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Remote and Direct Plasma Processing of Cells: How to Induce a Desired Behavior

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ABSTRACT: The interplay between plasma processes and the biological environment is a long and intriguing story that spans different applications, from surface modification of biomaterials to the direct interaction of plasma with cells. This makes plasma processes very powerful tools in such biomedical fields as tissue engineering and sterilization, which are much different than the typical field in which plasmas are used. *In vitro* cell culture experiments represent the best way to fully understand the more subtle and fundamental interactions between the chemical species produced by glow discharge and cells. Among the different kind of cells that can be used, cell lines allow high reproducibility and control of results. This article reviews 3 main items, ranging from low-pressure plasma modifications of 2- and 3-dimensional materials to dielectric barrier discharges directly on cells, with respect to the authors' scientific work.

I. INTRODUCTION

The *in vitro* study of cell behavior toward different material surface properties represents a necessary prerequisite in assessing the biocompatibility of materials intended to be used as medical devices.[1] To rapidly investigate the interaction between various cell types and these materials by means of *in vitro* experiments, the surface properties of a material can be tuned by creating well-defined topographical and chemical patterns on the surface. Because of their versatility in tailoring surface properties of materials in different applications, cold plasma processes are used to dictate the interactions of proteins, cells, and biological tissues with biomaterials, membranes, and biomedical devices to induce desired cell responses.[2,3]

Tailoring surface properties is of the utmost importance for both improving the performance of medical devices and studying cell-material interactions. This article describes different uses of plasma processes to induce different desired cell behaviors; processes span from low-pressure plasma modifications of 2-dimensional and 3-dimensional (3D) materials to dielectric barrier discharges (DBDs) directly on cells.

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Regarding material surface roughness, it has been widely demonstrated that microroughness contributes to cell attachment, spreading, and differentiation.[4] Low-pressure plasma processes fed with fluorocarbon gases, especially when they are carried out using a modulated power regime or when the samples are placed in the afterglow region, can deposit Teflon-like rough coatings. Such coatings have been characterized by different topographical features such as ribbons, bumps, or others[5–7] and were able to successfully tune the cell response.[8,9]

This article also provides 2 examples of how the surface chemistry and morphology of materials can be tuned by means of plasma processes. When the interactions of cells with different surfaces are investigated, it has to be considered that cells do not live in a flat environment; in fact they interact with other cells or with the extracellular matrix in a tridimensional world. Moreover the use of tridimensional substrates is of major importance in innovative fields like tissue engineering and regenerative medicine. In fact, although modern techniques for the transplantation of tissues and organs proved to be revolutionary and lifesaving, the major problem is the lack of enough tissues and organs for all patients who need them. Indeed, the efforts in modern scientific fields, such as biomaterials engineering, genetics, and stem cell biology, together with the engineering of 3D scaffolds, may ultimately develop into the treatment of human diseases.[10]

3D scaffolds may represent a new 3D cell culture model that allows cells to grow and adapt to their environment in a manner closer to that experienced by their native counterparts. It has been shown that materials used for the fabrication of 3D scaffolds do not appropriately interact with the biological environment. Thus the biocompatibility of such materials could be improved by modifying their surface properties, and plasma processes can reclaim their role in this task.

Apart from increasing the properties of cell adhesion to the material, the principal aim of surface modification of 3D substrates is to allow cell colonization of the whole substrate; greater cell colonization at the scaffold's periphery and inadequate colonization at its core are usually reported.[11] In this article we show an example of how plasma processes can help overcome this problem.

Modification of the surface of materials using plasma can be considered an indirect use of plasma in the biomedical field. A more "direct" approach focuses on plasma technology in the treatment of living cells, tissue, and organs.[12–14] In the past decade plasma processes have shown a very important role in directly modifying biological tissues. In fact, the direct exposure of living tissues to atmospheric pressure air plasmas have started to be used for therapeutic uses, for example, sterilization and decontamination of wounds and wound healing.[15–19] These different studies merged into the new innovative field of plasma medicine. According to the importance that these studies assume, the final contribution of this overview of the different roles that plasma processes have in the biomedical field concerns an *in vitro* study of the direct effect of atmospheric DBDs on different cell lines.

II. MATERIALS AND METHODS

A. Materials and Plasma Processes

1. Plasma Deposition of PEO-Like Coatings from Diethylene Glycol Dimethyl Ether

Crystalline native polystyrene (PS) substrate discs (12-mm diameter, 1.2-mm thickness; Goodfellow Corp., Coraopolis, PA) were used as substrates for depositing plasma electrolytic oxidation [Polyethilen Oxyde (PEO)]–like flat coatings. An asymmetric stainless steel reactor was used, with 2 vertical, parallel-plate, stainless-steel electrodes 6 cm apart.[20] The smaller electrode (8-cm diameter) was connected to a radiofrequency (13.56 MHz) power supply through a matching network unit, whereas the largest electrode (18-cm diameter) was grounded and used as sample holder. A root pump was used; the pressure, maintained at 400 mTorr, was monitored by a Baratron manometer (MKS Instruments, Andover, MA). Glow discharges, fed by a mixture of 0.4 sccm diethylene glycol dimethyl ether (DEGDME, CH3O(CH2CH2O)2CH3; Sigma-Aldrich, St. Louis, MO) vapors and 5 sccm argon, were used to deposit PEO-like coatings at different (5, 10, and 15 W) radiofrequency power values. In this article, PEO-like coatings are indicated with the corresponding power value used for the deposition (e.g., PEO5W are coatings deposited at 5 W). The discharge was performed for 1 hour to deposit PEO5W coatings and 30 minutes for PEO10W and PEO15W coatings. PEO5W and PEO10W coatings also were deposited on polycaprolactone (PCL) 3D scaffolds in the same experimental conditions used for the flat samples.

