Materials Research. 2013; 16(4): 695-702
DOI: 10.1590/S1516-14392013005000056

Benefits of Oxygen and Nitrogen Plasma Treatment in Vero Cell Affinity to Poly(Lactide-Co-Glycolide Acid)

Andrea Rodrigues Esposito [a,b]*, ***Camila Mika Kamikawa*** [a], ***Carolina Lucchesi*** [a],
Betina Mara Pereira Ferreira [a], ***Eliana Aparecida de Rezende Duek*** [a,b]

[a]*Laboratory of Biomaterials, Center for Medical and Health Sciences, Pontifical Catholic University of Sao Paulo – PUC-SP,, Sorocaba, SP, Brazil*
[b]*Department of Materials Engineering, Faculty of Mechanical Engineering, State University of Campinas – UNICAMP, Campinas, SP, Brazil*

Received: May 31, 2012; Revised: November 6, 2012

Cell adhesion on materials surface is critical because this phenomenon occurs before other events, as cell spreading, cell migration and cell differentiation. It is commonly accepted that the adhesion of cells on solid substrate is influenced by several substratum surface properties, such as wettability, surface charge, roughness and topography. Plasma technique is a convenient method for modifying surface properties of materials without affecting physical properties. In this study, poly(lactide-co-glycolide), PLGA, membranes were modified by oxygen and nitrogen plasma to improve polymer hydrophilicity and verify their effect on Vero cells culture. The PLGA membranes, which were characterized by SEM and contact angle, showed increased surface rugosity and narrower contact angles. Cell adhesion, cytotoxicity assay, SEM and cytochemistry analysis showed that plasma treatment was beneficial to cell growth by improving cell-polymer interaction.

Keywords: *biomaterials, plasma treatment, PLGA, cell culture*

1. Introduction

A research area that has received increasing attention is tissue engineering that restores tissue functionality from native or synthetic sources by applying engineering principles. Biomaterials play an important role providing scaffolds to guide tissue regeneration, means to deliver drugs and growth factors to stimulate tissue response, or by creating a new functional structure when damaged tissue cannot regenerate[1].

Whenever biomaterials are employed it is essential to understand their pattern of cell growth and differentiation and select the appropriate polymer, given that the latter may influence the development of bioresorbable polymers designed to stimulate the regeneration of damaged tissues[2]. The importance of cell culture methodology related to biomaterials research is that it enables fast evaluation of the performance of polymers. In addition, evaluation of cell-biomaterial interactions concerning the prediction of possible *in vivo* reactions to these polymers is invaluable when they are utilized as substitutes for body parts or as a stimulant for regeneration or repair of damaged tissues[1].

In tissue engineering, cell adhesion onto a surface is critical because it occurs before other events, as cell spreading, cell migration and differentiation of cell function[3]. Moreover, cell adhesion is closely related to surface properties of biomaterials. It is generally accepted that adhesion of cells onto solid substrate is influenced by several substratum surface properties, such as wettability[4], surface charge[5], roughness and topography[6]. Hence, surface modification techniques have been used to improve surface properties of polymers[7].

Biodegradable polyester, including poly(lactide-co-glycolide) (PLGA) with various lactide/glycolide ratios, is an important material for tissue engineering[8], despite its poor hydrophilicity and lack of natural cell recognition sites on its surface[9].

Plasma treatment is an effective and widely used method for modifying the surface of materials[10,11]. Moreover, reactive sites such as amine[12], sulfonic acid and carboxyl groups[13] on polymers (*i.e*. functional surfaces) can be created by using non-polymerizing gases. It has been shown that plasma processes can only proceed with localized surface treatment that does not change the bulk properties of the polymers[14].

Plasma processes have been used to increase the hydrophilicity of polylactide (PLA) and improve its cell adhesion[12]. Santos Junior et al.[15] and Ferreira[16] studied the adhesive force and affinity of Vero cells to PLLA and PHBV films, demonstrating that these polymers have poor cell-matrix interaction[16]. The characterization study indicated that the surface composition and the functional groups on the surface of PLLA and PHBV after plasma treatment had a great effect on cell affinity of these films[17].

In a comparative study, Croll et al.[17] and Hasirci[19] studied the plasma etching effect on PLGA. The results showed an increase of hydrophilicity of PLGA membranes. However, there are few reports on PLGA surface morphology after

*e-mail: aesposito@fem.unicamp.br

oxygen and nitrogen plasma treatment and the effect of surface morphology on cell adhesion.

Thus, the aim of this study was to investigate the effect of oxygen and nitrogen plasma treatment on the PLGA surface properties, including hydrophilicity and morphology, and to evaluate Vero cells affinity to treated PLGA.

2. Material and Methods

2.1. *Preparation of PLGA membranes*

Poly(lactide-co-glycolide), PLGA, was dissolved in methylene chloride (5% W/V) at room temperature. The solution was casting onto glass plates and dried for 24h. Plasma treatment was performed using a radio frequency Anatech LTD Ashing plasma equipment. Table 1 summarizes the plasma treatment conditions: N_2 or O_2 gas, power range of 50-100 W, pressure of 20 to 80 Pa, and time at 600s. The values of power, pressure and time were chosen based on preliminary studies. Previously, before cell culture, the membranes were sterilized by UV irradiation for 30 min and placed in 96-well plates with 100 µL of culture medium and maintained at 37 °C for 24 hours, according to ISO10993-1[18].

2.2. *Contact angle analysis*

Contact angles were measured in air, using a NRL CA goniometer Ramé-Hart Imaging System, model 100-00, and image acquisition was performed with Ramé-Hart Imaging 2001 software. Demineralized and deionized water and diiodomethane were used as probing liquid in order to obtain contact angles, free energies, and polar and dispersive components of the surfaces.

