EFFECT OF DEGRADATION MEDIA ON PHYSICAL PROPERTIES OF POROUS PLGA 85/15 SCAFFOLDS

[1]H.E. Naguib, [2]J.K. Perron, [3]J. Daka, [3]A. Chawla, [3]R. Wilkins

[1]Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada

[2]Department of Mechanical Engineering, University of Ottawa, Ottawa, Ontario, Canada

[3]Health Canada, Ottawa, Ontario, Canada

Statement of Purpose: This study investigates the effect of PLGA 85/15 scaffold on the cell growth and viability of a cell line, as well as the degradation of PLGA 85/15 scaffold in different media. The cell line used was human promyelocytic leukemia cells (HL-60). Three different media were considered: distilled water, a phosphate buffered saline (PBS) solution and HL-60 cell line. Porous PLGA 85/15 scaffolds were prepared with an optimized gas foaming/salt leaching technique.

Methods: For the scaffold fabrication, 85/15 poly (DL-lactide-co-glycolide) acid was used, that has a glass transition temperature between 50-55 ºC and a specific gravity of 1.27 g/ml. The fabrication of the scaffold also required sodium chloride (NaCl), dH_2O, and CO_2 gas. Human cell lines HL-60 were used for the cell viability and the degradation studies. The HL-60 were grown in complete RPMI-1640 medium (Invitrogen), supplemented with 10% fetal bovine serum (Sigma), 2mM $_L$-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. In addition, PBS solution and dH_2O were used in the degradation study. The scaffolds were sterilized with ethanol (70%), and fluorescence microscopy technique used in cell counting and viability investigations required ethidium bromide, fluorescein diacetate and Hank's Balanced Salt Solution. The gel permeation chromatography technique used to get the average molecular weight required the scaffolds to be dissolved using tetrahydrofuran as the solvent.

Scaffold made from PLGA 85/15 were fabricated by an optimized gas foaming/salt leaching technique, which is described in detail elsewhere[1]. PLGA 85/15 and NaCl particles had size ranges of 106 to 500 µm and 106 to 250 µm, respectively. The NaCl/polymer disks, having a total mass of 250 mg and using a NaCl/polymer mass ratio of 5, were prepared using a 12.8 mm diameter die. These solid disks were then saturated with CO_2, at a saturation pressure of 5.52 MPa for 12 hours. After foaming, NaCl/polymer samples were then placed in dH_2O for 48 hours in order to dissolve NaCl content.

Results: The HL-60 cell growth and viability in the presence of the scaffold were similar to HL-60 behaviors in the absence of the scaffold. Foreign material did not affect the cell growth for an incubation time of up to 7 days. Also, the percentage of cell viability was not affected by the scaffold for a period of 35 days, as shown in Figure 1. A longer study period is required to observe the effects of material release from the scaffold (i.e. lactic and glycolic oligomers) in the HL-60 cells medium. The degradation of the scaffold was performed in dH_2O, a PBS solution and HL-60 cells, and micrographs taken with a scanning electron microscope is presented in Figure 2. The degradation process initially occurred when water absorption by the scaffold caused the breakage of the polymer chains. As a result, the molecular weight, the volume and the porosity of the polymer decreased.[2] Smaller chains allowed the microstructure to be rearranged, leading to macroscopic shrinkage of the scaffolds. Throughout degradation, the mass of the matrix remained unaffected. However, a longer degradation period would have permitted larger amounts of water uptake, causing increased polymer chain cleavage. The molecular weight would decrease to a critical level, after which chains would have started to escape the matrix.

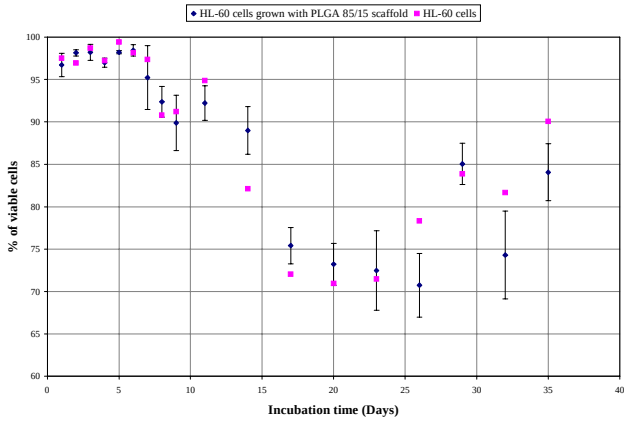

Figure 1. Cell viability of HL-60 cells.

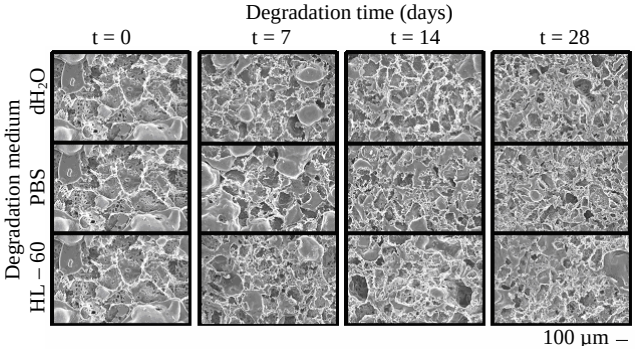

Figure 2. Micrographs of degraded samples.

Overall, the PBS medium appeared to have the most significant impact on the physical properties of the scaffolds during degradation. The degradation in dH_2O and HL-60 cells initially produced large variations in the scaffold's properties, but subsequent changes were minor. The average macropore size decreased significantly in all three media.

Conclusions: Overall, the scaffold's properties were affected differently depending on the degradation medium. Thus, the choice of the medium employed for *in vitro* studies is critical in order to replicate the actual conditions experienced by the scaffold. For the periods under consideration, the scaffold kept its integrity. The scaffolds are thus potential candidate for tissue growth.

References:

[1]J. Perron et. al, *ANTEC 2006,* pp 1371-75, 2006.

[2]S. Li, *J. Biomed. Mater. Res.,* 48, pp. 342-53, 1999.