2. Plasma Deposition of Rough Coatings from Hexafluoroacetone

Polyethylene terephthalate (PET) substrates (Goodfellow Corp.) were coated by deposition of a fluorocarbon precursor. A parallel plate plasma reactor was used, as described in a previous article.[21] First, PET samples were coated with films by glow discharges fed with hexafluoroacetone, at the following experimental conditions: hexafluoroacetone (40 sccm flow rate; 50 W power; 900 mTorr (0.120 kPa) pressure; 120 min deposition time; Fluorochem, Hadfield, UK). Samples were positioned in the reactor chamber at 8 and 18 cm from the gas inlet, in the afterglow region. Then, these morphologically different films and flat native PET substrates were further coated with a fluorocarbon coating from a discharge fed with tetrafluoroethylene (Fluorochem) at the following conditions: 6 sccm flow rate; 100 W power; 200 mTorr (0.027 kPa) pressure; 21 second deposition time.

3. Solvent Casting/Particulate Leaching to Produce PCL Scaffolds

PCL (molecular weight, 65 kDa) scaffolds were produced with the solvent casting/ particulate leaching technique, dissolving PCL in chloroform (20/80 wt/wt), with sodium

chloride (150–300 μm in size) as a porogen (5/95 wt/wt PCL/sodium chloride). Teflon molds were used to cast the polymer solution. The scaffolds then were dipped in ethanol to extract the solvent. The leaching process was performed in distilled water.

4. DBDs on Cell Lines

The plasma source consists of a DBD reactor, shown in Fig. 1, where a 6-cm-diameter Petri dish was used as the dielectric on the grounded lower electrode. A quartz disc was used as the dielectric on the top electrode.

All the discharges were operated at a peak-to-peak voltage value of 25 kV. Pulsed discharges were ignited in air, with cells positioned on the bottom of Petri dishes positioned on the ground electrode. A handle grip on the top of the reactor allowed the location of the Petri dish on the bottom electrode to be set 2 mm from the top one. Discharges were operated with a sinusoidal voltage of about 27.37 kHz and were fully modulated with a rectangular pulse (t_{ON} = 0.1 second; t_{OFF} = 1.9 seconds). Finite numbers of pulses, namely 1, 3, 9, and 27, were used.

B. Samples Surface Characterization

1. Chemical Characterization by X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) analysis was performed with a Theta Probe Thermo VG Scientific instrument (base pressure of 1×10^{-1} mbar), equipped with a hemispherical analyzer and a monochromatic Al Kα (hν, 1486.6 eV) X-ray source operating at 300 W. Photoelectrons were collected at a take-off angle of 53° (sampling depth, ~10 nm). Thermo Avantage 3.28 software (Thermo Electron Corporation, Madison, WI) was used either to determine the elemental composition from peak areas or to peak fit the high-resolution spectra.

2. Morphological Characterization by Scanning Electron and Atomic Force Microscopy

A Stereoscan 360 Cambridge scanning electron microscope (SEM), operating at 20 KV with a 50° tilt angle, was used to examine the surface morphology of the coatings and evaluate its distribution on the whole sample surface. Since samples were nonconductive, they were sputter-coated with a 10-nm-thick gold layer with a BioRad Polaron Division SEM Coating System with an E5100 Sputter Coater.

Height mode atomic force microscopy (AFM) investigations were performed in air at room temperature by means of a PSIA XE-100 SPM system operating in tapping mode. A silicon shock pulse method sensor for noncontact AFM (NanoWorld) was used, with a constant force of 42 N m^{-1} and a resonance frequency of 320 KHz. Topographic micrographs were collected from 6 areas per sample, with a scan area of 10×10 μm^2, by sampling the surface at a scan rate of 0.8 Hz and a resolution of 256×256 pixels. AFM