2.3. *Scanning electron microscopy (SEM)*

Scanning electron microscopy (SEM) was used to investigate the morphology of membrane surfaces. The membranes were stuck on a metallic support and coated with gold by using a Sputter Coater Bal-Tec SCD 050. The samples were observed with a JSM 5900-LV ARP scanning electron microscope.

2.4. *Cell culture*

Vero cells, a fibroblastic cell line established from the kidney of the African green monkey (*Cercopithecus aethiops*), were obtained from Adolfo Lutz Institute, Sao Paulo, Brazil. These cells were cultured in medium Meio 199 (EARLE) (Cultilab®, Campinas, SP, Brazil) supplemented with 10% fetal calf serum (FCS, Cultilab®) maintained in a gas-jacket incubator with 5% CO_2 at 37 °C. Vero cells, a recommended line of cells, were used for cytotoxicity and cell-substratum interactions with biomaterials studies[1,15,20-23].

Table 1. Parameters for PLGA treatment with O_2 and N_2 plasma.

Samples	Power (W)	Pressure (Pa)	Time (seconds)
PLGA (0)	-	-	-
PLGA O_2 (1)	50	20	600
PLGA O_2 (2)	100	80	600
PLGA N_2 (3)	50	20	600
PLGA N_2 (4)	100	80	600

2.5. *Cell adhesion and viability assays*

Identification of the effects of plasma treatments on early cell adhesion and cell viability was carried out using the MTT assay[23]. After incubation, 2×10^5 cells.mL^{-1} in 100 µL of medium Meio 199 (EARLE) supplemented with 10% FCS were seeded to the wells containing different PLGA membranes treated. The cells were cultured for 2 hours and 24 hours at 37 °C to enable cell adhesion and direct cell viability assays, respectively. Subsequently, the cells were washed twice with 0.1 M phosphate-buffered saline (PBS) pH 7.4 at 37 °C and incubated with 100 µL of medium, MTT assay mixture [10 µL per well, containing 5 mg.mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide – MTT, Sigma®] was added to each well, and cells were incubated for 4 h at 37 °C. After 4 hours, the culture medium in each well was substituted with 100 µL of Dimethyl Sulphoxide (DMSO, Sigma®) and 25 µL of buffered Glycine/Sorensen solution to dissolve formazan crystals. The absorbance was quantified spectrophotometrically at 570 nm, using a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). The culture plate itself (polystyrene) and phenol 1% were used, respectively, as the cell viability assay positive and negative controls. Polytetrafluoroethylene dishes (PTFE) and the culture plate itself were used as negative and positive controls for the cell adhesion test.

The absorbance of all experimental conditions was read without cells to control MTT reaction. ANOVA was used to perform comparisons of continuous variables of all groups and Tukey`s test was used to compare the groups ($p < 0.05$).

2.6. *Cell morphology analysis – SEM*

In order to carry out cell morphology analysis approximately 2×10^5 Vero cells.mL^{-1} of medium Meio 199 (EARLE) supplemented with 10% FCS were seeded in each well containing the membranes and cultured at 37 °C, 5% CO_2. As control, cells were cultured on glass cover slips in the same culture conditions. After 2, 48 and 168 hours, the samples were fixed in a fixative solution prepared by dissolving 2.5% paraformaldehyde, 2.5% glutaraldehyde, 0.03% picric acid, 1% tannic acid in 0.1 M cacodylate buffer and the same volume of medium Meio 199 (EARLE) for 1h at room temperature, washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide (Sigma®) in water for 1h at RT in the dark and washed in water. The samples were dehydrated with ethanol series, critical point dried (Balzers® CDT 030) and coated with gold in a sputter coater (Blazers® CDT 050). The coated specimens were observed with a JEOL 5800 scanning electron microscope.

2.7. *Cytochemical analysis*

For the cytochemical study 2×10^5 cells.mL^{-1} were cultured on PLGA membranes in medium Meio 199 (EARLE) supplemented with 10% FCS maintained in a gas-jacket incubator with 5% CO_2 at 37 °C. After 2, 48 and

168 hours in culture, the samples were fixed with Karnovsky fixative (4% paraformaldehyde, 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2), dehydrated in graded ethanol series at room temperature, cleared in xylene and stained with Toluidine Blue (TB) at pH 4.0, a basophilic dye, for the detection of DNA, RNA and glycosaminoglycans and with Xylidine Ponceau (XP) at pH 2.5, an anionic dye, for identification of cationic proteins[15]. Staining time was 15 minutes for TB and 20 minutes for XP, both at room temperature. The samples obtained were photographed under a Nikon® Eclipse E800 photomicroscope.

3. Results and Discussion

3.1. *Contact angle analysis*

Treatment of PLGA samples with nitrogen and oxygen plasma drastically narrowed the water contact angle as the result of increased surface hydrophilicity (Table 2). Hence, the notable increase of hydrophilicity on the sample surface is due to the enhancement of the polar component (γ_s^p) of the surface energy.

Table 2. Effects of O_2 and N_2 plasma treatment on PLGA surface energies.

Sample	Angle (deg)		Surface energy* ($mJ.m^{-2}$)		
	θ H_2O	θ CH_2I_2	γ_s^p	γ_s^d	γ_s
PLGA (0)	92.9	46.9	4.1	35.2	39.3
PLGA O_2 (1)	62.4	46.8	17.8	35.0	52.8
PLGA O_2 (2)	62.1	43.0	17.5	36.7	54.2
PLGA N_2 (3)	54.6	45.9	21.8	35.4	57.2
PLGA N_2 (4)	45.3	47.8	27.0	34.5	61.4

*γ_s = surface energy, γ_s^p = polar component, γ_s^d = dispersive component.

