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# Plasma Modified Textiles for Biomedical Applications

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## 1. Introduction

In the textile market industry, technical textiles are one of the fastest growing businesses. Part of that industry consists of textiles for medical and healthcare applications and are responsible for a continuous increase in its market potential [1]. Next to their need in hospital environments, there is a growing demand in other sectors such as the food and hotel industry, due to stricter hygiene regulations. In most cases biomedical textile meets a well-defined set of requirements such as minimizing non-specific protein adsorption, drug delivery coatings or the presence of active functional coatings and most importantly excellent biocompatibility (blood-, tissue- or cyto-compatibility) [2]. In general there are very few materials meeting all these characteristics, while at the same time offering the needed structural and mechanical properties. Furthermore, depending on the application, the production process has to be cost-effective and approved by local legislation.

In order to meet all these requirements, numerous modification techniques have been developed in the past [3-5]. Most of these techniques lead to the incorporation of extra/new functionalities and might lead to a change in surface free energy. For most biomedical applications, the preservation of material bulk properties such as elasticity, strength, ductility, structural integrity etc. is critical. For biomedical end-products, the use of solvents and chemicals based surface treatment techniques are reduced to a strict list approved by local legislation. Chemical-free techniques such as  $\gamma$ -radiation, UV treatments, corona discharges etc. have led to some excellent results in the field of tissue engineering [6, 7]. One of those solvent-free techniques that have been around for over a century, has more recently found its way into the biomedical field: non-thermal plasma technology.

Over time, it has extensively been proven that non-thermal plasma technology can profoundly change the surface properties of polymer films (PP, PET, PU, etc.) as well as material characteristics (adhesion, printability, dyeing etc.) of more complex substrates such as industrially

produced textile [8-14]. Alongside the growing interest in tissue engineering and the booming of the electrospinning industry at the end of last century, non-thermal plasma technology found its way into the biomedical field. Today non-thermal plasma treatment can be considered as a well-established technique for the surface treatment of (bio)materials.

Before the start of the 21<sup>st</sup> century, the majority of contributions to scientific literature was focussing on oxygen plasma treatments at low pressures and the corresponding response on cell adhesion, growth and proliferation. Although today there is still a steady stream of publications on these low pressure oxygen plasmas, there is a growing interest in atmospheric pressure plasma treatments as they offer a number of practical advantages. In the next chapter part, a detailed overview will be given on plasma technology in general and the different treatments possible. After that, the chapter will continue on the use of plasma technology for (bio)medical textiles, according to the application. At the end there will be a critical conclusion and a look forward to the possible future of plasma technology for the biomedical textile industry.

## 2. Non-thermal plasma technology

### 2.1. History and definitions

A plasma is a gaseous mixture of ions, radicals, electrons and neutrals. Plasma is often referred to as the fourth state of matter, as its properties fundamentally differ from solids, liquids and gasses and the change of state can be obtained by adding energy to a gas, similar to the transition from solid to liquid to gas. In 1929, Langmuir was the first to actually define a plasma, but already in the 19<sup>th</sup> century plasma was used on an industrial scale for the generation of ozone (Siemens) [15].

Plasma itself can be divided up into two categories: 1) thermal or equilibrium and 2) non-thermal or non-equilibrium plasma. Thermal or hot plasmas have temperatures of 4000 K or higher and are considered to be in a thermal equilibrium, meaning that both heavy ions and electrons have the same temperatures. Well known applications include plasma spraying, wide arc spraying, and thermal plasma chemical vapour deposition (TPCVD), thermal plasma synthesis of fine powders (nm), thermal plasma (toxic) waste destruction, thermal plasma densification of powders, thermal plasma metallurgy, thermal plasma extractive metallurgy etc. For non-thermal or cold plasma, only the electrons are accelerated via e.g. an applied electrical field, causing a thermal inequilibrium between the electrons and the heavy particles. This results in the formation of a plasma at lower temperatures. Due to this difference in operating temperature between thermal and non-thermal plasmas, they are often referred to as 'hot' and 'cold' plasmas respectively. Although referred to as cold plasma, temperatures of up to 1000 K can be reached. For biomedical applications, non-thermal plasma treatments are preferred with a degree of ionization of 1% or lower as this results in a discharge that can be sustained at room temperature (290-330 K), thus avoiding thermal degradation of thermo-sensitive materials. In the next paragraphs, the focus will be on the sources that are used to

drive the discharge, as they are an excellent way to distinguish between the ways a plasma can be generated, independent of the set-ups possible.