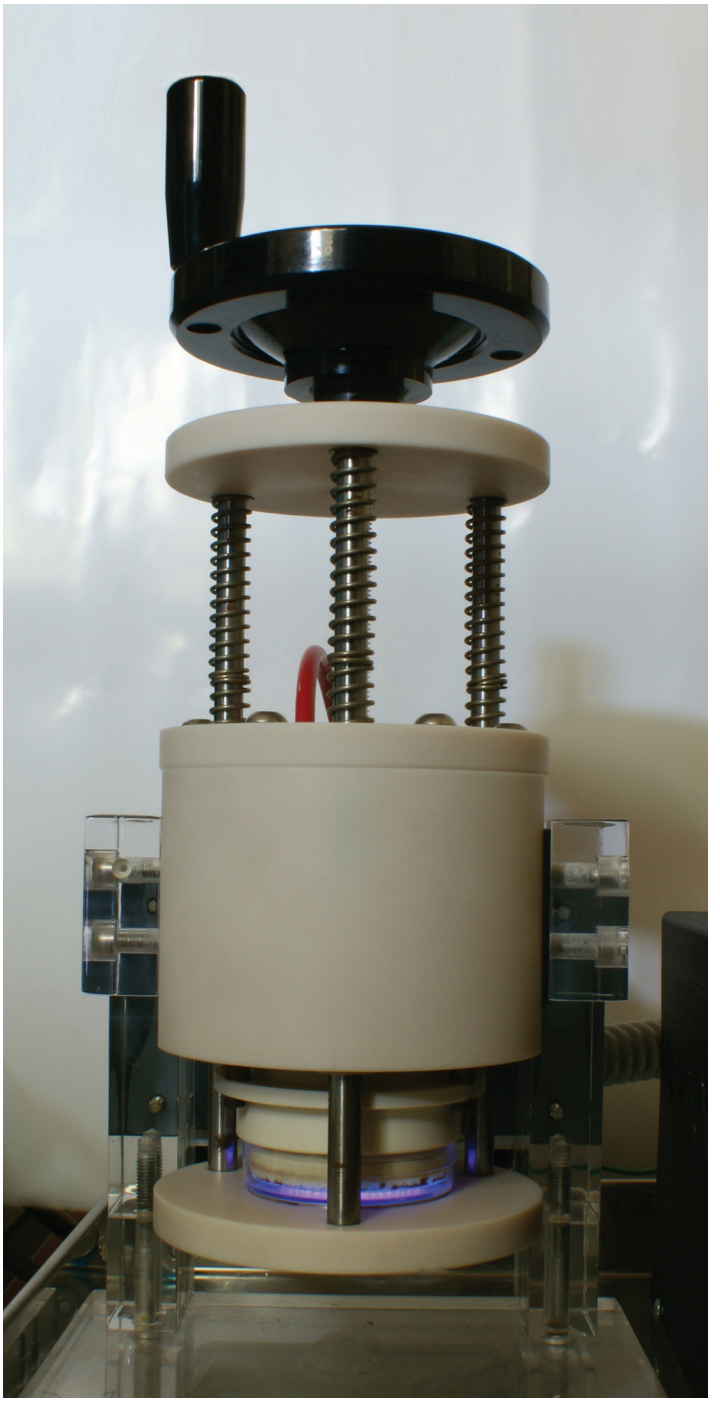


FIG. 1: A dielectric barrier discharge reactor used to directly treat cells seeded on a Petri dish.

images were processed using an XEI Program to flatten the topographic micrographs, to remove the slope and curvature artifacts produced by the scanning process, and to obtain statistical data as surface root-mean-squared roughness and mean height of sample features. The XEI software also was used to represent a 3D perspective of the sample surface with the original resolution of 256×256 pixels and to extract histograms showing the distribution of feature heights.

C. Cell Culture and Cyto-Compatibility Tests

We used 3 different cell types: 3T3 fibroblasts (ICLC, Genova, Italy), the osteoblast Saos-2 cell line (ICLC), and normal human dermal fibroblasts (NHDFs; Promocell, Heidelberg, Germany). Cells were routinely grown in Dulbecco's modified Eagle's medium (Sigma Aldrich, Milan,Italy); supplemented with 10% heat-inactivated fetal bovine serum, 50 IU/mL penicillin, 50 IU/mL streptomycin, and 200 mM glutamine; and maintained at 37°C in a saturated humid atmosphere containing 95% air and 5% carbon dioxide in 75-cm^2 flasks (Barloworld Scientific, Stone, UK). For cell culture experiments, cells were detached with a trypsin/EDTA solution (Sigma), resuspended in the appropriate medium, and seeded at the appropriate density on the different substrates according to the experimental protocols.

1. Analysis of Cell Proliferation by MTT Assay

Cell proliferation was studied after different time lapses with the MTT colorimetric assay based on the mitochondrial activity of cells. This test detects the conversion of 3-(4,5 dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, MO) to formazan. At each time point, the cells were incubated in a tenth of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide medium (5% carbon dioxide atmosphere at 37 °C for 3 hours) to allow the formation of formazan crystals. They then were dissolved in 10% Triton X-100 with acidic isopropanol (containing 0.1 N hydrogen chloride). Finally, the optical density was measured with a spectrophotometer (Jenway 6505; Keison International Ltd., Chelmsford, UK) at a wavelength of 570 nm, using 690 nm as the reference wavelength.

2. Analysis of Cell Adhesion and Proliferation by Coomassie Blue Staining

Cells seeded on the substrates and analyzed at different cell culture times were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) solution for 15 minutes and stained with a dye solution composed of 0.2% Coomassie Brilliant Blue R250 (Sigma, Italy), 50% methanol, and 10% acetic acid for 3 minutes. Cells on the samples were observed at different magnifications by means of a phase contrast microscope (Leica DM IL, Leica Microsystems, Buffalo Grove, IL). At least 15 images of a 3-mm^2 area per sample were acquired using a charge-coupled device camera (Leica DC100; Leica Microsystems). Images then were analyzed with ImageJ software (National Institutes of

Health, Bethesda, MD; available at: http://rsb.info.nih.gov/ij/) to evaluate the substrate area covered by cells.

3. Analysis of Cells' Cytoskeleton

To observe the actin cytoskeleton, cells were fixed in 4% formaldehyde/PBS solution at room temperature for 20 minutes and, permeated with PBS containing 0.1% Triton X-100, and incubated with Alexa Fluor488 phalloidin (Molecular Probes; Life Technologies) at room temperature for 20–30 minutes. After rinsing, samples were mounted in Vectorshield fluorescent mountant with DAPI (Vector Laboratories, Peterborough, UK) and then observed by means of an epifluorescence microscope (Axiomat; Carl Zeiss, Jena, Germany).