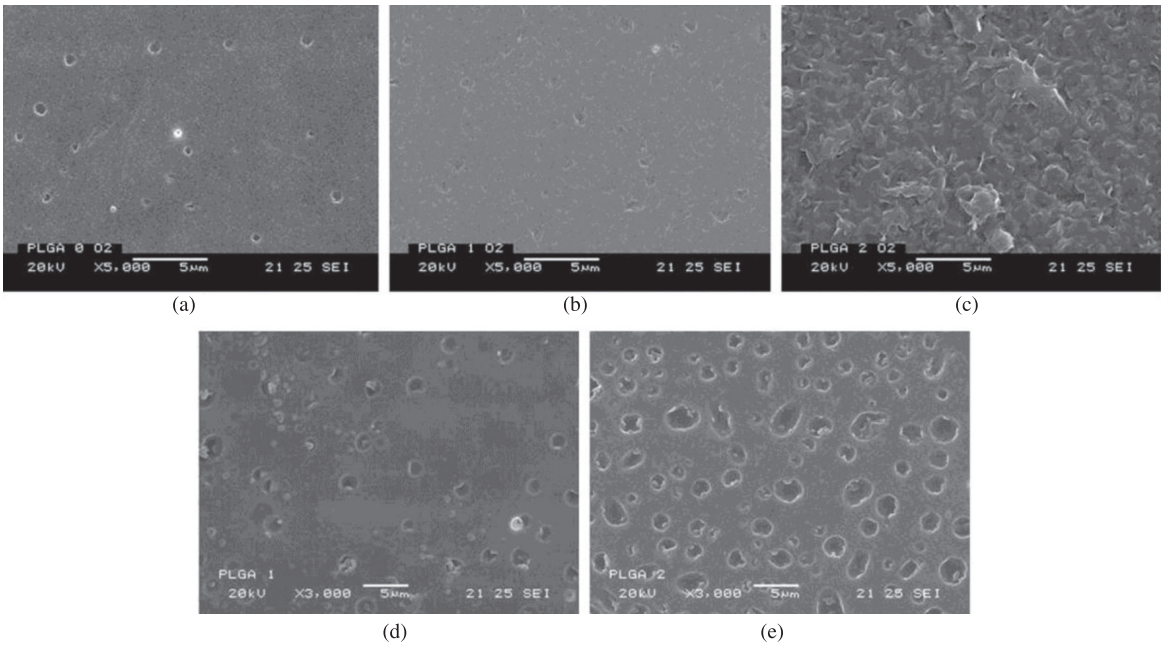
Hydrophilicity is one of the most important factors that influence biomaterial cytochemical compatibility. Cell adhesion and growth on the surface are considered to be strongly dependent on the hydrophilicity/hydrophobicity balance. Some authors have demonstrated that cells are preferentially anchored to hydrophilic surfaces[24-26]. The study has also shown that the cells adhere, spread, and grow easier on substrates with moderate hydrophilicity than on hydrophobic or highly hydrophilic substrates. In accordance with Yamaguchi et al.[9], showed that the majority of the cells presented good cellular adhesion on the surface with contact angles below 60°.

It was also observed that increased hydrophilicity is directly related to augmentation of plasma pressure, power and time (Tables 1 and 2). These results are corroborated by Chu et al.[14] who observed that hydrophilicity is related with the type of gas and treatment parameters.

3.2. *Scanning Electron Microscopy (SEM)*

Changes in the surface morphology are observed in Figure 1 which shows SEM micrographs of PLGA with and without plasma treatment. The untreated PLGA sample (Figure 1a) presents a flat slightly porous surface from solvent casting rate during the manufactory process[27], while PLGA samples treated had the surface morphology altered by oxygen showed rough morphology (Figure 1b-c) and nitrogen plasma presenting deep holes (Figure 1d-e). There were significant morphological surface changes as the plasma parameters increased.

According to Yang et al.[12], roughness is one of the reasons for hydrophilicity improvement in the plasma treated samples, given that the surface wettability is consequence



(a) (b) (c)

(d) (e)

Figure 1. SEM micrographs of PLGA: (a) untreated PLGA 0; (b) Oxygen plasma treated PLGA 1: (c) Oxygen plasma treated PLGA 2; (d) Nitrogen plasma treated PLGA 3; (e) Nitrogen plasma treated PLGA 4. White scale bar represents 5 µm.

of the surface roughness. Materials of lower surface energy (*i.e.* polymers) showed an increase in cellular adhesion associated with the increased surface roughness, whereas materials of higher surface energy (*i.e.* metals) demonstrated little change in cellular adhesion with increased surface roughness[28].

Briefly, physical and chemical properties of the polymer surfaces can be completely modified by plasma treatments. Not only the chemical changes of the surface, but also the topographic changes can contribute independently for modifications in PLGA hydrophilicity.

Cell morphology on treated and untreated membranes was quite different. The initial cell adhesion to the untreated PLGA membrane was poor. The cells were small, rounded and separated from each other (Figure 2a), which is in accordance with other studies using Vero cells seeded on untreated scaffolds[1,16,29-31]. In contrast, it was noticed that Vero cells cultivated for 2 hours on plasma-treated PLGA presented higher density of adhered cells in close contact with each other, spread on the membrane surface. The cells showed flat and elongated morphology and some vesicles on their surfaces (Figure 2b-d). On membrane surfaces where cells will likely complete their movement and adhere tightly, it was observed the development of focal contacts within a short cultivation time[32]. After 48 hours in culture, the cells seeded on PLGA membranes were anchored to the substrate and displayed a spread, flattened/elongated morphology with a large number of cells with phillopodes and lamellipods, similar to fibroblastic cells[33] (Figure 3).

According to Berry et al.[34], fibroblastic cells are sensitive to topography, which affects motility and proliferation. It was also observed processes through which cells were bonded to each other by prolongations and thin filaments budding from the basal portion of the cell (Figure 3c). Cell-cell contact is necessary for signaling and expression of particular tissue functions. These prolongations often formed a thin fibrillar reticulated material on the cell surface (Figure 3b).

