Surface Modification and Characterization of Polylactic Acid (PLA) 3D printed structures for Cell culture applications

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ABSTRACT

The main aim of this research is to understand the surface modification effects of 3D printed Polylactic acid (PLA) scaffolds to make them biocompatible and support the growth and proliferation of cells. Three different surface modification methods viz., Hydrolysis, UV/Ozone and using Gold thin film are studied with the objective of introducing useful levels of carboxylic acid or primary and secondary amine groups, respectively, onto the surface of PLA. PLA surfaces are also polished to study the effect of surface roughness on protein immobilization. Water contact angle measurement studies demonstrated increased wettability on unpolished PLA surfaces after hydrolysis compared to other surface modified techniques. Poly-L-lysine Labelled with FITC chromophore is immobilized on the surface modified PLA samples using EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] crosslinking technique and SATA linker solution in DMSO, following standard protocols. Fluoroscence microscopy shows denser protein attachment on polished hydrolyzed PLA surfaces. Raman Spectroscopy also revelas significantly higher intensities of $C_{C=O}/C_{C-O}$ after protein attachment on hydrolyzed PLA samples.

Keywords: 3D Printing, Poly-L-lactic Acid, Hydrolysis, Raman Spectroscopy, Fluoroscence Imaging

1 INTRODUCTION

Magnetic nanoparticle drug delivery, being one of the important active drug targeting approaches, is commonly used to increase the concentration of the drug at a defined target site and away from a reticular endothelial system, with the aid of an external magnetic field. However, the analysis and optimization of drug delivery to a targeted site through in-vivo experimental studies are quite complex, time- and cost- consuming. Hence, in-vitro model studies provide an efficient way of understanding the mechanisms of nanoparticle movement and prediction of its transport through the physiological system, for example, blood vessel. To make a realistic comparison, Lab-on-Chip (LOC) devices demonstrated their potential and benefits for many applications, including point-of-care diagnostics, genomic and proteomic research, analytical chemistry, enviromental monitoring and the detection of biohazards [1]. For example Park et al. [2] developed a two layered poly(dimethylsiloxane) (PDMS) LOC model to continuosly separate and gather bacteria from physiological samples such as cerebrospinal fluid and blood using positive & negative dielectrophoresis technique. In another effort, Ramalingam et al. [3] presented a real-time pathogen detection instrument incorporating polymerase chain reaction (PCR) assays in PDMS-glass microfluidic chips with no external pumps and valves.

In recent years, three dimensional (3D) printing has been drawing much attention from the research community for its ability to make complex structures with high resolution. However, there is very little literature on the use of 3D printing in LOC & microfluidics applications [4]. Biocompatible and biodegradable polymers, such as polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers which are used as filaments for 3D printers are extensively used as scaffolds in tissue engineering applications.

In this paper we propose few 3D printed LOC designs & models (Figure 1) and investigate the effect of surface modification of 3-D printed PLA structures for protein attachment and provide a critical analysis of the experimental results.

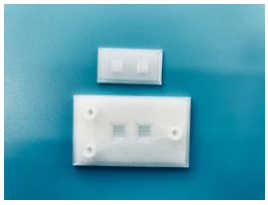
A]  B] 

Figure 1: Transparent PLA 3D Printed LOC devices A] Open T channel B] Closed T channel with reserviors and window plug-in.

2 MATERIALS AND METHODS

This study involves two important steps before the 3D printed PLA can be used for cell culture. These are surface modification (Hydrolysis, UVO and/or Gold film deposition) and protein immobilization.

2.1 Surface Modification

Surface modification (introduction of carboxyl side chain groups) is necessary to improve hydrophilicity of the surface of 3D printed bio-degradable plastics such as PLA for consequent cell culture and Lab-on-chip applications [5]. In this work, all studies were carried out using open channel structures. Surface modification was performed by chemical hydrolysis, UVO irradiation and Gold thin film deposition. All the above three surface modification techniques were tested on unpolished (Ra-21µm) & polished (Ra-2 µm) 3D printed surfaces to study the effects of surface roughness on Protein Immobilization.

Hydrolysis (Wet chemical etching): Three of each Unpolished & Polished PLA 3D printed samples were immersed in 1 M aqueous NaOH/Ethanol solution for 2, 4 & 6 hours respectively to observe the effect of etching time on wettability characteristics. Treated samples were then washed with deionized water, air-dried and kept in a vacuum desiccator [5].

