Surface modification with bioactive peptides through glycidyl-methacrylate-crosslinking to improve cell adhesion

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ABSTRACT

The extracellular matrix (ECM) environment consists of complex, well-defined molecular and topographical cues that significantly influence the structure and function of cells. The emerging nano-biotechnology enables researchers to create biomimetic microenvironments that could better guide cell response to a physiologically-relevant condition. In this study, a nanofabricated surface with a defined aligned nanopattern is used to guide the alignment of a myoblast cell line (C2C12) to mimic the highly aligned skeletal muscle structure in vivo. ECM is therefore necessary to conjugate on the surface to promote cell adhesion. However, the current methods for bioconjugation are inefficient and unreliable with NHS-NH_2 reactions due to the unstable NHS group. An alternate method to creating bilayers that is used in this experiment is instead using a epoxy-NH_2 reaction.

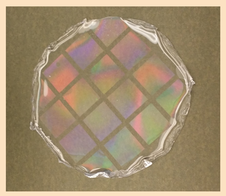
This research will explore alternative options to the unstable reaction of the $-NH_2$ group with N-hydroxysuccinimide (NHS) conjugation procedures. Because glycidyl methacrylate (GMA) is more stable than the NHS-NH_2 reaction, the epoxy group on the GMA should react with the $-NH_2$ group on the RGD-containing protein, and that will effectively attach the protein to the modified glass and allow cells to grow. The expected outcome of this research will determine if using GMA to conjugate RGD peptides to RGD-modified glass is a more cost and cell efficient crosslinking procedure than NHS crosslinking in research laboratory settings.

MATERIALS AND METHODS

Materials include acrylated glass coverslips (d=18mm), PUA precursor NOA 76 or 86 (Norland Optical Adhesive 76 or Norland Optical Adhesive 86) (only NOA 76 was used in this experiment but NOA 86 is also acceptable), GMA (glycidyl methacrylate, Sigma 151238-100G), and nanopatterned PDMS film.

Acrylation Protocol:

The glass slide must first be sonicated in detergent (30% by volume Contrad 70 solution) at 40°C for 30 minutes. After it is rinsed extensively by DI H_2O, the glass is soaked in piranha solution and is placed on a rocker at room temperature for at least one hour. The glass is once again rinsed with DI H_2O, and then with 200 proof ethanol. The glass is then soaked in 5 mL of190 proof ethanol. A volume of 100 µL of acetic acid is added to the 5 mL of ethanol, and then 0.5 mL to 1 mL of 3-(Trimethoxysilyl)propylmethacrylate (Aldrich) is added. This solution is placed on a rocker at room temperature overnight. Finally, the glass is washed with 200 proof ethanol and DI H_2O. It is then air dried. (These glass surfaces can be stored in a clean petri dish for months.)



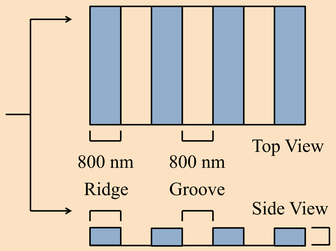
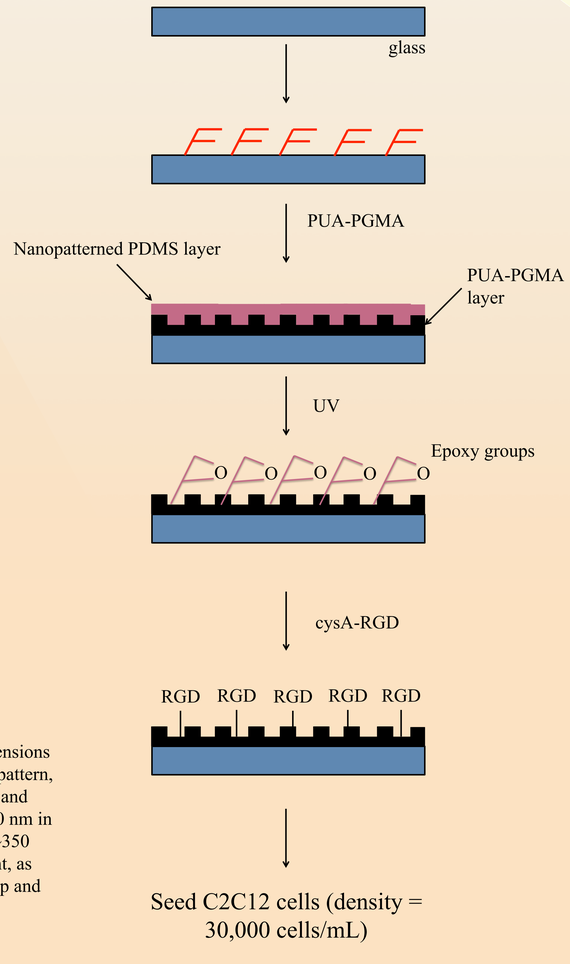


Fig 1. Dimensions of the nanopattern, with ridges and grooves 800 nm in width and ~350 nm in height, as shown in top and side view.