## 2.2. Plasma discharges

The different non-thermal or cold discharges discussed in the following parts have all proven their usefulness as well as their limitations. Over time, applications have been found for each different type of discharge in all branches of the industry: automotive, packaging, textiles, aerospace, catalysis, waste treatment, (bio)medical etc. [9, 13, 16-18]. The number of plasma reactor designs is nearly limitless and complete reviews have been written on that topic alone, as design changes are made to optimize the plasma treatment for their specific application [19]. Most of the designs available today can be linked to one of the plasma sources discussed here.

### 2.2.1. Corona and silent discharge

A corona discharge reactor typically consists out of a cathode wire and an anode, which is normally the material that needs treatment. The first developed systems were powered by a DC source working in a pulsed mode and were operated at atmospheric pressure. When turned on, the system generates a lighting crown build out of many streamers, hence the name corona [9]. Pulses are used that are shorter than the time necessary to form an arc, thus avoiding the transition to the spark regime. In the middle of 20<sup>th</sup> century, the first corona discharge systems were patented for the incorporation into industrial textile production systems [20]. Later on, the systems were adapted to work with high frequency sources (radio-frequency (RF), microwave (MW) and AC) and today a number of commercial systems are available from companies such as Tech Sales Company, Air Liquide, Acxys Technologies etc. These modern high-tech set-ups are able to quickly and efficiently treat delicate structures such as electrospun sheets. One of the main disadvantages of corona treatment is that the streamers always form at the same spots, resulting in an inhomogeneous treatment of the exposed surfaces. To solve this particular problem, there has been a shift to the usage of silent discharges [16].

A silent discharge, also known as a dielectric barrier discharge (DBD), is powered either via a high frequency AC or an RF source. What makes the DBD stand out against other systems, is its higher and broad pressure operating range (5-10<sup>5</sup> Pa) [21, 22]. In 1857, Siemens was the first to use a DBD in a successful attempt to generate ozone and to this day it remains one of the most important industrial applications of the DBD [15].

A DBD reactor typically consists of 2 electrodes, of which at least 1 is covered with a dielectric material such as glass, ceramic or quartz. The voltage used to drive the discharge can start as low as 0.5 kV and can be increased up to a few 100 kV. The generated plasma is a collection of many small micro-discharges or streamers. The dielectric material is able to limit the discharge current, giving cause to very short-lived micro-discharges (1-10 ns) that are distributed homogeneously across the electrode. In some specific cases, the streamers can be avoided altogether and a true glow regime can be obtained, which is considered the best case for homogeneous treatments [9].

DBD set-ups have one major advantage compared to most other systems: the possibility to operate in a higher pressure range makes it possible to avoid extensive vacuum equipment. This results in a lower operating cost and faster treatment cycles, thus allowing them to be implemented in industrial surface modification processes. The low heat generation at elevated pressures allows for a wider range of applications, including plasma chemistry, grafting, polymerization, cleaning... These applications are not always as easily feasible in systems powered with a different source.

It should be noted that occasionally in literature also the term corona discharge or corona treatment is used in connection with DBDs, although most authors prefer to use this term only for discharges between bare metal electrodes without dielectric.

### *2.2.2. DC discharge*

Non-thermal plasmas generated via a DC discharge are in most cases formed in a closed set-up between two electrodes at very low pressures ( $10^{-1}$  –  $10$  pa) [21, 22]. As the current is increased, different types of discharges can be obtained. The Townsend discharge is a self-sustaining discharge, typically characterized by a low current. A higher current results in a drop of voltage and a glow discharge is generated. The glow discharge regime is the desired regime for surface modifications, as it guarantees a homogeneous treatment all throughout the reactor. Increasing the discharge current still further results in a fast increase of voltage until an arc is formed, allowing for the charge to dissipate and the voltage drops almost completely. One of the biggest advantages today of DC discharges, is that it is a well understood process, allowing for a high control over the process and its different parameters.