The Vero cells had reached confluence in all samples after 7 days in culture appeared as layers covering the PLGA surface. Clear differences between the pressure and power applied were not observed (Figure 4).

The high number of Vero cells attached on the membranes should be result of cell proliferation in this period, showing that plasma treatment not only supports adhesion but also proper cell division and growth.

A key factor that influences the adherence of Vero cells is the substratum surface charges[1,15,35,36]. Oxygen and nitrogen plasma treatment improved the time span (2 hours) for interaction between Vero cells and the PLGA membrane by promoting anchorage to the substrate, spreading and proliferation.

This result is in accordance with those in which the surface chemical composition and its topography affect the interaction force that acts between the biomaterial and the biological medium, for instance, water and ion adsorption, protein adsorption, adhesion, cellular expansion and proliferation[15] as to improve cell adhesion and growth[8,9,12,24].

3.3. *Cell adhesion and viability assays*

Initial cell adhesion assay results showed that Vero cells have presented expected lowest adhesion on the negative control, independent of treatment conditions ($p < 0.05$). The capacity of cell adhesion to treated PLGA membranes was shown to be similar to untreated PLGA ($p < 0.05$). All samples presented a low capacity to stimulate cell adhesion when compared with the positive control ($p < 0.05$). The results are shown in Figure 5.

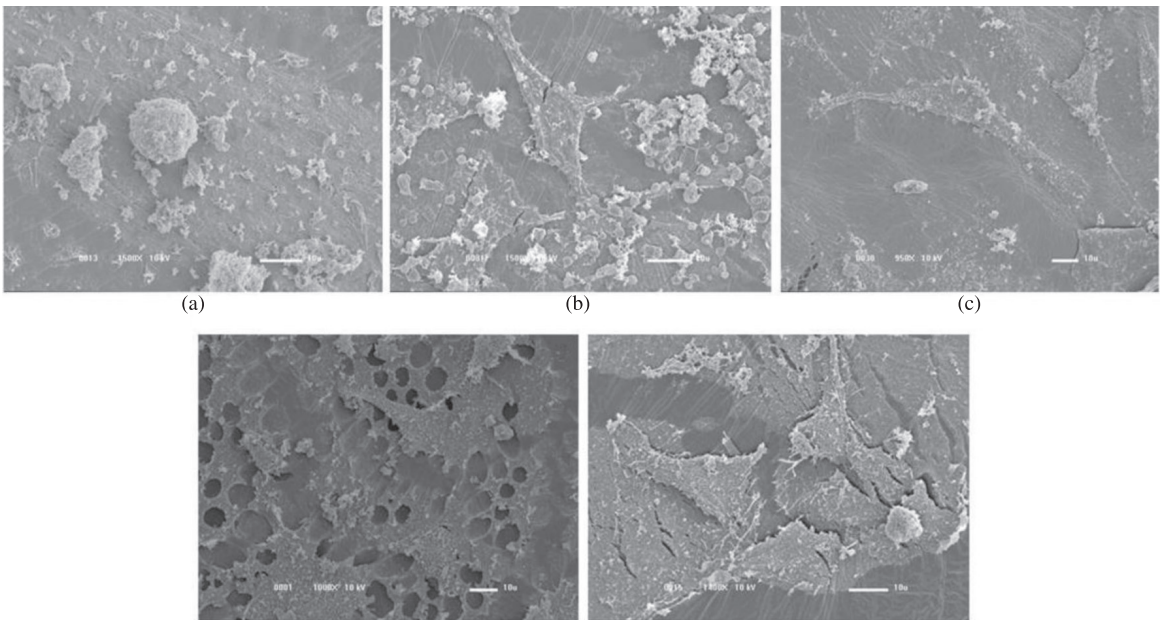
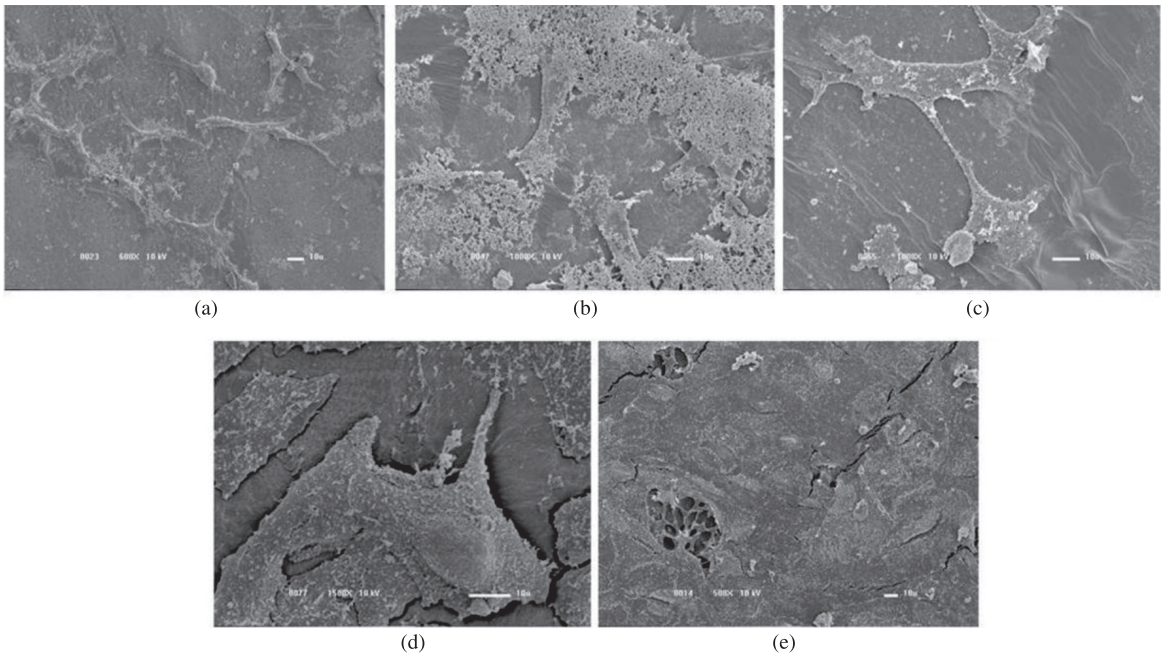