Polymerization Protocol[1,2]:

Due to the PUA solution containing a photo-initiator, all operations should be conducted in a dark place and the samples should be protected by foil wrapping.

GMA monomer (1% w/v) (Sigma Aldrich) was added to the liquid PUA precursor (NOA 76) and sonicated for one hour, and then hand mixed for 10 minutes. The solution was then degassed under a vacuum for 1 hour to remove air bubbles. PUA-PGMA pre-polymer (20 µL) is added to the d=18 mm acrylated glass. The PDMS template (face down) was then placed onto the glass. The film and coverslip were placed under 365 nm UV light for 1 hour to complete polymerization. (Polymerization is time dependent, and one hour gives 100% curing.) (The finished pattern coverslips may be stored in a vacuum desiccator, but downstream protein/polymer conjugation should be completed soon after polymerization because of the epoxy functionalized glass's reactivity.)



RESULTS

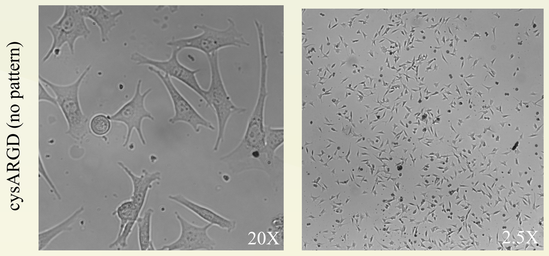


Fig 2. cysARGD-modified polymer with C2C12 cell adhesion at 20X and 2.5X magnification (left to right), no nanopattern

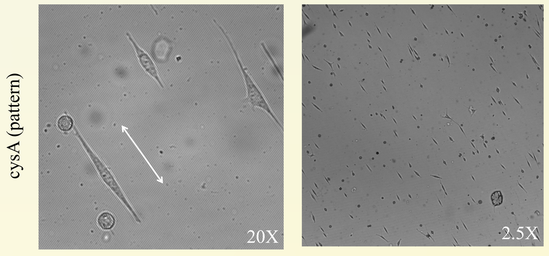


Fig 3. cysA-modified polymer with C2C12 cell adhesion at 20X and 2.5X magnification (left to right), nanopattern in the direction of the arrow

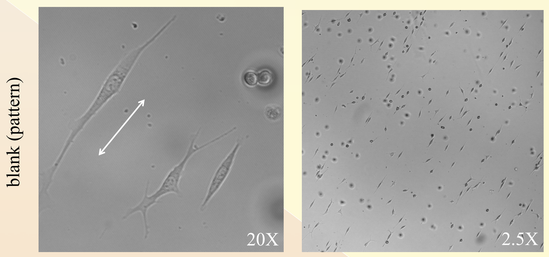


Fig 4. Unmodified polymer with C2C12 cell adhesion at 20X and 2.5X magnification (left to right), nanopattern in the direction of the arrow

The above images show the adhesion of C2C12 cells seeded onto the PUA-PGMA polymer modified glass. In Figure 1 and 2, the polymer has been modified with the bioactive peptides cysARGD and cysA. Significantly more C2C12 cells adhered to the polymer when modified with cysA-RGD as compared to cysA or unmodified polymer. Furthermore, the sample with no nanopattern on the polymer (Figure 1) showed chaotic adhesion while the samples with nanopattern (Figure 2) showed adhesion parallel to the nanopattern.

Fig 5. PUA-PGMA polymer on glass, shows the iridescence that indicates complete polymerization

CONCLUSIONS

While one conclusion drawn during the experiment includes the time dependency of the polymerization of PUA-PGMA due to incomplete polymerization after 10 minutes but full polymerization after one hour, other conclusions that can be drawn from the results of the trials include the fact that csyARGD is directly related to increased C2C12 cell adhesion and that C2C12 cells that have adhered to the polymer respond to the nanopattern and will grow parallel to the pattern. As shown in Figure 1 compared to Figure 2 and Figure 3 in the results, cysA-RGD also greatly improves cell adhesion when added to the polymer compared to just polymer modified with cysA and no protein.

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