A Novel Univarient Material for the Study of Cellular Response to Material Rigidity

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Statement of Purpose: Materials currently used for the study of cellular response to substrate rigidity include natural and synthetic hydrogels, and films of polyacrylamide (PA) or polydimethylsiloxane (PDMS). While these materials have helped shed light on the importance of material stiffness on cellular functions, they have inherent restrictions including a limited range of achievable stiffnesses, inconsistent surface chemistries, and/or limited cellular attachment.[1] The goal of the work presented here is to create model cell culture substrates that possess a wide range of stiffnesses while maintaining constant surface properties.

Methods: Fabrication: Monomers of nOM and DEGDMA were mixed with the photoinitiator Irgacure 2959 (0.25 wt%), injected into glass parallel plate molds, and exposed to UV light (λ = 365 nm) for 300 min. Crosslinked sheets (100 mm thick) cut into cylindrical samples (d = 6 mm). Samples for mechanical testing were fabricated in glass vials (d = 6 mm) and cut to a length of 12 mm after curing. Formulations of 3, 19, 25, or 33 wt% DEGDMA were examined.
Mechanical Properties: Bulk compressive modulus (n=5) was determined following ASTM 695-02A .
Surface Characterization: The sessile drop technique was used to determine the contact angle of samples (n = 4). To measure information on chemical functionalities, XPS survey and high resolution spectra for the 3 and 33% DEGDAM samples were obtained using Kratos Axis Ultra Spectrophotomer with monochromatic Al K(α) (1486.6 eV) source at 225 W. High-res spectra were obtained for two areas for each sample. Finally, atomic force microscopy (AFM) was employed to measure surface roughness at three random locations on samples of 3 and 33 wt% DEGDMA. Surface scans were performed at 2.50 µm per second at 0.2 Hz with a 40 nm radius cantilever tip while submerged in PBS at 37°C .
Cell Studies: MC3T3-E1 cells seeded at 25,000 cells/cm^2 onto solid crosslinked films (n=4). Attachment (6 hrs) and viability (96 hrs) were assessed using LIVE/DEAD Cell Viability Kit (Invitrogen®, L3224) per manufacturer's specifications.

Results: Compressive modulus was found to range from 25 ± 2 to 4700 ± 300 kPa (Figure 1). The material modulus of these networks can be tuned by changing the copolymer ratio. Contact angle measurements are a measure of the hydrophilicity of a surface, giving an indication of expected protein adsorption. All formulations exhibit contact angles of ~ 90° with no statistical differences observed. Further characterization of the 3 and 33 wt% surfaces was carried out using XPS. Survey scans showed consistent elemental compositions

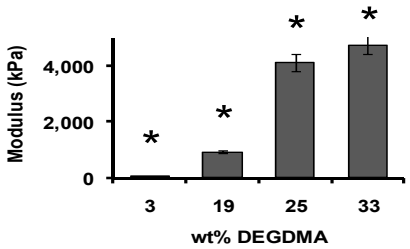

Figure 1: Compressive modulus, n = 5, *p < 0.01.

between formulations and within individual samples (data not included). Quantification of high resolution carbon and oxygen scans indicated minimal variations between functional groups present on the surfaces (Tables 1, 2).

Table 1: Carbon High Res Scan of 3 and 33wt% DEGDMA

	Experimental Values			
	O=C	C-O-C & O-(C=O)		H-O-H
DEGDMA wt%	532.3 eV[7]	533.9 eV	534.4 eV[7]	535.3 eV[7]
3%	34.6	53.1		12.4
33%	40.7	49.3		9.9

Table 2: Oxygen High Res Scan of 3 and 33wt% DEGDMA

	Experimental Values			
	C-C & C-(C=O)-O		C-O	C=O
DEGDMA wt%	285.0 eV[7]	285.7 eV[7]	286.8 eV[7]	288.8 eV[7]
3%	73.9		18.9	7.2
33%	70.9		18.3	10.8

AFM was used to measure the surface roughness at the nanometer scale. Root mean squared roughness was determined to be 14 ± 1 and 17 ± 6 nm for the 3 and 33wt% samples, respectively. Finally, Live/Dead staining gave a qualitative indication of cellular attachment and viability. On all formulations at both timepoints, viable cells were adhered to the surfaces, with a marked increase in cell number after 96 hrs (data not shown).

Conclusions: Here we present a copolymer system for the assessment of cellular functions on substrates with moduli ranging from 20 to 5000 kPa with no alterations to the surface features. This model platform can be used to screen a large range of moduli to identify appropriate rigidities for the design of biomaterials for tissue engineering. This platform will also find utility in studies of cellular biology associated with diseases characterized by a stiffening of the tissue, including many cancers. We have successfully created a model substrate which is the first to allow for the systematic study of cellular response to material stiffness independent of changes in other surface properties.

Reference

1) S. Nemir, J. West, Ann Biomed Eng, 2010:38:2-20.