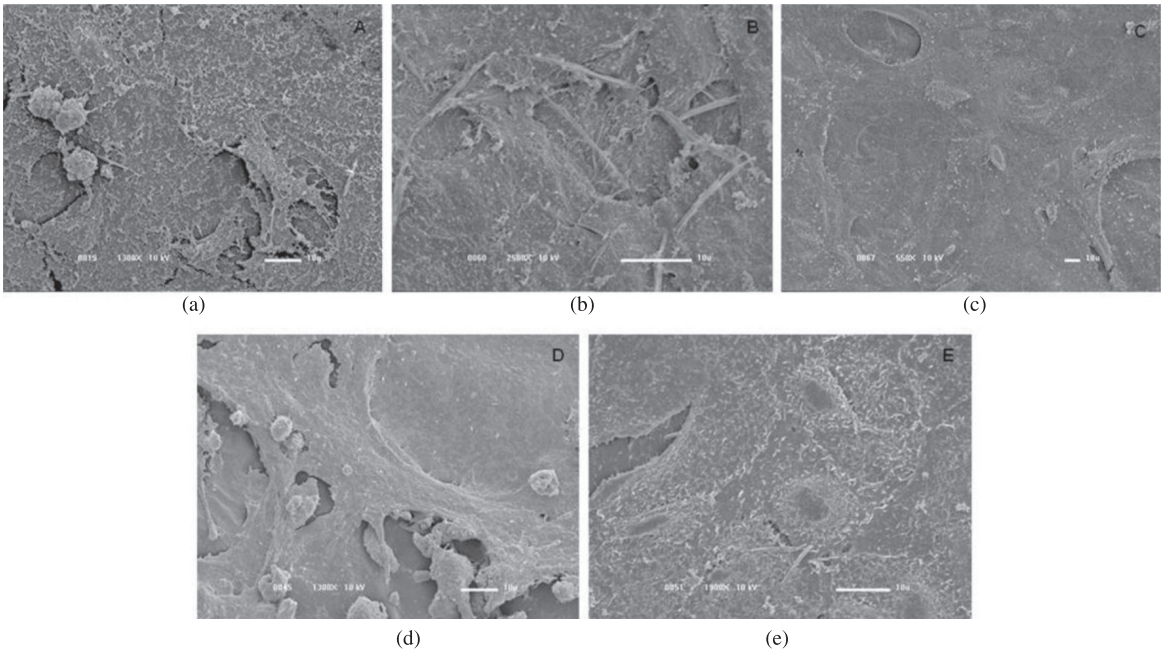
Figure 2. The SEM images show Vero cells on different PLGA membranes cultured for 2 hours. a) PLGA (0), b) PLGA O_2 (1), c) PLGA O_2 (2), d) PLGA N_2 (3), e) PLGA N_2 (4). White scale bar represents 10 µm.



(a) (b) (c)

(d) (e)

Figure 3. The SEM images show Vero cells on different PLGA membranes cultured for 48 hours. a) PLGA (0), b) PLGA O_2 (1), c) PLGA O_2 (2), d) PLGA N_2 (3), e) PLGA N_2 (4). White scale bar represents 10 μm.



(a) (b) (c)

(d) (e)

Figure 4. The SEM images show Vero cells on different PLGA membranes cultured for 7 days. a) PLGA (0), b) PLGA O_2 (1), c) PLGA O_2 (2), d) PLGA N_2 (3), e) PLGA N_2 (4). White scale bar represents 10 μm.

The polystyrene plate itself is commonly accepted as positive control once consists of the same material as the cells are usually culture *in vitro*, once it allows cell adhesion and proliferation as a gold standard. In the same vein, polytetrafluoroethylene dishes (PTFE) and phenol (1%) represent negative controls once are non-sticker material and toxic, respectively[1,15,22,27,31].

The cell viability assay results showed that all PLGA membranes are not cytotoxic to Vero cells when compared to the negative control (phenol) ($p < 0.05$), demonstrating the cytocompatibility improvement of the treatment. PLGA N_2 (3) and PLGA N_2 (4) membranes presented similar results regardless to the positive control ($p > 0.05$). While, PLGA O_2 (1) and PLGA O_2 (2) membranes have presented

intermediate analogous values independent of treatment conditions ($p > 0.05$). The results are shown in Figure 6.

Moreover, the positive and negative controls showed significant differences between means ($p < 0.01$), providing consistent data for standard controls.

MTT assay revealed that all materials in the study did not affect mitochondrial activity of Vero cells. Nitrogen and oxygen plasma proved to be more receptive to cell interaction after 24 hours in culture.

The main cell-biomaterials interaction parameters are toxicity, cell adhesion and proliferation[1,2]. Biocompatibility interaction is very closely related to cell behavior on the receiver recognition of the cell adhesion surface and the connections fields of the extracellular matrix proteins (fibronectin, collagen or vitronectin) that form a compound which can induce cell attachment, spreading and proliferation[25].

Cell adhesion on biodegradable polyester including PLLA, PLDLA and PLGA with various lactide/glycolide ratios is slow, despite their biocompatibility[26], possibly due to their poor hydrophilicity[29]. The difference observed in cell viability to PLGA membranes could be related to their chemical composition and topography variation.

As shown in the study conducted by Ignatius and Claes[37] on PLGA biocompatibility, the scaffolds did not present cytotoxic effects, given that the mitochondrial activities of cells were not affected. Although, toxic substances do not act at one specific level but affect several cellular functions[2], it was considered that the immediate measurement of the mitochondrial activity in terms of cell sensitivity would be enough to evaluate cell viability.