UV/Ozone cleaning: Ultra-violet ozone (UVO) plasma irradiation technique was used for ~14 min on one of each Unpolished and Polished 3D printed PLA samples using Jelight UVO cleaner-144AX system to gently dissociate the hydrocarbon contamination on the surface by exposure to UV radiation. The dissociated molecules react with the high energy oxygen to form volatile molecules which desorb from the surface. Radicals like *OH, COO* and CO* can also form on the surface of the substrate [5].

Gold thin Film deposition: The unique surface properties of Gold thin films make them ideal for use in many biocompatible based point-of-care devices. One of each Unpolished and Polished 3D printed PLA samples were plasma-cleaned followed by covering it with 15 nm layer of Titanium and then 50-nm of Gold thin films using magnetron sputtering. This gold layered PLA structures were subjected to the hydrolysis treatment and analyzed.

2.2 Protein Immobilization

The hydrolyzed and UVO-treated PLA surfaces contain carboxylic (COOH-) groups which can be activated and then linked to the primary/secondary amine groups and couple them with the proteins. This was carried out using EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] crosslinking technique [6]. 1% EDC phosbhate buffer (ph 5.4) solution was dropped on hydrolyzed & UVO treated PLA samples and incubated for 12 hrs at room temperature. EDC is a powerful tool for crosslinking peptides and proteins and its ability to crosslink primary amines to carboxylic groups makes this technique suitable to functionalizing an artificial polymer "blood vessel" with proteins and polypeptides specific for endothelial cell culture.

The Gold thin film coated PLA samples which did not have COOH- functionalities were functionalized using SATA linker solution in DMSO, following standard protocols [7].

Poly-L-lysine Labelled with FITC chromophore was used as a protein since it contains amino groups which can readily react with COOH- functional groups activated on the functionalized PLA surfaces. 5% Poly-L-lysine phosbhate buffer solution (ph 5.4) was dropped on the PLA surfaces and incubated for 12 hrs. at room temperature. After incubation, samples were thoroughly washed in deionized water to remove unbound protein.

3 RESULTS AND DISCUSSION

3.1 Wettability Measurements

Water Contact Angle (WCA) Measurements were carried out using First Ten Angstroms Dynamic Contact Angle Analyzer FTA200. The contact angle results of each of the hydrolyzed PLA samples which were kept for 2, 4 & 6 hours of etching time respectively to observe the effect of etching time on wettability characteristics of Unpolished and Polished PLA surfaces are shown in Figure 2. This clearly indicates that 6 hours of etching time resulted in reduced contact angles from 72^o & 83^o to 36^o & 53^o of Unpolished & Polished PLA samples (Figure 3) respectively which are also in good agreement with studies done by Kaiyong Cai et al.[8]. Interestingly this trend continued on all samples with increased etching time. This clearly demonstrates that longer etching time helps in improving the wettability of these PLA surfaces. We hypothesize that the lower wettability on unpolished PLA surfaces after hydrolysis could be due to its 3D printed meshed/porous structures which makes the water seep into it thus reducing contact angles. This also demonstrates that hydrolysis on unpolished PLA surfaces is more effective way of improving hydrophilicity without influencing its bulk properties.

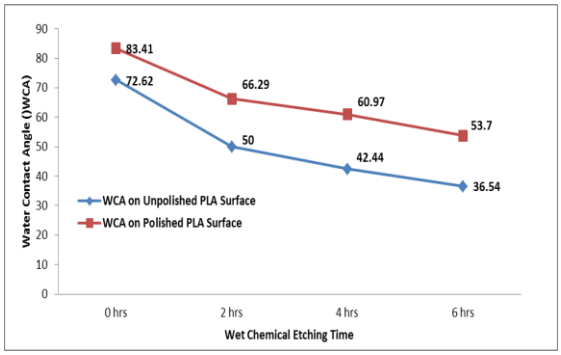


Figure 2: Comparison of Water contact angle measurements on Unpolished vs Polished Hydrolyzed PLA surface

Contact angle measurements on one of each Unpolished & Polished UVO treated PLA samples also displays a reduced contact angle from 72^o & 83^o to 53^o & 55^o respectively after ~14 min of treatment time (Figure 4).

