In vitro Characteristics of Self Assembled Polycaprolactone Matrices in Aqueous Media

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INTRODUCTION: Polycaprolactone (PCL) is the biocompatible polyester which is explored in forming various medical devices, templates in tissue regeneration and drug delivery systems. Typically used halogenated hydrocarbon solvents such as Chloroform have been shown to generate a hydrophobic surface with smooth surface characteristics. We have discovered that PCL can be dissolved in glacial acetic acid (AA) which allows spontaneous aggregation of PCL upon contact with water. The aim of this study was to compare the characteristics of self assembled matrices to those formed using chloroform. Formed matrices were evaluated for surface characteristics, tensile properties, effect of alkaline etching at nanoscale, cellular interactions and effect of mixing gelatin in the same solution.

MATERIALS AND METHODS: From each MW PCL (80kDa and 10kDa Mn from Sigma Aldrich, St. Louis, MO, 46kDa from Polysciences Warrington, PA), 10% (wt/v) solutions were prepared in glacial acetic acid. Blend solutions containing different MW PCL were prepared by mixing equal volumes of individual solutions using factorial design of experiments, and 80 kDa PCL solution was prepared in chloroform to compare the effect of solvent and charge distribution. PCL matrices were made in 5 cm diameter Teflon by dispensing 3 to 4 mL PCL solution drop-wise on the top of a water bath. For etching the surface, formed matrices were immersed in 1N NaOH for 10 min.
Formed matrices were also analyzed using a scanning electron microscope (SEM) and an atomic force microscopy (AFM-DI Nanoscope V Multimode Scanning Probe Microscope) for surface roughness and charge distribution. Tensile properties were assessed in both dried and hydrated conditions using INSTRON 5842. Human Foreskin Fibroblasts (American Type Culture Collection Walkersville, MD) cells were seeded uniformly onto control and PCL matrices. They were stained for actin fibres using Alexa Fluor 546 phalloidin (Molecular Probes) and counterstained with DAPI following vendor's protocol (Invitrogen Corp).

RESULTS: 80 kDa PCL solution in water bath formed matrices quickly. 10 kDa PCL and mixture of 46 kDa and 10kDa PCL solutions did not produce a stable matrix despite precipitation, suggesting that PCL molecular weight plays a role in the matrix formation. No further testing was done with the ratio of 10 kDa PCL and mixture of 46 kDa and 10kDa PCL solutions.
AFM analysis showed a significant increase in nanoscale roughness relative to chloroform-casted matrices (**Figure 1**). Matrices formed in acetic acid showed different surface charge characteristics relative to chloroform casted membranes (**Figure 2A**). Further etching process had a significant different effect. However, mixtures of different MW did not have a significant effect of etching process on the surface roughness (**Figure 1**). In the dry state, results showed a significant decrease in the tensile stress of blended matrices relative to 80 kDa matrix. When matrices were tested in hydrated condition, tensile strain range increased remarkably. Although tensile stress range decreased in both 80 kDa PCL and mixture of 80 kDa and 46 kDa, no significant difference was observed in matrixes containing 10 kDa PCL.
Cellular activity showed that the PCL structure promotes cell attachment although the cell spreading area decreased significantly (**Figure 2B**). There was no significant toxicity of the materials in all the samples.

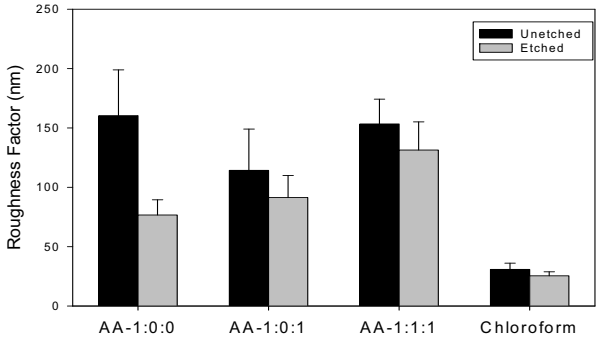

Figure1. Effect of blending and etching different MW of PCL on surface roughness

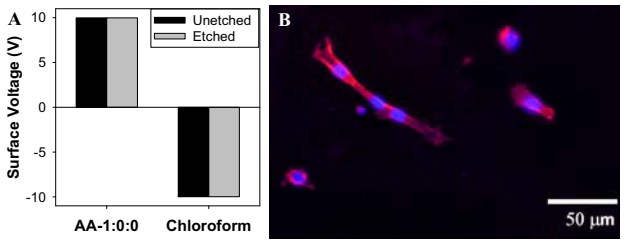

Figure2. Surface properties of 80 kDa PCL (A) Difference in surface charge and (B) fluorescent micrograph showing fibroblast spreading on the membrane.

CONCLUSIONS: Self assembled PCL matrices showed a significant difference in the surface roughness characteristics. Different MW PCL can be blended and matrices can be formed. Immersion in NaOH decreased roughness. Tensile properties decreased relative to chloroform casted membranes. Cell growth on the scaffolds shows that the novel process is non toxic to cells. Fibroblasts did attach and spread, similar to tissue culture plastic.