4. Morphological Characterization of Cells by SEM

For a SEM observation of cell structure and distribution on the samples, the cells were fixed with a 2.5% glutaraldehyde/0.1 M sodium cacodylate solution and dehydrated using ethanol/water solutions (20%, 40%, 50%, 70%, 90%, and 100%). Finally, cell cultured samples were air-dried under a biological hood and sputter-coated with a 10-nm-thick gold layer for visualization using SEM.

D. Statistical Analysis

Each experiment was performed at least in triplicate; final data are presented as the mean of at least 3 values + standard deviation. Statistical analysis was performed using a 2-way analysis of variance test within groups, followed by a Bonferroni post test, in Prism software version 4.00 for Windows (GraphPad Software, San Diego, CA; available at: www.graphpad.com). Differences were considered statistically significant at $P < 0.01$.

III. RESULTS AND DISCUSSION

A. Low-Pressure Plasma Modifications of Bidimensional Surfaces to Tune Cell Reaction to Surfaces with Different Chemistry or Topography

PEO-like coatings were deposited by DEGDME to modulate the input power, and surfaces with different wettability were obtained (data not shown); this effect was dependent on the retention of the so-called PEO character, that is, the density of the etilene oxide (-CH2CH2O-) moieties in the coating[22] (Fig. 2).

Once surfaces with different chemistries were obtained, we investigated whether those differences were able to induce different cell responses. The results of the growth of 3T3 fibroblasts on the 3 different PEO-like surfaces for different periods of time are represented in Fig. 3. As this graph shows, surfaces with 40% and 60% of PEO character

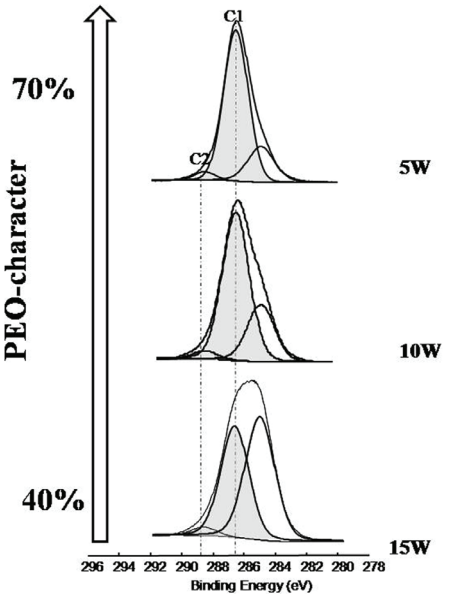


FIG. 2: C1s X-ray photoelectron spectroscopy spectra of plasma electrolytic oxidation (PEO)–like films deposited at different power values. In grey is the C1 component of the best fit, attesting to the PEO characteristic.

allow a different degree of cell adhesion, whereas very few cells adhere to a surface characterized by more than 70% of PEO character.

The differences in cell adhesion are strikingly important after 96 hours of culture. It is hard to find any cells on PEO5W coatings, even after 96 hours, which confirmed their good cell-repulsive, nonfouling property. Another striking effect of the different chemistries on cell behavior is the degree of their spreading on the different surfaces, as shown in the SEM images in Fig. 4.

Since spreading indicates a good affinity of fibroblasts for the substrate, we can see that the PEO15W coating provides surfaces with a good cell affinity. The difference in cell adhesion and spreading between PEO10W and PEO15W coatings can be attributed to the different PEO character of the 2 surfaces.

Cell spreading can be considered a sign of good affinity between the cell and substrate. Nevertheless, it has been observed that, for certain cells, spreading represents a sign of de-differentiation, that is, a loss of peculiar characteristics of a particular cell type. For this reason it is important to tune the chemical composition of the adhesive substrate to obtain a range of cell behaviors (adhesion, spreading, and differentiation) for different cell types. For example, for fibroblasts, spreading is necessary for cell division since fibroblasts that cannot adhere and spread on the surface can become apoptotic.[23] On the other hand, when hepatocytes are grown on a highly dense matrix of fibronectin or collagen, 2 cell adhesion proteins of the

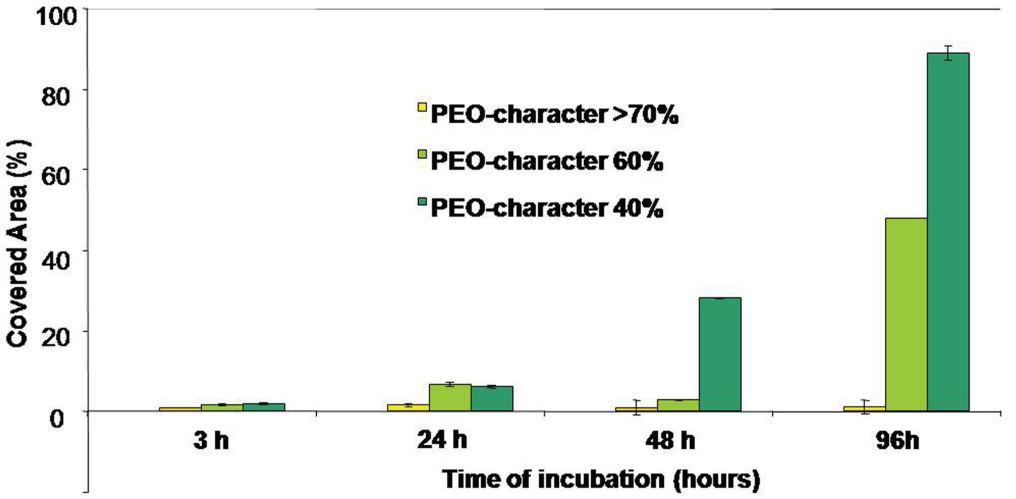


FIG. 3: Percentage of substrate area covered by 3T3 cells grown on different plasma electrolytic oxidation (PEO)–like surfaces for 3, 24, 48, and 96 hours. Data are expressed as means ± standard deviations of data calculated from at least 20 optical images.

