CHARACTERIZATION OF SOLID UV CROSS-LINKED PEGDA FOR BIOLOGICAL APPLICATIONS

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ABSTRACT

This paper reports on solid UV cross-linked Poly(ethylene)-glycol-diacrylate (PEGDA) as a material for microfluidic devices for biological applications. We have evaluated biocompatibility of PEGDA through two separate means: 1) by examining cell viability and attachment on cross-linked PEGDA surfaces for cell culture applications, and 2) by determining if cross-linked PEGDA inhibits the polymerase chain reaction (PCR) processes for on-chip PCR. Through these studies a correlation has been found between degree of curing and cell viability, attachment, as well as on PCR outcome.

INTRODUCTION

Poly(ethylene)-glycol-diacrylate (PEGDA) is a liquid polymer that can be cross-linked into a solid form by exposure to UV light in the presence of a photoinitiator. Recently, solid UV cross-linked PEGDA has been reported as a material for microfluidic fabrication [1-4]. It offers advantages over traditional polydimethylsiloxane (PDMS) techniques, including lower nonspecific adsorption of bio-molecules [1], greater stiffness, and unique fabrication potential, such as high aspect ratio structure fabrication [5] and lithographic patterning [4]. In previous work, we have demonstrated a fabrication process capable of producing an enclosed microfluidic channel and high aspect ratio structures, using PDMS soft molds [3]. As UV cross-linked PEGDA has a lower non-specific adsorption than PDMS, it is a promising material for fabrication of microfluidic devices for biological applications.

One of the more common uses of PEGDA is in the form of hydrogels as biocompatible tissue engineering scaffolds because of their similarity in structure to natural tissue [6, 7]. A PEGDA hydrogel is obtained when the liquid polymer is dispersed in an aqueous medium prior to cross-linking. Its use as a tissue scaffold material implies high biocompatibility, and studies of cell viability encapsulated in PEGDA hydrogels with different photoinitiators have been previously reported [8, 9].

However, the differences between PEGDA as a hydrogel and as a solid material, in both fabrication method and application require further characterization, which cannot be inferred directly from hydrogel studies alone. For example cell encapsulation in hydrogel involves the radiation of UV light on both cells and polymer simultaneously, limiting the total exposure dose that can be acceptable to avoid cell damage. Another difference is the effect of oxygen as an inhibitor of the cross-linking reaction of PEGDA [10], which is less significant in the case of hydrogels since the reaction naturally occurs in a low oxygen environment, and more

significant in the case of solid UV cross-linked PEGDA. The importance of having minimal amounts of prepolymer to ensure good biocompatibility has been previously reported for solid UV cross-linked PEGDA; however, results have been only qualitative [2]. In this paper, we report quantitative analyses on biocompatibility of solid PEGDA in microfluidic devices applications.

SAMPLE FABRICATION

Test samples were fabricated following a process previously published by Foulds' group [3]. This process enables the fabrication of microfluidic devices for biological applications, such as cell trapping and culture devices [11], as shown in Figure 1, using solid UV cross-linked PEGDA.

Figure 1: Cell-trapping microfluidic device from previous work [11] but fabricated using UV-cross-linked PEGDA instead of PDMS. Left: Microscope photograph of channels. Right: Macro scale photograph of chip.

The fabrication processes are relatively simple. Liquid 258 PEGDA mixed with photoinitiator is poured into a polydimethylsiloxane (PDMS) soft mold. The mold is covered with glass slides, compressed between two clamps and cured using a UV light box (7mW/cm²). Samples are then demolded by peeling off the PDMS soft mold and rinsed with isopropyl alcohol (IPA) and DI water. The photoinitiator used was 2,2-Dimethoxy-2-Phenylacetophenone (DMPA, Sigma Aldrich 306312). Samples of 1 inch x 3 inch size were fabricated under different fabrication conditions shown in Table 1.

Table 1: List of samples tested.