3.4. *Cytochemical analysis*

In the culture period of 2, 48 and 168 hours, we detected cells stained by TB or XP, which are basophilic and acidophilic dyes, respectively (Figures 7 and 8). The cells were able to reach confluence on all PLGA substrates.

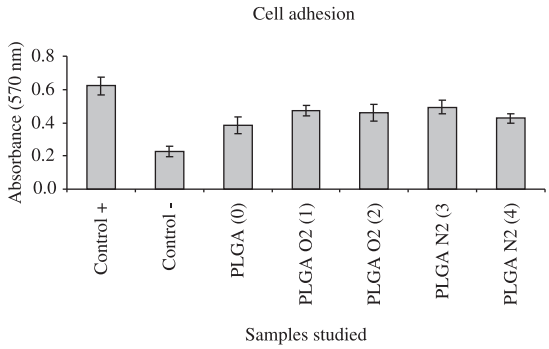


Figure 5. Adhesion of Vero cells cultured on different PLGA membranes after 2 hours. Mean ± sd ($n = 6$). All treatment results are higher than the negative control ($p < 0.05$).

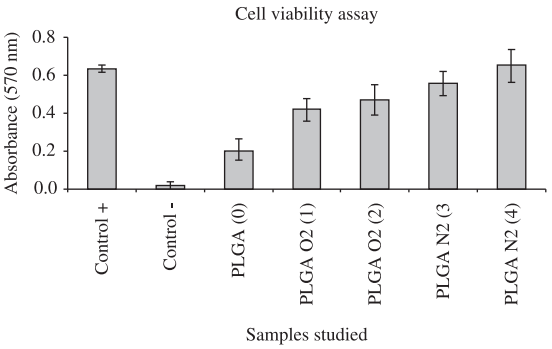


Figure 6. Vero cell viability cultured onto different PLGA membranes after 24 hours. Mean ± sd ($n = 6$). All treatment results are higher than the negative control ($p < 0.05$).

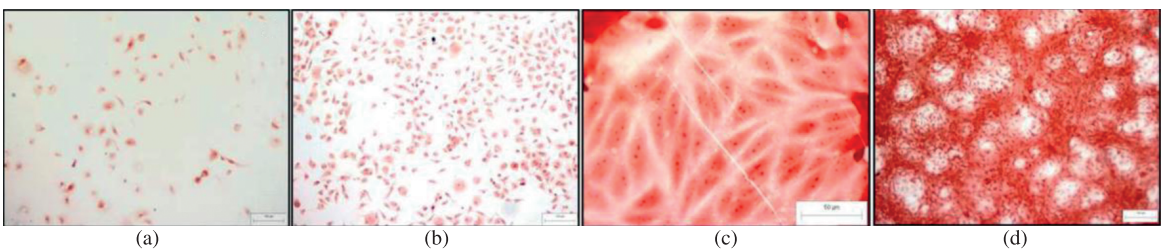


Figure 7. Vero cells cultured on different PLGA membranes and stained with Xylidine ponceau (XP): a) PLGA O2 (2) after 2 hours, 200×; b) PLGA N2 (3) after 2 hours, 100×; c) PLGA O2 (1) after 168 hours, 400×; d) PLGA N2 (4) after 168 hours, 100×.

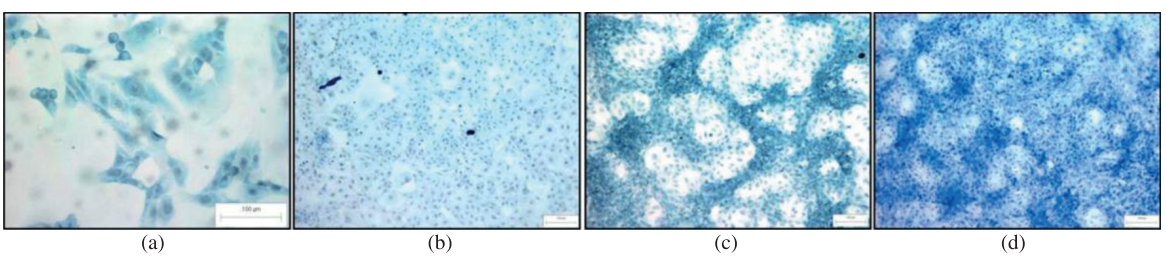


Figure 8. Vero cells cultured on different PLGA membranes and stained with Toluidine blue (TB): a) PLGA O2 (1) after 48 hours, 200×; b) PLGA N2 (4) after 48 hours, 100×; c) PLGA O2 (2) after 168 hours, 100×; d) PLGA N2 (3) after 168 hours, 100×.

Cytochemical alterations induced by the different treated and non-treated PLGA membranes on which the cells were cultured were not found. Basophilic cells stained for detection of nucleic acids and glycosaminoglycans with TB pH 4.0 were found in all samples. The basophilic cytoplasm was confirmed by using XP – a stain for protein groups.

According to the results obtained by Santos[1,31] the basophilic cytoplasm suggests that the cells are enabled to activate protein synthesis. These results agree with the description that different cells are able to produce extracellular matrix on PLGA copolymers[30,38].

The surface modifications were successfully induced to improve cell interaction. The results of Vero cell *in vitro* culture showed that plasma treatment of PLGA surface provided favorable conditions for cell adherence and spreading due to interaction improvement between the membrane polar groups and the cell surfaces. Consequently, the cells could grow and proliferate naturally in a short lapse of time. Cytochemistry did not show alterations in cell behavior induced by different PLGA treatments. Therefore, it can be concluded that the substrate surface charge greatly influences the growth of Vero cells in adhesive experiments.

4. Conclusions

Oxygen and nitrogen plasma treatment promoted narrower contact angles and consequently increased hydrophilicity and rugosity of PLGA samples.