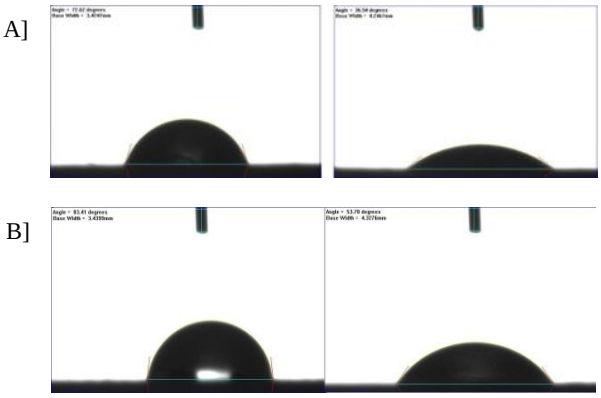


Figure 3: A] Water contact angle on Unpolished PLA surface before (72^o)/after (36^o) Hydrolysis surface modification technique. B] Water contact angle on Polished PLA surface before (83^o)/after (53^o) Hydrolysis surface modification technique.

It is clear from the above measurements that UVO exposure yields in significantly low wettability characteristics on either of the Unpolished & Polished PLA surfaces compared to Hydrolysis treatment. Further, the effect of the hydrolysis/UVO treatment on the protein attachment is analyzed using Fluoroscence microscopy.

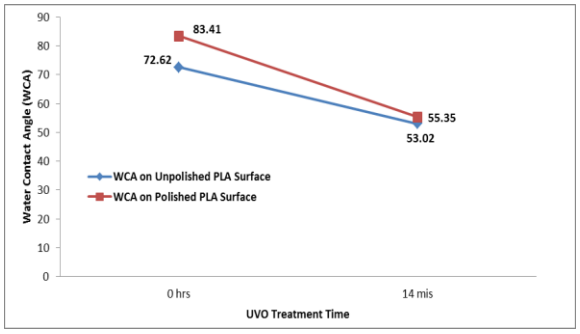


Figure 4: Comparison of Water contact angle measurements on Unpolished vs Polished UVO treated PLA surface

3.2 Protein attachment on modified PLA surfaces

Protein attachment on the modified PLA surfaces was analyzed using Fluoroscnece imaging methods. Laser scanning confocal fluorescence microscope, Zeiss LSM 510 Meta was used to visualize and verify successful immobilization of a FITC-labelled protein on the functionalized PLA samples. Fluorescence reflectance mode was used to capture images of the Unpolished and Polished hydrolyzed with protein attached PLA samples (Figure 5). By looking at the following images one can clearly state that protein immobilization on Polished surfaces is much better compared to Unpolished surfaces. Image J analysis software was used to quantify the mean intensity (mean grey value) of the given images and as a result we found that for a given 0.02345 cm^2 area only 5.43% (0.0013 cm^2) area of the Unpolished PLA surface was covered with Fluorescence compared to 100% on the Polished PLA surface.

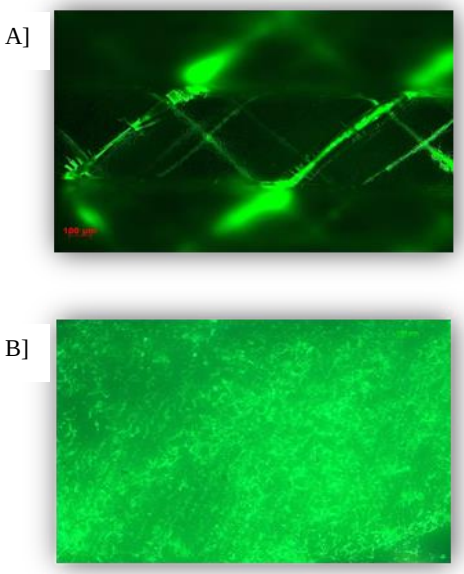


Figure 5: A] Fluorescence image of protein attached (focused on inside channel) on Unpolished Hydrolyzed PLA surface. B] Fluorescence image of protein attached on Polished Hydrolyzed PLA surface.