The DC current can be driven through the system in a continuous manner, or it can be pulsed. For biomedical applications in general, there are two advantages in doing the latter: first of, higher discharge powers can be applied without the otherwise inevitable thermal damage caused by the heating of the electrodes and secondly, if used for the coating applications, it renders a more homogeneous coating. One of the main disadvantages of the DC driven systems is the direct exposure of the electrodes to the plasma environment, making them prone to corrosion if exposed to certain reactive monomers.

### *2.2.3. Radiofrequency and microwave discharges*

Radiofrequency (RF) and microwave (MW) discharges are generated using high frequency electromagnetic fields [21-23]. RF discharges have a relatively wide frequency operating range between 1 – 100 MHz, but in most cases a fixed frequency of 13.56 MHz is applied. Concerning the operating pressure, a wider range, compared to DC systems, ( $1$ - $10^3$  Pa) is possible, but with the exception of a few, high-vacuum equipment is needed, which is expensive, drastically increases treatment times and are hard to implement in continuous production processes. For the treatment of biomedical materials, it is most likely the most applied discharge, as it is the plasma treatment technique of choice for the popular oxygen plasma treatments and several systems are commercially available.

Microwave discharges are operated at a higher frequency range, usually fixed at 2.45 GHz. The pressure range is more versatile compared to RF and DC discharges, with a range between 1 Pa and  $10^5$  Pa. Higher pressures lead in most cases to an increase of heat transfer from the electrodes to the substrate, making it a less than ideal situation for the treatment of textiles and nonwovens. This limitation results in the same treatment restrictions as the previously discussed discharges.

#### 2.2.4. Atmospheric pressure plasma jets

To finalize this chapter part on plasma technology, some special attention will be given to atmospheric pressure plasma jets (APPJ's). Operating a plasma in a confined space has certain advantages when it comes to the control of the physics and chemistry taking place, but sometimes there are cases where it would be more desirable if the plasma could be free from any geometrical confinements. APPJ's, also referred to as plasma plumes are an ideal solution and are excellent tools for the treatment of geometrically larger and more complex surfaces such as textile fibers [24].

APPJ's can be powered with any of the sources discussed before, but all deal with the same problem: how to avoid the transition from glow to arc. For DC sources this can either be achieved via the use of hollow cathode discharges with sub mm dimension or the use of resistive barrier discharges. For the DBD systems driven by high frequency AC sources, the dielectric barrier itself is the solution, as it prevents the discharge current to increase to the point of arcing. Under some special circumstances the DBD's can generate an uniform diffuse plasma that is filament free. For the RF powered APPJ, either a set-up similar to the DBD set-up can be used, or the metal electrodes are left bare. For the latter, cooling of the electrodes is required, as well as an excellent control of the flow rate in order to minimise the risk of arcing [24]. Finally it is also possible to generate a plasma plume, using a microwaves to drive the plasma, but it is limited to a strict set of geometrical parameters which has been described in more detail by Park et al. [25].

It would be possible to give an extended description on the different set-ups available today, but it would lead to far out of the scope of this chapter. Laroussi and Akan already wrote a complete review on the different set-ups available. Also Shütze et al. wrote a compact review on the physics behind several set-ups [22]. Since that time also a number of commercial systems have become available on the market (crf *Plasmatreat*<sup>®</sup>, *PlasmaSpot*<sup>®</sup>, *PlasmaStream*<sup>®</sup>...). The applicability of the APPJ for the treatment of biomedical textile will be covered in the following chapter part 3: Plasma and textile: the biomedical applications.

### 2.3. Plasma-material interactions

In order to have an understanding of what is happening at the plasma-material interface, it is critical to have a basic knowledge about the possible effects the different active species have on a substrate exposed to them.

### 2.3.1. Plasma cleaning and etching

During the production process and storage of (bio)materials, they can be exposed to a number of solvents, greases, volatiles components etc. These contaminants will adsorb and accumulate on the material surface over time, resulting in an altered, non-reproducible surface with a likely reduced product performance. A typical example in the biomedical field, is the adsorption of low molecular weight carbon species onto a pristine titanium sample, when exposed to ambient air. When used as an implant material, this surface pollution results in a reduced cell adhesion, proliferation and growth and in some cases even results in cell death [26, 27].

