughness in the nano scale quantified by image analysis in the corresponding plot. The propensity
of these amphiphilic block copolymers to be suitable surfactants for oil-in-water emulsions may cause instabilities at the oil-water interface to produce surface roughness. Such roughness may be described as divinylbenzene latex particles nucleating at the interface that have not bud off to form discrete particles. Although the formation of particles from oil-in-water polymerization is undesirable, surface roughness was effectively controlled by varying emulsion polymerization temperature while maintaining macro-porosity of the foams. This provides additional control over the hierarchical structure of such 3D substrates.

3.2 Surface chemistry and wettability

X-ray photoelectron spectroscopy (Figure 2) revealed the integrity of the COOH group of the PS-PAA block. The C 1s peak is split with a major peak with a slight shoulder at 287 eV and a smaller peak appearing at higher energy 289 eV corresponding the C 1s in the C=O double bond. The major (averaged) peak consists of 3 separate signals. The biggest signal at 286 eV corresponds to C 1s in hydrocarbons (PS matrix), the smaller peak overlapping at 286 eV is from the C 1s of the C-OH single bond of the acid. Note that this peak intensity was made to correspond with the peak at 289 eV and is justified by the fact that the signal is coming the same C atom. Finally, the shoulder appearing at 287 eV corresponds to the C 1s from the α carbon (C-COOH).copolymer compared to the presence of the bulk C-C and C-H matrix of the polystyrene/divinylbenzene backbone when span 80 is used (not shown). Since the ether functionality of the PEO is masked by the broad C-C and C-H signals of the underlying bulk matrix, a change in surface chemistry was characterized by contact angle measurements obtained at pH7 as well as pH2.2 (Figure 3) for both PS-PAA and PS-PEO. At a pH below the pKa of the PAA block (pKa ~4), the acid groups are protonated making them hydrophobic while at a pH above the pKa, the reverse is true making the copolymer hydrophilic. Surface functionalization using block copolymer surfactants was possible due to their high molecular weights, that enables them to be incorporated into the back bone of the polystyrene/divinylbenzene matrix through chain entanglement.
To understand the formation of block copolymer domains, chemical force spectroscopy was carried out on corresponding 2D films. 2D block copolymer films consisted of the same compositions and showed similar surface roughness to those of the 3D foams. By changing only the dimensionality of the surface, validates chemical force spectroscopy as a method to detect the differences in coulombic forces between PAA, PEO and divinylbenzene bulk chemistry and the functionalized AFM tip with PLL. Since all scans were taken were 2 µm x 2 µm at a resolution of 32 pixels x 32 pixels, each pixel corresponds to roughly 60 nm. In other words, the resolution of our data is roughly 60 nm. Our findings show that films composed of DVB have the lowest adhesion around 0.5nN while PEO 100 films show an average adhesion of 1nN and PAA 1.5nN. From these controls, the adhesion due to PEO and PAA in the block copolymer blends were mapped (Figure 4) identifying regions or domains formed by PS-PEO and PS-PAA as a result of block copolymer phase separation at the oil water interface.

3.3 Detecting block copolymer phase separation

Figure 2. XPS of divinylbenzene foams with the block copolymer surfactants a) PS-PAA showing the distinctive COOH signal and b) PS-PEO.

Figure 3. Contact angle measurements on 3D foams at pH 7 and pH 2.2.
4 CONCLUSIONS

We have shown that amphiphilic block copolymers can successfully be used in the high internal emulsion process and such block copolymers remain anchored to the surface thereby controlling surface chemistry. Furthermore, block copolymer domains formed by the mixing of disimilar block copolymers were detected and mapped by chemical force spectroscopy.

5 REFERENCES

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