Plasma processing of scaffolds: perspectives for Tissue Engineering

F. Intranuovo[1], R. Gristina[2], M. Domingos[3], G. Camporeale[1], E. Sardella[2], P.J. Bartolo[3], P. Favia[1,2,4]

[1]*Department of Chemistry, University of Bari "Aldo Moro", Bari, Italy*
[2]*Institute of Inorganic Methodologies and Plasma (IMIP) CNR, Bari, Italy*
[3] *Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, 2430-028 Marinha Grande, Portugal*
[4]*Plasma Solution Srl, Spin off of the University of Bari, Italy*

intranuovo@chimica.uniba.it

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Since the mid-1980s, Tissue Engineering has been proposed as a potential tool in addressing the repair, replacement, and/or regeneration of vital organs. Thus this field is now an interesting alternative to artificial prosthesis and confronts the transplantation crisis, i.e., the shortage of donor tissues and organs available for transplantation[1].

This new emerging field is based on the use of cells (particularly stem cells) seeded in a three-dimensional (3D) scaffold (most often made og a biodegradable polymer), that provides the initial structural integrity and organizational backbone for cells to assemble into a functional tissue[2]. Thus, scaffolds, characterized by high porosity, proper pore size, shape, surface area, suitable biodegradability and biocompatibility, stiffness and mechanical integrity, are required. These 3D structures have to allow the necessary support for cell attachment, proliferation, differentiation, leading to a correct tissue regeneration in advanced applications.

Nowadays, several fabrication techniques have been employed to realize scaffolds with suitable biocompatibility[3-5]. For instance, Solvent Casting/Particulate Leaching (SC/PL) is the most easy, low time-consuming and cheap conventional scaffolding technique, used by material engineers. It allows the control of micro-structural characteristics such as total porosity and total pore size[6]. Another known conventional technique includes the supercritical carbon dioxide ($scCO_2$) as porogen, that is advantageous because it does not require organic solvents and leaching process to eliminate the porogen[7]. Also, with this technique it is possible to control the total porosity and pore size, even if the pores are randomly distributed inside the scaffold.

Unlike conventional machining, computer-aided technologies, like Solid Free-Form fabrication (SFF) and Rapid Prototyping (RP), are able to build scaffolds by selectively adding materials, layer-by-layer, as specified by a computer program, creating a highly reproducible architecture along the 3D construct[8].

Surface properties influence cell-material interactions in the tissue engineered structures. Generally, when cells are seeded *in vitro* into 3D scaffolds, cell adhesion is favoured at the peripheries of the constructs, resulting in poorly populated inner parts, because the external surfaces of the scaffolds are more accessible than the inner ones. Thus, by controlling scaffold surface chemistry by some surface modification techniques, it should be possible to control adsorption of proteins from the cell culture medium and, in turn, to enhance cell

adhesion and motility, inside/outside the scaffolds, in a homogeneous way[9].

Cold plasma processes at low and atmospheric pressure can be used to tailor the surface composition of scaffolds . Recent advances in radiofrequency plasma processes (RF, 13.56 MHz) Glow Discharges for biomedical applications, include the achievement of functional surfaces for direct cell growth and biomolecules immobilization, the deposition of non-fouling coatings, the deposition of nano-composite bacterial resistant coatings and the synthesis of nano-structured surfaces[10,11]. Even though many approaches for the modification of surface chemistry of polymers have been described, it remains an interesting challenge to understand their efficiency and penetration when applied to complex 3D structures.

Recently, we have described low pressure plasma modification processes applied to poly-(lactic acid, PLA) scaffolds, fabricated with a conventional scCO_2 technique, using allyl amine/hexane plasma depositions. This technique is based on the generation of gas bubbles within a polymer and the use of supercritical CO_2 as porogen, through a three phases cycle (plasticization, nucleation and vitrification). The low pressure plasma depositions were able to create chemical gradients (hydrophobic outside and hydrophilic inside the scaffolds) attracting cells inside the polymeric structures[12].

A low pressure O_2/H_2 plasma treatment has been applied to polycaprolactone (PCL) scaffolds, produced by means of SC/PL technique. This scaffolding technique consists of dispersing a porogen (NaCl crystals at proper diameter range and NaCl/polymer composition ratio) within a polymeric solution, fixing the structure and removal of the porogen, to result in a porous scaffold. We observed that by varying the plasma parameters (e.g. pressure, power, gas feed composition), the hydrophilicity of the scaffolds varied in a controlled way, obtaining different water absorption kinetics, that could address the behaviour of different cell lines[13].

Further, ethylene/N_2 mixtures were used to plasma deposit cell-adhesive coatings to increase the affinity of osteoblast-like cells of PCL scaffolds produced with the same SC/PL technique[14]. Good nitrogen content penetration was achieved inside the porous constructs, with a good adhesion of osteblast-like cells.

The same low pressure plasma deposition has been applied to PCL scaffolds fabricated with a Fused Deposition Modeling (FDM) technique, by means of the BioCell Printing instrument[8]. This technique is based on the extrusion of a thermoplastic material through a nozzle, where the filament material is supplied by an extrusion head, controlled by a computer, that follows a programmed path which is based on a pre-defined CAD model. The plasma depositing species were able to uniformly coat the PCL filaments of these scaffolds, from the top to the bottom surfaces. Osteoblast-like cells followed the nitrogen-based thin film, adhering on all the scaffold thickness[15].

Although in the biomedical field at low pressure plasma processes are utilized more often rather than atmospheric pressure, the latter are gaining popularity, especially in the field of Plasma Medicine. This emerging area is based on the use of atmospheric plasmas directly on living tissues, wound healing, cancer treatments and other therapeutic purposes[16]. Among the atmospheric pressure plasma sources, Dielectric Barrier Discharges (DBD) are mostly utilized in this field. Few works have been devoted to the application of these plasmas to tissue engineered scaffolds. Safinia *et al.* treated poly-(lactic/glycolic acid) scaffolds with an air atmospheric pressure plasma process, grafting O- and N-containing chemical groups on the scaffold surfaces, improving wettability and affinity with eukaryotic cells[17]. In our laboratory, we have applied air plasma DBD treatments on 2D polystyrene substrates and PCL scaffolds fabricated with the SC/PL technique. Increased hydrophilicity was assessed after the plasma treatments and for 2D substrates, the behavior of osteoblast-like and NHDF fibroblast cells was also studied.

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