2.5D Constructs for Surface Evaluation of 3D Scaffolds in Tissue Engineering

Jolanta Marszalek[1], Alamgir Karim[1], Alexei G. Basnakian[2], Carl Simon[3]
[1]University of Akron, Akron, OH 44325;
[2]University of Arkansas for Medical Sciences, Little Rock, AR 72205
[3]Polymers Division, NIST, Gaithersburg, MD 20899-8540

Background: There is an abundance of information on how cells grow and proliferate on flat, film surfaces. Many tissue engineering optimization trials are conducted on 2D polymer films because of the ease of fabrication and surface analysis. However, it is uncertain if these 2D models provide accurate data for cell growth in more physiologically applicable 3D environments of scaffold-like materials. In addition, methods of 2D film preparation vary significantly from preparations of three-dimensional constructs. Alternatively, due to fabrication difficulties, 3-D scaffolds are often made without biologically relevant micro and nanostructure even though it has been determined that the micro and nanoscale physical environment encountered by cells can influence adhesion, proliferation and differentiation. It has been shown that the phase separation process induces nanotopology into the polymer blend films on a roughness scale comparable to biological environment that promotes cell growth.[1] Salt leaching is one of the methods for manufacturing of scaffolds in tissue engineering. However, the fragile 3D constructs are very difficult to analyze, especially when one wants to determine surface characteristics.

Objective: This work address a novel tissue engineering approach to processing that influences microstructure developed from phase-separated polymer blend in pseudo-2.5D constructs. The main question stated was the following. Is phase separation sustained in the 3D scaffold and if so, how does the micro- and nano-topology of scaffold walls appear? Can we infer this data by studying 2.5D constructs?

Methods: Poly(ε-caprolactone) , PCL, (Mw = 80,000, Aldrich) and poly(D,L-lactide), PDLA, (Mw = 107,300, LACTEL) were obtain from commercial supplier and used without additional purification. Films were spin-coated on glass substrates from 5 wt % chloroform solutions. Salt crystals with different average sizes (<53 μm, <106 μm, <250 μm) were sprinkled on the surface. Samples were placed in preheated oven and annealed. Samples were pulled out at different times and allowed to quench at room temperature. Next samples were immersed in DI water for five days to dissolve salt and vacuum dried. The surface analysis was done with Atomic force microscopy (AFM) and various microscopic methods. Osteoblast and chondrocyte growth is evaluated on 2.5D constructs.

Results: The surface of polymer films is easily evaluated with various surface methods. The opposite is true for surfaces that develop under salt crystals in leached 3D scaffolds. Thus, 2.5D constructs were prepared as an intermediate structure between 2D films and 3D scaffolds. These transitional structures were prepared from thicker films that were coated with salt crystals and annealed at temperatures previously determined to cause phase separation.[2] When film samples are annealed the salt crystals are imbedded into the polymer blend and create structures within the thicker films. These structures approximate the appearance of the true three-dimensional scaffolds from a salt-leach method of preparation. In order to observe how salt crystals affected the phase separation of the polymer blends they were removed via dissolution in water in order to look under the salt structures. The samples were annealed at different temperatures and for various annealing times for manipulation of phase separation.
The first observation was that the phase separation occurs not only in spaces between salt crystals, but also, as shown on Figure 1B under the salt crystals. It was detected that, larger domains were observed not only at longer annealing times and at higher annealing temperatures, but also when smaller crystals were used.

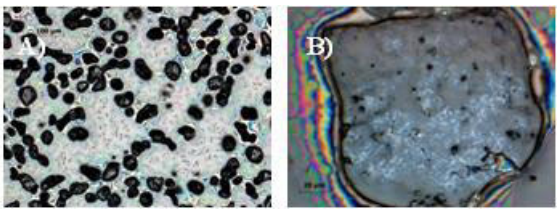

Figure 1. Polarized microscopy of 2.5D construct's surface: (A) salt crystals size <53μm, mag. 100X; (B) vacancy after salt crystal, size <250 μm, mag. 500X.

Conclusions: A pseudo-2.5D constructs were prepared for surface analysis in approximation of 3D scaffolds. It was observed that phase separation of biodegradable polymer blend can be manipulated through processing parameters even in presence of salt crystals that could have restricted its manifestation. Annealing of the blend at longer times and at elevated temperatures allows more extensive phase separation to occur. In addition, size of salt crystals sprinkled on the surface of the films influences the degree of phase separation and domain size.

Acknowledgement: Wextend our gratitude to Renee Horn from St. Vincent-St. Mary High School, Akron, OH, who worked on a preliminary aspects of this research.

References:

1. Meredith JC; Sormana J-L; Keselowsky BG; García AJ; Tona A; Karim A; Amis EJ. J Biomed Mater Res 2003: 66A: 483-490.
2. Meredith JC; Amis EJ. Macromol Chem Phys 2000: 201: 733-739.