Similarly, Fluorescence images of Protein attached Unpolished and Polished UVO treated PLA samples were captured to visualize the amount of protein immobilization (Figure 6). As a result using Image J analysis software we found out that only 0.6851% (0.00016 cm^2) of the total given area (0.02345 cm^2) was covered with protein possibly due to smaller amount of activated *COOH groups present on the UVO treated PLA surface.

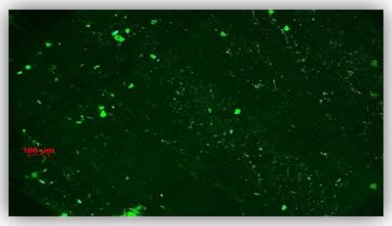
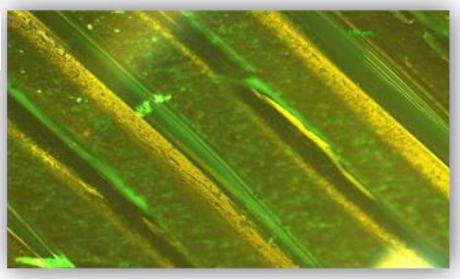


Figure 6: Fluorescence image of protein attached on Unpolished UVO Treated PLA surface.

Contrast fluorescence mode was used to capture images of the Protein attached Unpolished Gold Coated samples. Due to high background (Yellow Color) of the Gold coating Image J was not very useful to measure total area covered with Protein but by simply looking at the images (Figure 7) which were focused first on the outer surface and later zoomed in on the inner meshed surface, one can clearly state that protein was successfully attached to the Gold coated PLA surface.

A]



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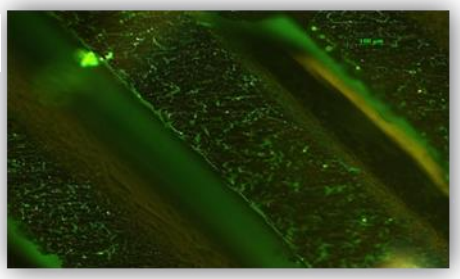

Figure 7: A] Fluorescence image of protein attached (focused on outer surface) on Unpolished Gold coated PLA surface. B] Fluorescence image of protein attached (focused on inner surface) on Unpolished Gold coated PLA surface.

3.3 Raman Spectroscopy Anaylsis

Renishaw inVia system was used to gather Raman spectra of the Virgin vs functionalized PLA samples. 633 nm laser at 5% of total laser power was used to collect Raman spectra. All the raman spectra of the protein attached samples were taken immediately after the protein was dropped on the functionalized PLA surfaces.

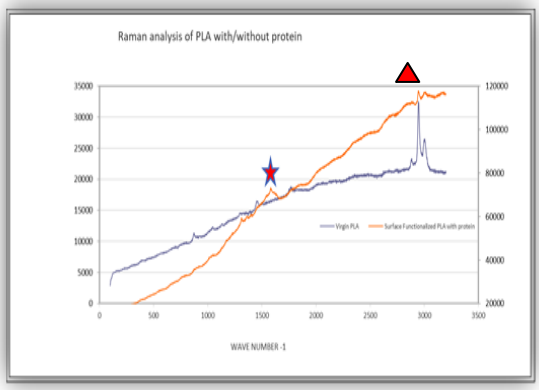

Figure 8: Raman Spectra of PLA with/without protein attachment; ★ Amide I bond peak at 1650 cm^{-1}, ▲CH bond peak at 2994 cm^{-1} (PLA)

The higher intensities of the peaks found at 1650 cm^{-1} on the protein attached samples (Orange color spectra) compared to Virgin (non-protein attached) blue color spectra clearly indicates the presence of the amide I bonds (C=O stretch) on the functionalized surfaces (Figure 8), proving the successful coupling of the Poly-L-lysine protein to the pre-treated surface of 3D printed PLA samples. The other significant peak found at 2994 cm^{-1} in both the Virgin and Protein attached PLA Raman spectra's denote –CH stretch which is one of the assigned Raman peaks for symmetric structures of PLA material [9].

4 CONCLUSIONS

This study clearly demonstrated the efficacy of the surface modification techniques such as Hydrolysis for protien attachment on 3-D printed PLA surfaces. The comparative anaylsis indicates that the UVO surface modification alone is not sufficient for protein attachment.

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