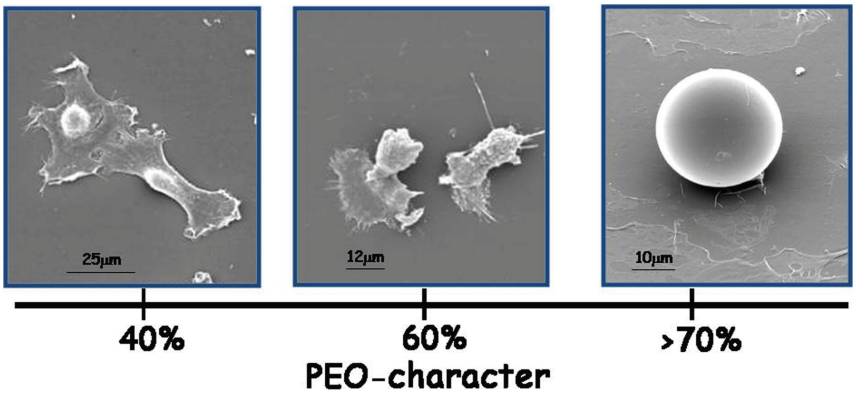


FIG. 4: Scanning electron microscopy images of fibroblasts grown on plasma electrolytic oxidation (PEO)–like coatings with different PEO characteristics. (Adapted from Sardella *et al*.[22])

extracellular matrix, they display a well-spread morphology associated with growth and de-differentiation.[24]

Can this difference in cell behavior also be found for cells facing substrates with different roughness? In our laboratory, Teflon-like micro-/nanostructured coatings have been deposited from radiofrequency glow discharges fed with different fluorocarbon gases in continuous and modulated discharges. By varying the fed gas, coatings with

different chemical and morphological nano- and microfeatures (ribbon-like, desert rose–like, domes) were obtained.[25]

For cell culture experiments, particular care was devoted to obtaining surfaces with different roughness but with the same chemical composition to disentangle the morphological effect on cell adhesion and growth from the chemical one. Toward this aim, plasma technology helps us with the production of a thin, conformal, homogeneous film that can be applied to any geometrical surface to impart a different chemical composition.

Fig. 5 shows how films with different chemical and morphological structures were obtained by varying the sample position in the plasma reactor with respect to the gas inlet. The fluorinated structure that is shown was obtained by means of deposition discharges fed with hexafluoropropylene oxide.

The topography of the obtained substrates is very different, depending on the position of samples downstream with respect to the plasma zone, with flat morphology on samples placed in the glow zone, domes on samples in the afterglow zone 8 cm from the gas inlet, and very rough surfaces with a petal-like morphology on samples at 18 cm in the afterglow zone. We refer to them as G1, AG8, and AG18, respectively. AFM

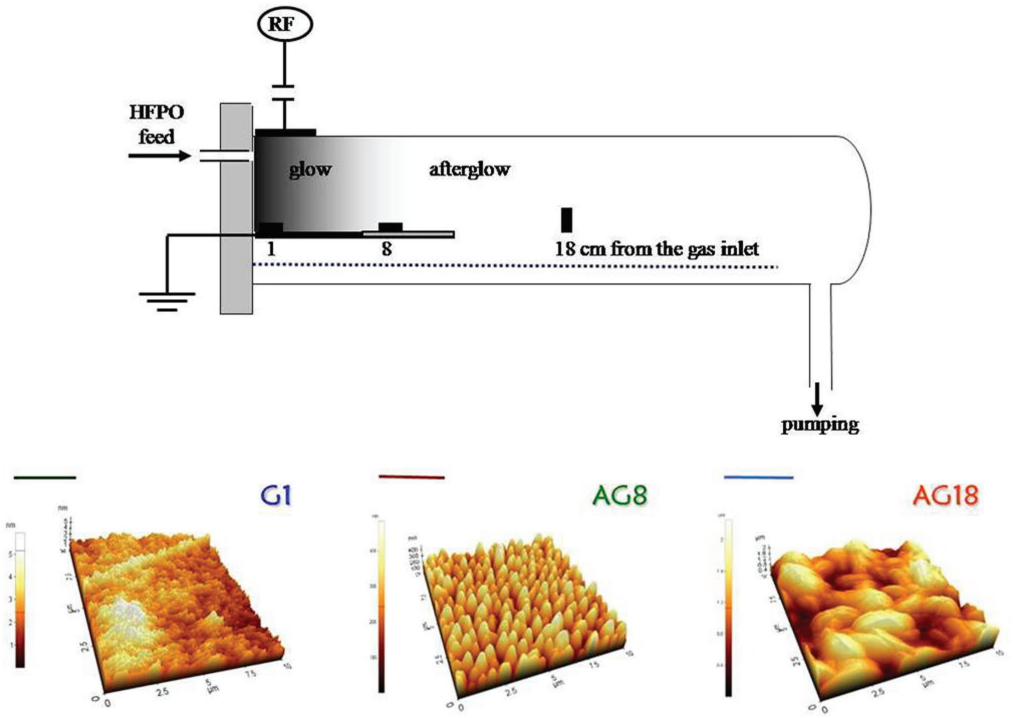


FIG. 5: Schematic representation of the plasma reactor showing sample positions along the reactor axis (top) and atomic force microscopy pictures of the obtained surfaces (bottom).