Any volatile surface contamination that is exposed to a non-thermal plasma, will be removed in a few seconds [28]. Prolonged exposure to the plasma will not only result in the removal of the adsorbed contamination but will cause etching of the top layers of the material surface [29-32]. Depending on the density and hardness of the exposed material, more intense discharges and/or extended exposure are required to obtain a notable effect. As (biomedical) textiles are in most cases build out of relatively soft materials, the etching effect cannot be overseen and will introduce a certain nano-roughness on the fiber surface. For in-vitro and in-vivo applications this change in surface topography can have a benign effect, as it can amplify the other effects plasma has on cell adhesion and proliferation [33-35].

### 2.3.2. Plasma activation

Plasma activation or plasma treatment is the exposure of a surface to the reactive particles present in the plasma. This mixture of reactive particles will result in the incorporation of radical sites on the surface, up to the depth of a few 10 nm. Depending on the gas used to maintain the plasma, these sites will react (in)directly with other radicals present, recombining into a broad variety of functional groups. These new functional groups have a high impact on surface properties such as wettability and surface free energy, which in turn might have a positive effect on material-material and material-cell interactions.

In most cases an increase in hydrophilicity is pursued to enhance the materials histological performance. For some applications such as the surface of heart valves, the insides of needles and tubes or artificial stents, any adhesion of cells and proteins is highly unwanted, as it can lead to blockages resulting in premature failure of the biomedical device. Instead of using typical gas feeds for plasma treatment (noble gasses, oxygen, dry air, nitrogen...), fluorinated gasses such as  $CF_4$  are used which result in the formation of super hydrophobic surfaces with water contact angles of  $150^\circ$  and higher. These fluorinated surfaces prevent cells and proteins from effectively adhering on the surface and thus guaranteeing an optimal performance of the implant material [11, 36, 37].

Plasma activation is definitely not the only technique available for the introduction of new functional groups onto a surface, but as it is non-invasive and chemical-free, it guarantees the preservation of even the most delicate structures.

### 2.3.3. Plasma grafting and polymerization

Non-thermal plasmas are not only applied for plasma treatments, but can also be used as an initiation medium for radical polymerization, resulting in the deposition of a wide variety of

thin films. In order to optimize the bonding between the thin film and the biomaterial, the deposition process is preceded by a plasma treatment, introducing radical sites that allow covalent bonding of the polymer to the substrate surface. The polymerization process itself can happen via two different reaction pathways: plasma polymer grafting simply uses the radical sites introduced via plasma treatment to initiate the chain reaction. In other words, during the polymerization process itself, no plasma is used and the monomer is not exposed to the plasma. This results in the incorporation of the monomer as such, thus preserving its functional groups.

For plasma polymerization this is not the case. The plasma is used as an initiation medium and remains active during the entire polymerization reaction. This has as a consequence that the monomer is exposed to the reactive plasma, forming initiation sites on both the substrate surface as well as on the monomer. In contrast to chemical initiation, plasma is not as specific as to where the radicals are formed, using any functional groups of the polymer precursor as well to initiate the chain reaction. This results in a highly cross-linked, pinhole free and completely amorphous thin film that significantly differs from its traditional counterpart and adheres to almost any surface. Varying the discharge power gives a high control over the amount of functionalities preserved in the film. From a biomedical viewpoint this is an interesting application, as functional group density plays a critical role in the growth and proliferation of cells and differs for the type of cells used.

