Atmospheric Plasma-Grafted Thermally Responsive Coatings for Cell-Sheet Engineering

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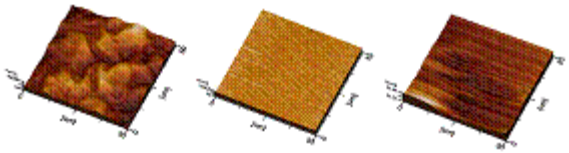
Statement of Purpose: There is an urgent, unmet need for expansion bioreactors capable of culturing and then releasing adherent cell types. Current bioreactor designs are adequate for limited expansion and sustenance of adherent cell types, but cell release requires an enzymatic degradation process that results in inferior cell product, and disrupts cell focal contacts, problems that substantially diminish bioreactor effectiveness. Thermal detachment from PNIPAM grafted surfaces represents an alternative to enzymatic cell/tissue recovery, and yields cell sheets with intact intercellular junctions [2] and differentiated functions [3]. These sheets of cells can be used as is, or stacked to form homo- or heterogeneous multi-layered tissue constructs [1].

Flexible porous tissue culture substrates for cell sheet engineering functionalized by PNIPAM represent a new mechanism for promotion of large areas of single cell, cell sheets, and eventually, 3-D tissue expansion. Atmospheric plasma grafting of functional polymer surfaces is a scaleable technology for graft polymerization of fabrics and films [4, 5]. Investigation of an atmospheric plasma grafting technique for thermally responsive PNIPAM is described below, using PS tissue culture plates as an initial model substrate.

Methods: Six-well non-tissue culture treated polystyrene (PS) plates (Falcon) were used as the substrates for graft polymerization. N-isopropylacrylamide (NIPAM) (Kohjin) was recrystallized using hexane prior to grafting. The PS plates were treated in oxygenated helium (99% He, 1% O_2) in a capacitively coupled atmospheric plasma system (4.8 kW, 5 kHz). The He gas flow rate was approximately 10.18 L/min. The PS plates were exposed to plasma pretreatment for 2 min. to activate the surface. 150 µl of a solution of 55% NIPAM in 2-propanol was added into the PS plates and spread evenly. The plates were immediately exposed to plasma for a 5 min. plasma grafting treatment. The treated samples were rinsed with agitation in distilled water and dried at room temperature.

Surface topography of the PNIPAM-grafted PS was examined using atomic force microscopy (JEOL JSPM-5200) under Tapping Mode in dry (vacuum) and in wet phase at room temperature (22 °C) and 40 °C. Human Epidermal Keratinocytes (HEK's) were plated in plasma grafted six-well culture plates (9.6 cm^2 well area). The HEKs were seeded at 100K cells in 2ml of media per well. The media was replaced after 24 hr in a manner that assured the temperature of the plates and medium was maintained at 37ºC. After four days, the grafted plates were removed from the incubator, placed on ice, and observed periodically under the microscope.

Results / Discussion: The PNIPAM-grafted surface topography at various temperatures and in wet and dry conditions is shown in Figure 1. Dry at 22°C (Figure 1a), atmospheric plasma PNIPAM-grafted PS has a highly convoluted surface, indicating a condensed polymer network. In water at 22°C, the surface convolutions disappear, and the surface appears smooth (Figure 1b). Under these conditions, the AFM tip easily penetrates the entire grafted layer and small defects in the underlying plasma treated films are visible. When the temperature increases from 22°C to 40°C in water, the surface became convoluted and more viscous; drag on the AFM tip results in visible horizontal striations (Figure 1c).



HEK's grew to approximately 70% confluency prior to evaluation. Upon cooling, cells detached as either single cells, large aggregates, or in sheets (Figure 2). However, cellular release was not homogeneous on the plates. This may be due to inconsistency in graft thickness.

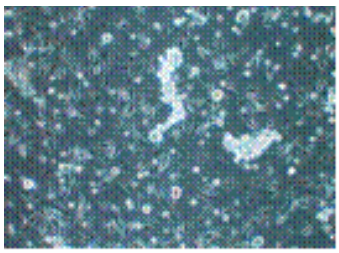


Figure 2. HEK's release from surface upon cooling.

Conclusions: Temperature-responsive PNIPAM was grafted onto PS plates by atmospheric plasma treatment of NIPAM monomer coated surfaces. AFM images show that the PNIPAM-grafted PS surface is highly mobile and watery at room temperature, and becomes a condensed viscous gel at 40 °C. HEK's adhere and proliferate on PNIPAM grafted PS surface at 37 ºC. As temperature decreases to below the LCST, the HEK's spontaneously detach from the surface as cell aggregates, sheets, or single cells. Atmospheric plasma PNIPAM grafting shows promise as a technique for creating novel textile substrates for cell sheet engineering.

References:

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