clearly showed that roughness changes the different surfaces and, as a consequence, also changes the surfaces' wettability.[21]

As stated before, for cell culture experiments, particular care has been devoted in obtaining surfaces with different roughness but with the same chemical composition. XPS data show that we obtained 3 types of surfaces with different morphologies but the same chemistry by means of a conformal homogeneous plasma coating obtained with tetraflurorethylene.[26]

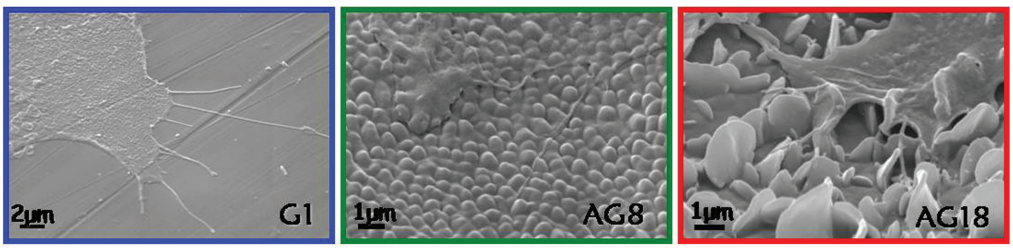
From SEM analysis we can observe that cells show different interactions with the single micro/nano surface features: On G1 substrates there is good cell adhesion through long and thin filopodia. Otherwise, on AG8 and AG18 rough substrates, cells mainly interact with the structures by lamellipodia. We found that filopodia facing AG8 substrates are longer than those interacting with the petal-like features on AG18 samples.

Furthermore, many cells had filopodia in contact with the surface structures, and their length appears to be correlated to the dimension of the micro-/nanofeatures. In Fig. 6, the profile of the tall petal-like structures below the cell body could be observed. Thus, cells attach to the AG18 substrate, even if very rough, by anchoring with thin lamellipodia and short filopodia.

This SEM study clearly demonstrated that cells interacted directly with the micro-/nanofeatures, confirming the strong dependence of the cell behavior on surface topography. Since all surfaces were produced with the same surface chemical composition, as previously reported, the observed differences in cell response are supposed to be exclusively dependent on micro-/nanoscale roughness.

B. Low-Pressure Plasma Modifications of Tridimensional Scaffolds

In vivo, cells do not live in a bidimensional world; rather, they interact with other cells or with the extracellular matrix in a tridimensional world. To analyze this interaction *in vitro*, we need a 3D environment, which can be achieved through the use of 3D scaffolds.



(A) (B) (C)

FIG. 6: High-magnification scanning electron microscopy images of Saos-2 cells cultured for 24 hours on flat **(A)**, AG8 **(B)**, and AG18 surfaces **(C)** after the C2F4 deposition to show the cell-material interactions and characteristics of each kind of surface: long cell filopodia are seen on the flat surface, lamellipodia are seen on the AG8 surface, and short filopodia and lamellipodia are seen on the AG18 surface.

In our laboratory we use the technique of solvent casting/particulate leaching to produce PCL scaffolds for *in vitro* cell culture experiments.[27] Sodium chloride crystals in the range of 150–300 μm are used as a porogen. Most of our *in vitro* experiments have been performed by means of scaffolds with a diameter of 10 mm and a height of about 4 mm.

Based on the fluorescence images of cells' actin in Fig. 7, it is clear that when we seed cells on a PCL scaffold there is a gradient of cell density from the top to the bottom of the scaffold, with poor colonization inside. So any modification of 3D scaffolds aims to achieve better cell colonization within the scaffold core. Our strategy (Fig. 8) consisted of the use of a PEO-like coating obtained at 5W and 10W in the same conditions used for coating flat samples and was able to impart different cell adhesive properties to the material surface.

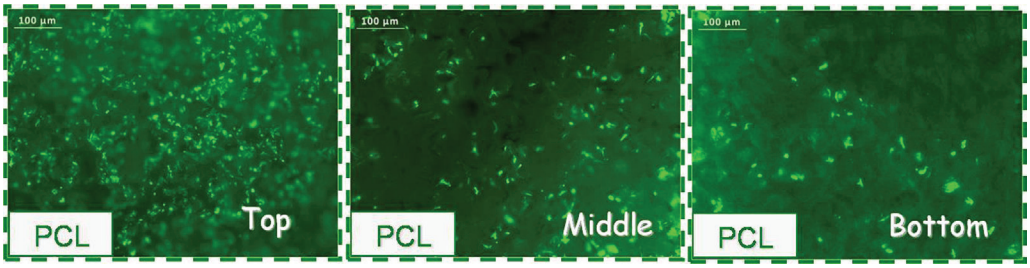


FIG. 7: Fluorescence microscopy images of Saos-2 cells cultured for 120 hours on untreated polycaprolactone (PCL) scaffolds. Saos2 cells were fixed in 4% formaldehyde/phosphate-buffered saline solution and incubated with Alexa Fluor 488 phalloidin, allowing the observation of the cell actin (green). Scaffold were cut and images of the top, middle, and bottom of the sample were taken.