### **3. Plasma and textile: The biomedical applications**

#### **3.1. Wound dressing**

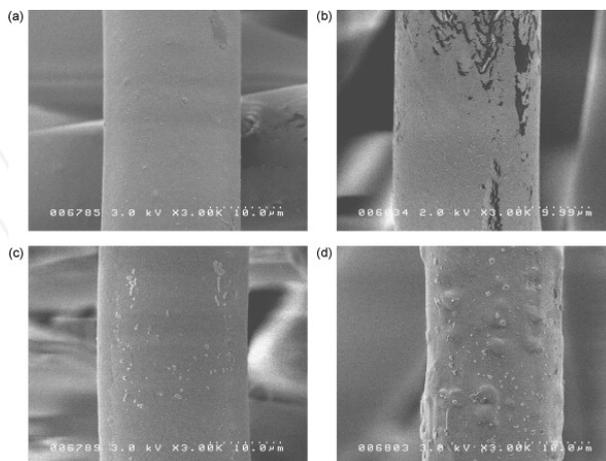
Optimal modern wound dressings should assure a moisture wound bed, help drainage, remove debris of the wound surface, provide optimal thermal stability, might be removed without trauma of the wound bed and wound edge, be antiallergenic and without immunogenicity [38]. Over the years wound dressings have experienced a continuous development stimulated by a better understanding of wound healing and bacterial growth mechanisms. In more recent years research has shifted to targeted therapy by including different pharmaceutical compounds (e.g., antiseptics, analgetics, or growth factors) in to wound dressings. The continuous presence, or controlled release of active substances, can tremendously stimulate the healing process.

Non-thermal plasma technology has been part of this development process in many different aspects of wound healing treatments. The review of the literature dealing with the use of plasma technology for the enhancement of wound dressings will be divided according to the purpose of the treatment: wound monitoring, enhancement antimicrobial properties, intermediate bonding, and adhesion.

##### *3.1.1. Antimicrobial properties*

Successful wound treatment cannot be achieved without keeping its two major aspects in mind: maintaining a decent hydrophilicity and a high antimicrobial efficiency.

In two different papers Persin et al. compared a few different treatment methods on viscose fibers to address both of these aspects [39, 40]. In a first reaction pathway, a two-step process consisting of an oxygen plasma treatment, followed by the immobilization of AgCl particles, is followed. The alternative pathway consists of a single-step treatment of the cellulose fibers with an ammonia plasma. The single-step treatment resulted in a 30-fold increase in water uptake while the immobilization of the silver particles only had a marginal effect. For the antimicrobial properties of the wound dressings, the reverse trend was found. The single-step treatment only had an effect on Gram-positive bacteria, while the steady release of silver ions resulted in a quasi-complete destruction of both Gram-positive and Gram-negative bacteria. The authors therefore suggest to use a different treatment for different wound-healing applications, depending on the risk of infection. In a third paper from the same authors, a closer look is taken on the ageing of the plasma treated surfaces and the effect on the water up-take [35]. The study showed that 4 days after the treatment, the water contact angle increased with  $15^\circ$  and the oxygen concentration decreased with less than 1%. These numbers suggest that there is a limited shelf-lifetime of the modified wound dressings. Hacker et al. followed an alternative strategy for the immobilization of Ag particles by plasma polymerizing PEG onto electrospun PU mats, followed by soaking in  $\text{AgNO}_3$  and UV treatment, resulting in the incorporation of metallic silver [41]. Water-uptake tests revealed a significant increase of hydrosorption after 24 hours. The steady release of silver particles had a detrimental effect on the viability of both Gram-positive and -negative bacteria while at the same time no cytotoxic effects were noted on the adhesion and proliferation of fibroblasts. Further on in the chapter, other strategies will be discussed to obtain antibacterial properties for other applications, but it is already clear that the incorporation of silver particles with the help of plasma technology is a successful pathway for the improvement of wound dressings.



**Figure 1.** SEM micrographs (3000 $\times$ ) of the (a) plasma modified nonwoven, (b) AAC coated nonwoven, (c) PP-g-collagen nonwoven, and (d) PP-g-collagen-g-PNIPAAm nonwoven [126].

### 3.1.2. Adhesion and intermediate bonding

Next to increasing the hydrophilicity of surfaces, plasma are also known to promote the adhesion between layers. Gajanan et al. published two papers where plasma technology was used to improve the adhesion between an electrospun material and a woven support structure [42, 43]. In the first article chitosan was used as a raw material for the electrospinning process. In the second article, the chitosan was mixed with silk fibroin. For both cases a 100% cotton gauze was used as support material. Out of the different DBD plasma treatments, using He +1% O<sub>2</sub>, the combination of a pre-and post-treatment gave the best results. Flex durability tests showed that there was a 4-fold increase in adhesion between the electrospun material and the cotton gauze. On top of that SEM images showed that the plasma treatment resulted in a reduction of the fibers' delamination after repetitive flexing.

