In vitro Characterization of Polycaprolactone Matrices Generated in Aqueous Media

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Statement of Purpose: Polycaprolactone (PCL) is a biocompatible polyester with low melting point (60°C), explored in forming various medical devices and tissue regneration. Further, mechanical properties and non-enzymatic degradation (by hydrolysis) of PCL can be altered by regulating its molecular weight. PCL is typically processed into matrices after dissolving in halogenated hydrocarbons such as Chloroform, which typically generate a hydrophobic surface in addition to concerns of carcinogenicity.

We hypothesized that by avoiding usage of hydrocarbons, the PCL surface is more hydrophilic and the process of making two and three dimension structures is simplified. To test the hypothesis, a novel process of dissolving PCL in acetic acid was explored which spontaneously formed membranes upon contact with water. To understand the process better, different molecular weights (80 kDa, 42.5 kDa and 16 kDa MWn) were used individually and after blending. Formed matrices were evaluated for surface characteristics, tensile properties, and biological properties. Since small diameter (<6 mm) blood vessels are in great demand for use in heart bypass surgeries (with over 500,000 surgeries being performed in the United States last year alone), the process was extended to form blood vessel-prototypes.

Methods: 10% (w/v) PCL was dissolved in 94% acetic acid. Blood vessel prototypes were made by coating a ¼ inch Teflon rod in PCL and submerging in water while membranes were created for tissue culture and tensile strength testing by coating Teflon dishes in PCL and spontaneously generating in water.

Formed blood vessel prototypes (5 mm diameter and 5 to 6 cm long) were exposed to pressures between 0 to 250 mmHg in closed-loop flow system built in-house, during which dimensional changes were quantified and compliance was calculated sing the equation $C=(\Delta V/V)/\Delta P*100$ where ΔV is the change in volume for the corresponding change in pressure.

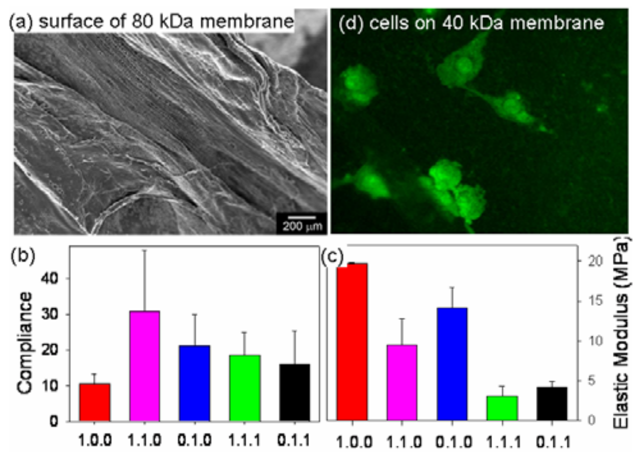
Tensile properties of 2-D scaffolds were analyzed in wet conditions at body temperature (37°C), while compliance testing was done in dry conditions.

To determine the changes in the crystal properties, formed matrices were analyzed by differential scanning calorimetry. Further, a four-week degradation study on 80 kDa matrices at 37°C in 5% CO2/95% air, in phosphate buffer solution (pH=7.4).

In vitro cell cultures were conducted using mouse embryonic fibroblasts and tracked through day 4. Resazurin assay was conducted to measure growth of the cells on the scaffolds. Cytoskeletal staining was done with Alexa 688 Phalloidium to see the attachment of cells to scaffold.

Results/Discussion: SEM analysis indicated that all matrices had an increased surface roughness (**Figure a**) relative to membranes formed after dissolving in Chloroform. However, matrices could not be made from 16 kDa MW. DSC analysis showed no change in the melting point of PCL. Degradation study indicated no major changes in weight.

Compliance testing (**Figure b**) of blood vessel prototypes showed that (abbreviation is in the order of 80:42.5:16 kDa and numbers indicate the volume proportions) decreasing the molecular weight increased the compliance. The trend was validated by the tensile testing results (**Figure c**) as the tensile strength decreased with blending of the weights.



Cell culture results showed that formed membranes are not toxic to cells. Further, cells attached and grew on matrixes, though there is no significant difference in the specific growth rate constant (calculated assuming exponential growth phase) between different MWs and their blends.

Conclusions: PCL membranes and small diameter blood vessels can be generated using the novel technique. Different MW PCL can be blended and matrices can be formed which could help in optimizing degradation rate as low MW PCL degrades significantly faster than high MW PCL. Compliance of the blood vessel prototypes indicated an increase with decrease in MW. Elastic modulus decreases with decreased MW, validating the observed trend in compliance measurement. Novel process is not toxic to cells. .

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