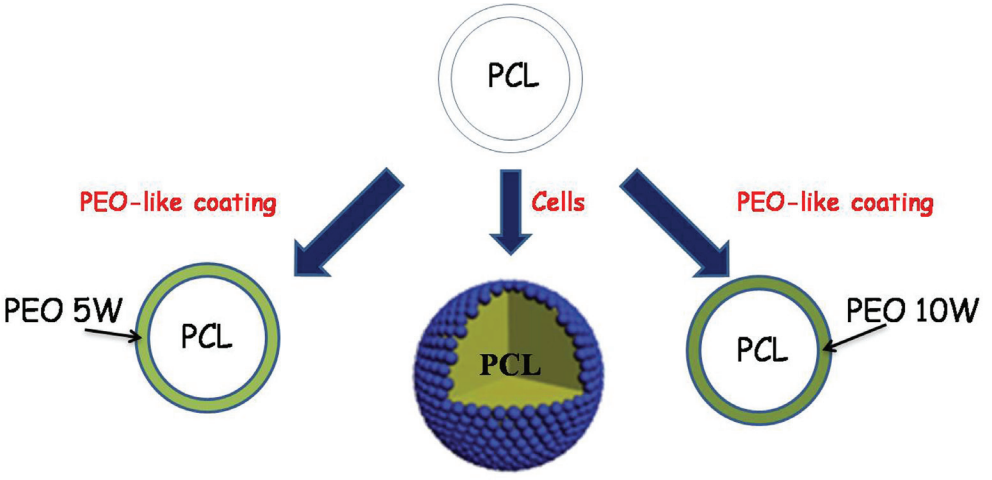


FIG. 8: Scheme of the strategy adopted to increase cell colonization within the scaffold core by means of plasma electrolytic oxidation (PEO)–like sheaths. PCL, polycaprolactone.

The aim was to obtain a scaffold with either a PEO5W or a PEO10W sheath to direct cell adhesion toward the scaffold core. XPS analysis clearly revealed that on both kind of scaffolds only the periphery was coated with PEO-like surfaces.[28] In fact, surface chemistry of different slices within the scaffold was found to be different, with a PEO5W coating that penetrated only for few microns and a PEO10W coating that penetrated through the first 200 μm of the scaffold.

To analyze how cells colonized the different types of scaffolds, they were stained by means of phalloidin conjugated to AlexaFluor 488 to observe the actin cytoskeleton. Pictures of both the surface and internal slices of scaffolds were acquired and analysis using ImageJ software was used to quantify the surface area covered by cells.

Indeed, when we look at cell distribution on the 3 different typologies of scaffolds, we can see how the best internal cell colonization was achieved by means of the scaffold with the PEO10W shield. In particular, after 5 days of cell culture, scaffolds coated with PEO10W had the highest cell colonization both on the top and in the core of the scaffold with respect to native PCL and PEO5W-coated samples (Fig. 9).

C. DBDs Directly on Cells

In the different example of plasma modification of materials shown before, low-pressure plasma sources were used. Several authors recently reported the use of atmospheric plasma for the modification of biomaterial surfaces.[29,30] Atmospheric plasma is also the pro-

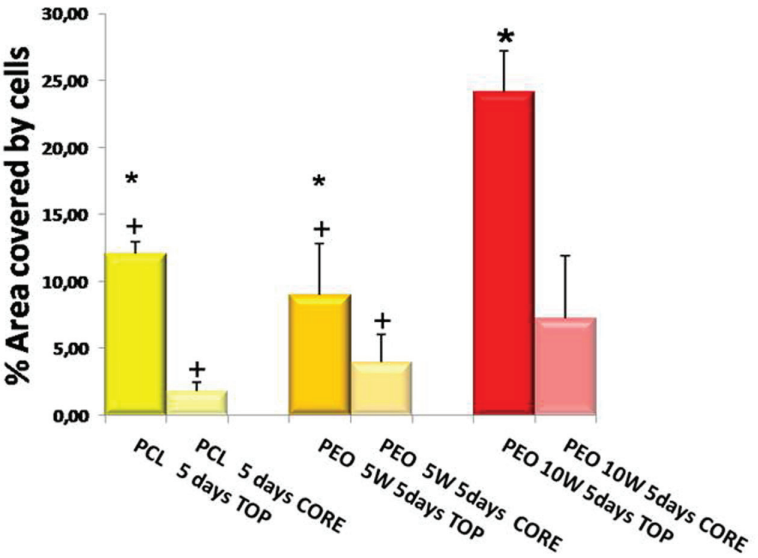


FIG. 9: The mean percentage of area covered by Saos-2 osteoblast cells on the top surface and the core of the scaffolds after 5 days of cell culture using 2-way analysis of variance and Bonferroni post test *$P < 0.05$ vs. CORE; +$P < 0.05$ vs. PEO10W. PCL, polycaprolactone; PEO, plasma electrolytic oxidation.

tagonist of the very innovative field of plasma medicine.[13,14] In this field the "material" is represented by living matter that spans from cells to bacteria to tissues. In our laboratory in the past few years we performed *in vitro* preliminary experiments aimed to observe how different kinds of eukaryotic cells react directly to the action of plasma discharges.

