Effects of Polymer Surface Chemistry on Endothelial Cell Attachment

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Introduction: Glow-discharge gas-plasma (GP) treatment is a surface treatment that enhances surface roughness and alters the surface chemistry of polymers, improving cell adhesion and proliferation. Previous studies have shown improved attachment of cells after gas plasma treatment. It is not known if the effects of gas plasma are due primarily to increases in surface roughness or the change in surface chemistry. This study examines cell attachment to polymers treated with oxygen, argon, and nitrogen gas plasma. By treating films cast on coverslips, changes in surface roughness are avoided so only surface chemistry changes are made. This will determine if the improvement of cell attachment are primarily due to the increase in surface roughness or to surface chemistry changes.

Methods: Poly (d,l-lactic) acid (DURECT, Pelham AL) films were prepared by casting a 4.1wt% PLA acetone solution on glass molds with and without 15mm coverslips. The films were GP treated for 3 minutes at 100W (PDC-32G, Plasma Cleaner/Sterilizer; Harrick Scientific Inc.) under Oxygen (O_2), Argon (Ar), or Nitrogen (N_2). Controls were untreated PLA films. Contact angles were measured using a goniometer (AST Products Inc., *VCA Optima* XE, Boston, MA). XPS analysis was also performed. Films on coverslips were seeded with human umbilical vein endothelial cells (HUVEC) and allowed to attach for four hours (n=3). Cell attachment was measured using AlamarBlue (Invitrogen, Carlsbad, CA). Statistical analysis was performed using student's t test.

Results: There were significant differences in contact angles between control films and all treated films and between Ar and N_2 gas plasma treated films. (Fig. 1)

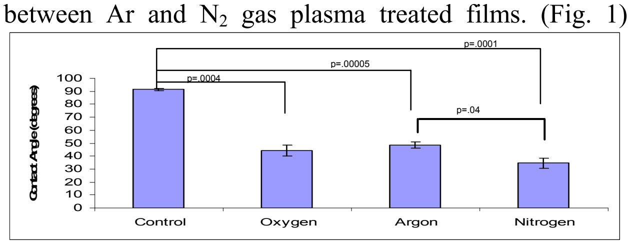

Figure 1. Contact angles of PLA films without coverslips

Cell attachment was similar between Ar and N_2 treated films and between control and O_2 treated films. There was a significant difference between control films and the Ar and N_2 treated films and between O_2 and N_2 treated films ($p<0.05$). (Fig 2)

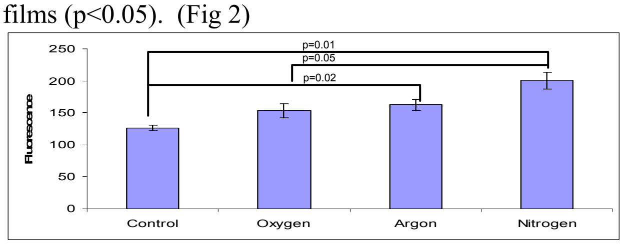

Figure 2. Cell attachment to PLA films with coverslips

XPS showed changes in surface chemistry in all gas plasma treated films compared to control films. A significant decrease in the molar concentration of C-C and a significant increase in the molar concentrations of C-O and C=O bonds were observed for gas plasma treated PLA when compared to control PLA. The XPS oxygen 1s spectrum of control and gas plasma treated PLA showed a significant increase in the molar concentration of C-O and a significant decrease in the concentration of C=O. This suggested the incorporation of more C-O-H groups on to the gas plasma treated surfaces. The atomic concentration of Carbon and Oxygen was similar between the treated films, but there was more Nitrogen groups present on Ar and N_2 treated films. (Fig. 3)

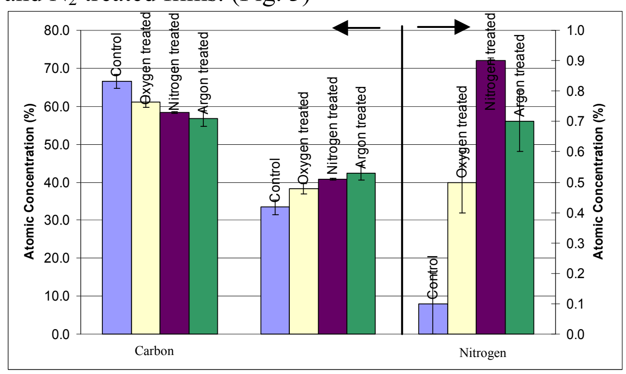

Figure 3. Atomic concentration of carbon, oxygen and nitrogen on PLA films (blue:control, beige:O_2 treated, purple:N_2 treated, green:Ar treated)

Conclusions: After removing gas plasma surface morphology alterations, the effects of surface chemistry changes could be evaluated. PLA films treated with N_2 and Ar plasma showed increased cell attachment compared to O_2 treated films and control films. There was no significant difference in cell attachment between O_2 treated and control films even though there was a significant difference in contact angles. XPS analysis showed that the greatest surface chemistry differences between the Ar and N_2 treated films versus O_2 treated and control films was the atomic concentration of Nitrogen. Nitrogen containing functional groups may have greatest effect on cell attachment. Ammonia gas plasma also deposits nitrogen functional groups and its effects on cell attachment can be investigated in future studies.

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