Plasma-treated surfaces provide better cellular adhesion, enabling cell growth, spreading and proliferation independently of the gas-type treatment, demonstrating the biocompatibility of the samples.

This work may effectively contribute to the development of biomaterials with different properties and increased feasibility of cell/polymer interaction.

Acknowledgements

The authors would like to express their appreciation to PIBIC-CNPq for financial support, the Brazilian agency FAPESP (project no. 03/02188-5), the Brazilian Synchrotron Laboratory for giving us permission to use the plasma, SEM, AFM, and XPS equipment, and São Paulo State University, Sorocaba, for the goniometer.

References

1. Santos Junior AR, Ferreira BMP, Duek EAR, Dolder MAH and Wada ML. Use of blends of bioabsorbable poly(L-lactic acid)/poly(hydroxybutyrate-co-hydroxyvalerate) as surface for Vero cell culture. *Brazilian Journal of Medical and Biological Research*. 2005; 38(11):1623-1632.
2. Hubbell JA. Biomaterials in tissue engineering. *Biotechnology*. 1995; 13(6):565-576. PMid:9634795. http://dx.doi.org/10.1038/nbt0695-565
3. Saltzman MW. Cell interactions with polymers. In: Principle of tissue engineering. Landes Company: Academis Press, 1997.p. 225-246.
4. Kaminska A, Kaczmarek H and Kowalonek J. The influence of side groups and polarity of polymers on the kind and effectiveness of their surface modification by air plasma action. *European Polymer Journal*. 2002; 38(9):1915-1919. http://dx.doi.org/10.1016/S0014-3057(02)00059-9
5. Hasirci V, Tezcaner A, Hasisci N and Süzer S. Oxygen plasma modification of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) film surfaces for tissue engineering purposes. *Journal of Applied Polymer Science*. 2003; 87(8):1285-1289. http://dx.doi.org/10.1002/app.11532
6. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford Junior J et al. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). *Journal of Biomedical Materials Research*. 1995; 29(3):389-401. PMid:7542245. http://dx.doi.org/10.1002/jbm.820290314
7. Ma Z, Mao Z and Gao C. Surface modification and property analysis of biomedical polymers used for tissue engineering. *Colloids and Surfaces B*: Biointerfaces. 2007; 60(2):137-157. PMid:17683921. http://dx.doi.org/10.1016/j.colsurfb.2007.06.019
8. Wan Y, Qu X, Lu J, Zhu C, Wan L, Yang J et al. Characterization of surface property of poly(lactide-co-glycolide) after oxigen plasma treatment. *Biomaterials*. 2004; 25(19):4777-83. PMid:15120524. http://dx.doi.org/10.1016/j.biomaterials.2003.11.051
9. Yamaguchi M, Shinbo T, Kanamori T, Wang P, Niwa M, Kawakami H et al. Surface modification of poly(L-lactic acid) affects initial cell attachment, cell morphology, and cell growth. *Artificial Organs*. 2004;7(4):187-93.
10. Morent R, Geyter N, Desmet T, Dubruel P and Leys C. Plasma Surface Modification of Biodegradable Polymers: A Review. *Plasma Processes and Polymers*. 2011; 8(3):171-190. http://dx.doi.org/10.1002/ppap.201000153
11. Rasal RM, Janorkarc AV and Hirt DE. Poly(lactic acid) modifications. *Progress in Polymer Science*. 2010; 35(3):338-356. http://dx.doi.org/10.1016/j.progpolymsci.2009.12.003
12. Yang J, Bei JZ and Wang SG. Improving cell affinity of Poly(D,L-lactide) film modified by anhydrous ammonia plasma treatment. *Polymers for Advanced Technologies*. 2002;13(3-4):220-226 http://dx.doi.org/10.1002/pat.177
13. Inagaki N, Tasaka S and Miyazaki H. Sulfonic acid group-containing thin films prepared by plasma polymerization. *Journal of Applied Polymer Science*. 1989;38(10):1829-1838. http://dx.doi.org/10.1002/app.1989.070381005
14. Chu PK, Chen JY, Wang LP and Huang N. Plasma-surface modification of biomaterials. *Materials Science and Engineering Review*. 2002; 36(5-6):143-206. http://dx.doi.org/10.1016/S0927-796X(02)00004-9
15. Santos Junior AR, Ferreira BMP, Duek EAR, Dolder H, Wada RS and Wada MLF. Differentiation Pattern of Vero cells cultured on Poly(L-Lactic Acid)/Poly(Hydroxybutyrate-co-hHydroxyvalerate) Blends. *Artificial Organs*. 2004; 28(4):381-389. PMid:15084200. http://dx.doi.org/10.1111/j.1525-1594.2004.47199.x
16. Ferreira BMP. *Obtenção*, *caracterização*, *estudo in vitro e in vivo de blendas de PLLA/PHBV*. [Thesis]. Campinas: Faculdade de Engenharia Mecânica; 2011.
17. Ferreira BMP, Pinheiro LMP, Nascente PAP, Ferreira MJ and Duek EAR. Plasma surface treatments of poly(l-lactic acid) (PLLA) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV). *Materials Science and Engineering*: C. 2009; 29(3):806-813. http://dx.doi.org/10.1016/j.msec.2008.07.026