Air DBDs in pulsed mode have been directly performed toward different cell lines to study how the effect of different "doses" of atmospheric air plasma can influence their behavior. For this purpose a very simple DBD reactor was assembled (Fig. 1), and the effect of plasma on different cell lines was studied.[31] Discharges had a sinusoidal voltage of about 27.[37] kHz and were fully modulated with a rectangular pulse (t_{ON} = 0.1 second; t_{OFF} = 1.9 seconds). A finite number of pulses, namely 1, 3, 9, and 27, was used. The discharge parameters are summarized in Table 1.

The same process was applied to different cell lines derived from tumors, Saos-2 and MG63 osteoblasts, HepG2 hepatocytes, and a primary fibroblast cell line (NHDFs). For cell culture experiments, cells were resuspended in the appropriate medium at a concentration of 5×10^4 cells/mL, and 1 mL was seeded on Petri dishes. After at least 20 hours of growth, adhered cells were exposed to different DBD air plasma pulses. Before the exposure to plasma at different pulses, the culture medium was aspirated from the dishes under a biological hood, so cells were plasma-processed without the medium. The medium was added again after exposure to the plasma. To exclude the influence of medium deprivation on cell proliferation, control samples were deprived of medium for the maximum time of plasma exposure (i.e., 54 seconds = 2 seconds × 27 pulses) and showed no influence on cell proliferation.

The proliferation of plasma-treated cells was compared with that of control (unexposed) cells 24 and 72 hours after plasma exposure by means of the MTT proliferation essay. The results obtained by the MTT proliferation assay after 24 and 72 hours of cell growth after plasma exposure for the 3 different cell types derived from tumors clearly showed that 9 and, in particular, 27 pulses were able to decrease cell adhesion and growth. Figure 10 shows a graph of the MTT data of Saos-2 cells exposed to different pulses.

TABLE 1. Experimental Conditions of Atmospheric Plasma Treatments

Parameters	Values
Frequency	27.37 Hz
Voltage, peak to peak	25 kV
Power	120 W (30W/cm^3)
Duty cycle	5%
T_{ON}	0.1 s
T_{OFF}	1.9s
Energy/pulse	12 J
Number of pulses	3, 9, and 27

A different and very interesting result was observed when the primary fibroblast cell line was used for our experiments. In fact, an increase in cell proliferation was found for cells exposed to 3 and 9 pulses of plasma. Moreover, if a decrease in cell adhesion was present for cells exposed to 27 pulses, the effect did not impair their growth during the 72-hour time lapse. This last result characterizes most of the experiments on the primary fibroblast cell line, while the increase in proliferation strictly depends on the cell line lot. Fig. 10 compares the different MTT results obtained for the Saos-2 cells and the NHDFs.

The obtained results clearly show that a different cell response to the same dose of plasma is strictly related to the cell type. Recent studies have clearly shown that reactive oxygen species and the closely related reactive nitrogen species often are generated in such types of discharge.[32,33] Future studies will be necessary to analyze why the different behavior observed in tumor-derived cells and primary fibroblasts can be attributed to reactive species produced in the plasma discharge.

IV. CONCLUSIONS

A brief journey through the use of plasma discharge both at low and atmospheric pressure to induce desired cell behavior has been undertaken through the use of *in vitro* cell culture experiments, showing the ability of plasma-modified bidimensional surfaces to tune cell adhesion and morphology and how our strategy, with an "unfouling" sheath, was successful in inducing cell colonization within tridimensional scaffolds. Finally, the same DBD was able to induce different behaviors in different types of cells. The results obtained for cell lines derived from tumors suggest that this type of discharge can be useful in cancer treatment. Otherwise, the observed effect on the primary fibroblast cell lines can be of help in the fields of wound healing and tissue regeneration. This overview clearly demonstrates the deep and wide penetration of plasma technologies in life sci-

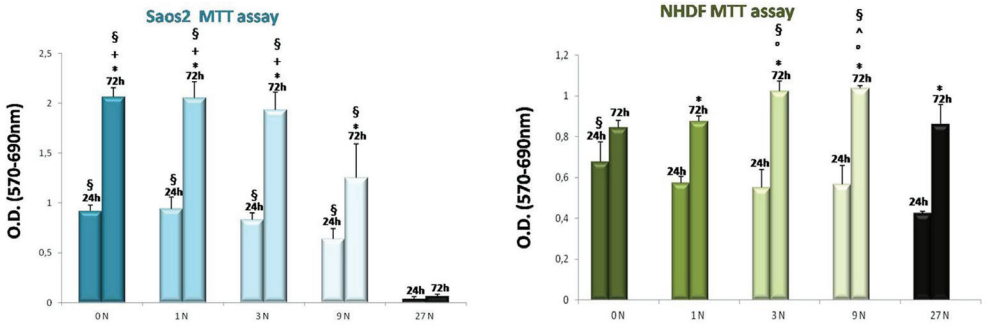


FIG. 10: MTT results (optical density [O.D.]) for Saos2 cells (left) and normal human dermal fibroblasts (right) processed with 3, 9, and 27 pulses of plasma and grown for 24 and 72 hours after the dielectric barrier discharges. 0N indicates control cells not processed with plasma. Two-way analysis of variance and Bonferroni post test indicate **P* < 0.01 vs. 24 hours; °*P* < 0.01 vs. 0N; ^*P* < 0.01 vs. 1N; +*P* < 0.01 vs. 9N; and §*P* < 0.01 vs. 27N.

ence to resolve both technological problems, helping us to understand the basic interactions between the biological environment and the surface of materials.

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