As explained earlier in section 2.3, plasma can be used to graft new side chains on polymer substrates. The goal of grafting can either be to introduce new functional groups and use their properties as such, or to introduce them as intermediates that can be used in consecutive reactions. In biomedical surface engineering the latter is quite popular as the list of products that can be used is limited, meaning that in most cases there is a sub-optimal affinity between coating and substrate. Chen et al. used a PP-non-woven as a substrate on which acrylic acid was grafted. The newly introduced carboxylic acid functionalities were used to either covalently bond collagen or chitosan, on which the thermo sensitive polymer PNiPAAm was immobilized of which each step is depicted in figure 1. Above 32° C, the wound dressing becomes hydrophobic and releases the stored moisture, which in turn resulted in an enhanced wound healing [44, 45]. Lin et al. used an oxygen low pressure plasma to activate a PE non-woven substrate, followed by the grafting of N-isopropyl acrylamide [46]. This intermediate was then used for the covalent bonding of bovine gelatin. In-vivo tests revealed that the covered wound healed completely (reached the maturation phase) and much faster compared to the PE control.

The results discussed above show that plasma technology can be a valid, solvent-free alternative for the permanent fixation of technically advanced layers onto standard substrates. Most of the studies on adhesion and intermediate bonding are relatively new and more research is needed to see if the obtained results can be extrapolated to other materials and applications.

### 3.1.3. Wound monitoring

In modern society the development of 'smart' materials is a hot topic (crf smartphones, smartwatches, smart-TV's etc.). In the wound care industry similar developments are taking place and several groups are doing research on so-called 'smart bandages'. The idea behind smart bandages is that they autonomously could monitor the wound and signal in case of infection or other irregularities. A few groups working on biosensors have successfully incorporated plasma technology in their biosensor production process.

Phair et al. used a corona discharge in ambient air to activate carbon fiber threads in order to improve their electro-analytical performance [47]. Via a redox probe set, the ion exchange rate was determined as a function of plasma treatment time. The results showed that the incorpo-

ration of carboxyl groups resulted in better electron transfer kinetics. Exposing the prototype bandage to whole blood proved that they were capable of detecting urates.

Zhou et al. developed a biosensor based on self-quenching fluorocarbons [48]. These fluorocarbons were stored in lipid vessels which were immobilized on a pp-non-woven that was plasma coated with maleic anhydride using a commercial RF discharge system. Upon contact with both Gram positive and negative bacteria, the lipid vessels released the fluorocarbons, allowing for a visual infection detection mechanism. In combination with the simultaneous release of antibacterial products using the same release system, they believe it is possible to make a quasi-autonomous or 'smart' bandage.

The collection of reviewed papers shows that non-thermal plasma technology can play an important role in the development of smart bandages, allowing for low-cost personalized wound care treatment.

### 3.2. Antimicrobial functionality

Within the section on wound dressings already a part on antibacterial properties of plasma treated fibers has been included, but the applicability of these modified textiles goes much further than wound treatment alone and is highly wanted for other (bio)medical applications such as surgical gowns, sutures etc. as well as in the food industry and catering business [49]. The study on antimicrobial finishes of fibers and meshes has been quite extensive and between 25 and 30 papers will be reviewed here. A wide variety of substrates has been modified, ranging from natural-based products such as cellulose and wool to PP, polyesters, polysulfons, polyamides, carbon composites etc.

#### 3.2.1. Ag immobilization

As for the modification itself, a distinction can be made between alterations involving the immobilization of metal nanoparticles (Ag, Cu) and all other treatments. To this day, the controlled release of silver particles is by far the most effective technique to inhibit the attachment and growth of both Gram positive and negative bacteria [50-65]. Most of the research groups use a non-thermal plasma to increase the wettability and nano-roughness of the textile substrate in order to enhance the uptake of silver nanoparticles (either via  $\text{AgNO}_3$  or  $\text{Ag}_n$ ) or a plasma polymer coating with controlled release properties. Antibacterial tests involving micro-organisms such as E. Coli, S. Aureus and C. Albicans show in most cases a bactericidal efficiency of more than 99.9%.