Material	Fabrication Parameters		
	Photoinitiator, Concentration w/w %	Oxygen atmosphere	Curing Time
PEGDA	DMPA, 0.1%	Low/High	30 min
PEGDA	DMPA, 0.1%	Low/High	5 min
PEGDA	DMPA, 0.1%	Low/High	1.5 min
PEGDA	DMPA, 1.0%	Low/High	5 min
PDMS	Sylgard curing agent, 10%	High	24 hour (65C)
PEGDA	DMPA, 0.1%	Low	2 hour
PEGDA	DMPA, 0.1%	Low	6 hour
PEGDA	DMPA, 0.1%	Low	12 hour
PEGDA	DMPA, 0.1%	Low	24 hour

A correlation between curing time and cell viability has been clearly observed. As oxygen inhibits the cross-linking of PEGDA [10], curing the samples in an air atmosphere showed adverse effects on biocompatibility. This effect can be easily visualized in looking at the 3-day cell viability study in Figure 2. SUM159 cells were cultured on two distinct PEGDA surfaces, those cast from PDMS molds and those cast off flat glass surfaces.

Figure 2: Graph showing 3-day viability cell density vs. curing factor (photoinitiator concentration times curing time) of UV-cured PEGDA samples in air atmosphere.

During fabrication, the top surface of the PEGDA sample is in contact with a glass slide, while the bottom surface is in contact with the PDMS mold. As PDMS is more gas permeable than glass, the liquid PEGDA adjacent to it is more exposed to oxygen, which results in a lower curing degree and therefore reduced cell viability. With the same curing intensity (quantified as curing agent concentration multiplied by curing time), surfaces from the glass surface showed significantly higher viability. As such, we modified our further fabrication to include “low” oxygen environments as shown in Table 1.

Curing in an oxygen free atmosphere, for example carbon dioxide or nitrogen [10], has been shown to minimize the effects of oxygen inhibition, and our fabrication process was modified accordingly. An additional 30 minute vacuum step was added before covering the mold with a glass slide, to minimize the amount of oxygen present in the liquid PEGDA and PDMS mold. Additionally, a nitrogen chamber that could fit on top of the samples during UV exposure was fabricated.

Figure 3: Increased normalized cell attachment with plasma and ECM surfaces treatments. Cell attachment normalized to attachment rates on PS plate at same seeding density.

Prior to exposure, the chamber was filled with nitrogen and run for 30 minutes to ensure all oxygen was displaced. Flow of nitrogen was maintained throughout the entire procedure.

CELL VIABILITY AND ATTACHMENT

To assess cross-linked PEGDA as a cell culture material, cells (SUM159 breast cancer cells) were seeded onto the sample surfaces and evaluated for their surface attachment and viability. Prior to loading, cells were cultured on tissue culture treated petri dishes in DMEM + 10% FBS (Invitrogen) culture media. Cells were trypsinized and re-suspended at 5×10^6 cells per ml for loading onto samples. PEGDA surfaces were fabricated with the various parameters described above, and in some cases, further modified. PEGDA surfaces were treated with oxygen plasma (80W for 60 sec) and then submerged in D-PBS or .1% collagen solution (to create an extracellular matrix, ECM, coating). The resulting cell attachment and viability was observed and compared to untreated PEGDA surfaces as well as to polystyrene (PS) and PDMS surfaces.

First, cell surface attachment percentage was analyzed by assessing cellular morphology 4 hours after initial loading. Figure 3 shows marked increased in cell attachment after surface treatment with both plasma and ECM coating. Increased PEGDA curing time lead to more effective cell attachment, and with the various surface treatments at 24 hours of curing, PEGDA outperformed similarly treated PDMS surfaces in enabling cell attachment. Sample cell images can be seen in Figure 4, and they show the very different cell morphologies obtained depending on curing time and surface treatment.

Stable cell viability must be maintained for reliable cell assays, and as such cellular viability was assessed over and 8 day time period. Traditional cell Live/Dead stains (Invitrogen) were used to visualize cellular viability, and PEGDA surfaces were compared to PDMS and PS, as a control. The resulting 8 day viability (Figure 5) was shown to be lower than that presented in PS but comparable to PDMS when cured for at least 24 hours. There is a clear correlation between curing degree and cell health.