18. Croll TI, O'Connor AJ, Stevens GW and Cooper-White JJ. Controllable surface modification of poly(lactic-co-glycolic acid) (PLGA) by hydrolysis or aminolysis I: physical, chemical, and theoretical aspects. *Biomacromolecules*. 2004; 5(2):463-73. PMid:15003007. http://dx.doi.org/10.1021/bm0343040
19. Hasirci N, Endogan T, Vardar E, Kiziltay A and Hasirci V. Effect of oxygen plasma on surface properties and biocompatibility of PLGA films. *Surface and Interface Analysis*. 2010; 42(6-7):486-491. http://dx.doi.org/10.1002/sia.3247
20. International Organization for Standardization - ISO. *ISO 10993-1/5: 1992 (E*). Biological evaluation of medical devices-Part.5 - Test for cytotoxicity: in vitro methods. Geneva; 1992.
21. Kirkpatrick CJ. Biological testing of materials and medical devices - A critical veiw of current and proposed methodologies for biocompatibility testing: cytotoxicity in vitro. *Regulatory Affairs*. 1992; 4(1):13-32.
22. Santos Junior AR, Babanti SH, Duek EAR, Dolder H, Wada RS and Wada MLF. Vero cell growth and diferentiation on poly(L-lactic acid) membranes of different pore diameters. *Artificial Organs*. 2001; 25(1):7-13. http://dx.doi.org/10.1046/j.1525-1594.2001.025001007.x
23. Lucchesi C, Ferreira BM, Duek EA, Santos Junior AR and Joazeiro PP. Increased response of Vero cells to PHBV matrices treated by plasma. *Journal of Materials Science*: Materials in Medicine. 2008; 19(2):635-43. PMid:17619989. http://dx.doi.org/10.1007/s10856-007-0169-3
24. Wan Y, Yang J, Yang J, Bei J and Wang S. Cell adhesion on gaseous plasma modified poly- (L-lactide) surface under shear stress field. *Biomaterials*. 2003; 24(21):3757-3764. http://dx.doi.org/10.1016/S0142-9612(03)00251-5
25. Ma Z, Gao C, Gong Y and Shen J. Chondrocyte behaviors on poly-L-lactic acid (PLLA) membranes containing hydroxyl, amide or carboxyl groups. *Biomaterials*. 2003; 24(21):3725-30. http://dx.doi.org/10.1016/S0142-9612(03)00247-3
26. Tezcaner A, Bugra K and Hasirci V. Retinal pigment epithelium cell culture on surface modified poly (hydroxybutyrate-co-hydroxyvalerate) thin films. *Biomaterials*. 2003; 24(25):4573-83. http://dx.doi.org/10.1016/S0142-9612(03)00302-8
27. Mas BA. *Imobilização de colágeno em arcabouços de poli* (*L-co-D,L ácido lático*). [Dissertation]. Campinas: Faculdade de Engenharia Mecânica; 2011.
28. Hallab NJ, Bundy KJ, O'Connor K, Moses RL and Jacobs JJ. Evaluation of metallic and polymeric biomaterial surface energy and surface roughness characteristics for directed cell adhesion. *Tissue Engineering*. 2001;7(1):55-71. PMid:11224924. http://dx.doi.org/10.1089/107632700300003297
29. Silva DR, Scapin SM, Joazeiro PP, Alberto-Rincon MC, Luciano RM and Duek EA. In vivo interaction of cells on poly L-(lactic acid) membranes containing plasticizer. Journal of materials science. *Journal of Materials Science*: Materials in Medicine. 2002; 13(3):327-32. PMid:15348633. http://dx.doi.org/10.1023/A:1014027405057
30. Lombello CB, Santos Junior AR, Malmonge SM, Barbanti SH, Wada ML and Duek EA. Adhesion and morphology of fibroblastic cells cultured on different polymeric biomaterials. *Journal of Materials Science:* Materials in Medicine. 2002; 13(9):867-74. PMid:15348552. http://dx.doi.org/10.1023/A:1016552413295
31. Santos Junior AR, Barbanti, SH, Duek EAR and Wada MLF. Analysis of the growth pattern of Vero cells cultured on Dense and Porous Poly (L-Lactic acid) scaffolds. *Materials Research*. 2009; 12(3):257-263. http://dx.doi.org/10.1590/S1516-14392009000300002
32. Köse GT, Ber S, Korkusuz F and Hasirci V. Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) based tissue engineering matrices. *Journal of Materials Science*: Materials in Medicine. 2003; 14(2):121-126. PMid:15348483. http://dx.doi.org/10.1023/A:1022063628099
33. Genari SC, Dolder MA and Wada ML. Scanning and transmission electron microscopy of transformed Vero cells, with altered in vitro growth characteristics. *Journal of Submicroscopic Cytology and Pathology*. 1996; 28(4):565-72. PMid:8933740.
34. Berry CC, Campbell G, Spadiccino A, Robertson M and Curtis AS. The influence of microscale topography on fibroblast attachment and motility. *Biomaterials*. 2004; 25(26):5781-8. PMid:15147824. http://dx.doi.org/10.1016/j.biomaterials.2004.01.029
35. Luciano RM, Zavaglia CA, Duek EA and Alberto-Rincon MC. Synthesis and characterization of poly(L-lactic acid) membranes: studies in vivo and in vitro. *Journal of Materials Science*: Materials in Medicine. 2003; 14(1):87-94. PMid:15348543. http://dx.doi.org/10.1023/A:1021509722296
36. Eisenbarth E, Linez P, Biehl V, Velten D, Breme J and Hildebrand HF. Cell orientation and cytoskeleton organisation on ground titanium surfaces. *Biomolecular Engineering*. 2002; 19(2-6):233-7.
37. Ignatius AA and Claes LE. In vitro biocompatibility of bioresorbable polymers: poly (L, DL-lactide) and poly(L-lactide-co-glycolide). *Biomaterials*. 1996; 17(8): 831-839. http://dx.doi.org/10.1016/0142-9612(96)81421-9
38. Ishaug-Riley SL, Crane GM, Gurlek A, Miller MJ, Yasko AW, Yaszemski MJ et al. Ectopic bone formation by marrow stromal osteoblast transplantation using poly (DL-lactic-co-glycolic acid) foams implanted into the rat mesentery. *Journal of Biomedical Materials Research*. 1997; 36(1):1-8. http://dx.doi.org/10.1002/(SICI)1097-4636(199707)36:1<1::AID-JBM1>3.0.CO;2-P