#### 3.2.2. Plasma activation, grafting and intermediate bonding

Other research groups followed less known pathways with mixed success. Yao et al. did an Ar low pressure plasma pretreatment of PVDF-HFP/PU fibrous membranes, followed by the UV grafting of 4-vinylpyridine and a quaternization of the introduced pyridine group using hexyl bromide [66]. Anti-bacterial essays showed a killing efficiency of 99.9999 % for both E-Coli and S. Aureus strains (See figure 2). Despite the excellent performance of the fibers, the

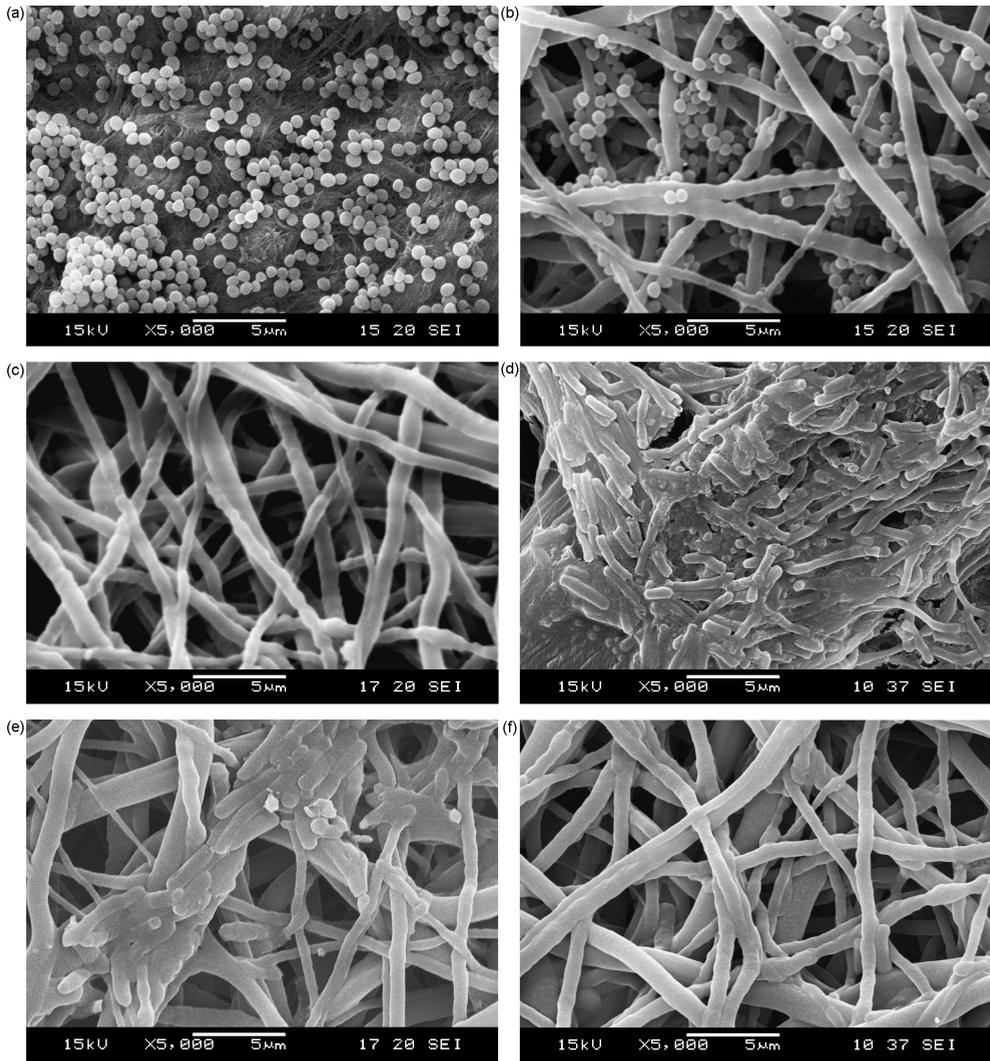
commercial applicability remains limited due to relatively expensive production process in a competitive business environment.