Figure 4: SUM159 breast cancer cells attaching to PEGDA surfaces with different exposure times and surface treatments. The rounded morphology shown in the top left images is indicative on an un-attached cell. However, as the cells adhere, their morphology becomes more elongated and spread as seen in the bottom right

While PEGDA surfaces still underperform PS, further steps can be taken to improve long term viability such as even longer curing and better selection of a biocompatible photoinitiator (further discussed in the concluding remarks) and will be discussed in the concluding sections.

PCR INHIBITION

The PCR compatibility test of our PEGDA samples was done using a previously developed method [12]. A PCR master mix was prepared, using male saliva genomic DNA as a template. The master mix composition and cycling conditions are described in [13]. The primers were used to amplify a gene fragment from the Sex-determining region Y (SRY), with an expected amplification product size of 93 base pairs. Presence of amplified product indicates male saliva; no amplification indicates saliva of female origin.

The material samples to be investigated were cut with a blade into pieces of approximately 1.4 mmx 1.4 mm x 1 mm, and the same amount of pieces were introduced into individual PCR microteter plate wells. This was done to be able to have comparable results among the different tubes,

Figure 5: Graph showing 8-day viability cell density vs. curing time of UV-cured PEGDA samples in Nitrogen atmosphere.

given the fact that the inhibitory effect on PCR is surface-related. 30 μ l of the master mix was pipetted into each of the wells containing material samples, and additionally two positive controls that did not contain any material were included. The tubes were incubated in ice for 30 minutes prior to the thermocycling process.

The PCR outcome was quantified using an Agilent 2100 Bioanalyzer, running DNA 1000 assays, which can produce a gel-like image for quick result assessment, as shown in Figure 6. Quantitative concentration results for the band of interest are also identified and presented in Figure 7. A successful PCR reaction will produce a greater quantity of DNA; therefore the intensity of the fluorescent band on the gel-like image is stronger. Because of the length (measured in base pairs) of the DNA being amplified in this experiment, a stronger band in the region of 100 base pairs (bp) will indicate successful amplification, also reflected in a higher concentration

When measuring and quantifying the values of concentration at the 100 bp band, virtually no signal (< 0.01 ng/ μ l) was detected for the PEGDA samples cured for less than 30 minutes. Increased exposure times and the addition of the nitrogen atmosphere during fabrication show a decrease in PCR inhibition as compared to the control. Cured PEGDA shows comparable inhibitory effects to PDMS at 6 hours min curing time, but for 12 hours shows better performance than PDMS.

Figure 6: Gel-like Bioanalyzer image of different materials tested. Presence of band at 100 base pairs (bp) range indicates successful DNA amplification. Concentration of amplified DNA at 100 bp band is indicated. Sufficient curing of PEGDA shows improved performance over PDMS.

Figure 7: Concentration of amplified DNA at 100 bp band for different materials tested.

DISCUSSION

The cytocompatibility of different UV photoinitiator types and concentrations has been reported by Bryant et al. [8] for cell encapsulation in hydrogels. Short term UV exposure of cells with photoinitiators in solution was used to evaluate cytocompatibility, and they find the photoinitiator DMPA at 0.1% concentration as one of the least cytocompatible. Despite of this, our work, which uses DMPA at 0.1% has shown good 8-day cell viability, so an improved performance is expected if a more cytocompatible photoinitiator is used.

Our method of testing accurately represents cell culture assays, more closely resembling the conditions that a microfluidic device for biological applications would encounter. Future work includes on-chip cell culture, in a device similar to the one shown in Figure 1.

A correlation between degree of curing and cell viability, cell attachment and PCR inhibition was clearly shown. Solid UV cross-linked PEGDA shows improved performance over PDMS as long as exposure time and surface chemistry are adequately controlled.

For high exposure times, PEGDA shows a statistically significant improvement over PDMS for cell attachment when both are treated with oxygen plasma and 0.1% collagen solution. Cell viability is found to be equivalent to PDMS for high curing times. Inhibition on PCR outcome is reduced with higher exposure times, outperforming PDMS when cured for times longer than 12 hours. Our studies show that solid UV cross-linked PEGDA is a promising microfluidic material for biological applications.

ACKNOWLEDGEMENTS

This work was funded in part by the NIH T32 Microfluidics in Biomedical Sciences Training Program at the University of Michigan.

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