Different research groups did a plasma pretreatment on cotton and polypropylene (PP) nonwovens respectively, followed by the immobilization of natural products [67-69]. Vaideki et al compared the uptake of neem leaf extract before and after RF air plasma treatment and found that both the increase in wettability as well as the surface etching resulted in a superior adsorption of the extract [67]. Nitkyakalyani et al made a mix of herbal plant parts that were dried and grinded after which they were dissolved so the PP could be soaked in it [68]. A wide variety of both Gram positive and negative bacteria were tested and excellent antimicrobial activity was noted. Although the use of natural products is a pro in some cases, it is difficult to obtain a certain consistency in the production process and there is always a certain risk of product pollution. Strnad et al. treated cellulose fibers with an RF O<sub>2</sub> plasma to increase the adsorption of chitosan [69]. The antimicrobial tests revealed a modest effect against *S. Aureus* and no effect against *E-Coli*. Antifungal tests using several fungi strains resulted in modest antifungal behavior.

Some groups simply applied a plasma treatment as such, using reactive gasses such as CF<sub>4</sub> and hydrazine to obtain the desired antimicrobial effect. Uygun et al. did a pretreatment of chitosan powder using a RF hydrazine plasma [70]. Chemical analysis shows a significant increase in the number of primary amines present. Using the modified chitosan for the electrospinning of nano-fibers results in a nonwoven that has a better moisture uptake and an enhanced antibacterial effect for Gram-positive bacteria. Canal et al. and Virk et al. used an Ar-CF<sub>4</sub> post discharge plasma with mixed results. The treatment of wool, polyamide and Sontara<sup>®</sup> resulted in a heightened bacterial resistance, while the treatment of cotton had no effect at all [71, 72].

Just as described in the intermediate bonding paragraph of the wound treatment section, plasma is used for the grafting of intermediate layers that are used for the immobilization of antibacterial components. Degoutin et al. used a low pressure RF Ar plasma to graft acrylic acid onto a PP nonwoven [73]. The carboxylic acid functionalities were used for the immobilization of gentamicin, which is known to be 99% bactericidal as was confirmed by the testing with *E. Coli*. Gawish et al. used a He plasma for the grafting of glycidylmethacrylate, which was used for the covalent bonding of cyclodextrines [74]. These macromolecules are known for the controlled release of active components, in this case biocides with antimicrobial and insect repellent properties. These experiments confirm that plasma grafting is a versatile technique that forms the basis for subsequent reactions.

In general it can be concluded that the incorporation of silver micro-and nano-particles is still the golden standard for the production of antibacterial textiles. Non-thermal plasma technology can be a useful tool for the immobilization of the metal particles and plasma deposited coatings are ideal for the controlled release. Plasma treatments as such can generate highly antimicrobial surfaces, as was proven, amongst others, by Yao et al. [66], but only after the right selection of precursors and discharge gasses.



**Figure 2.** SEM images of (a) and (d) filter paper (control), (b) and (e) pristine, and (c) and (f) modified PU fibrous membranes after immersed in PBS suspension of (a)–(c) *S. Aureus*, or (d)–(f) *E. Coli* at  $10^7$  cells/mL for 4 h. PU fibrous membranes were electrospun from 10% (w/v) solutions in THF and DMF (1:1, v/v) [127].

### 3.3. Cell culture and soft tissue engineering

Technical textiles for tissue engineering applications are one of the youngest branches in the textile industry, but in a just a few decades they have become a major player on the biomedical market and the number of publications dealing on the theme of tissue engineering applications has exponentially grown. The idea behind tissue engineering is that (stem) cells are extracted

from a patient with a malfunctioning organ. The retrieved healthy cells are seeded on a culture plate and grown to a full culture [3, 4, 75]. Once enough cells can be harvested, they are seeded onto a 3D nanofibrous scaffold. After a certain incubation period, allowing the cells to grow and differentiate into the scaffold, the nonwoven is implanted into the patient in order to restore the organ functionality or replace the organ as such. By treating patients with their own cells, immuno-response can be reduced to an absolute minimum and it is considered to be a constructive solution for the transplant waiting list issue.

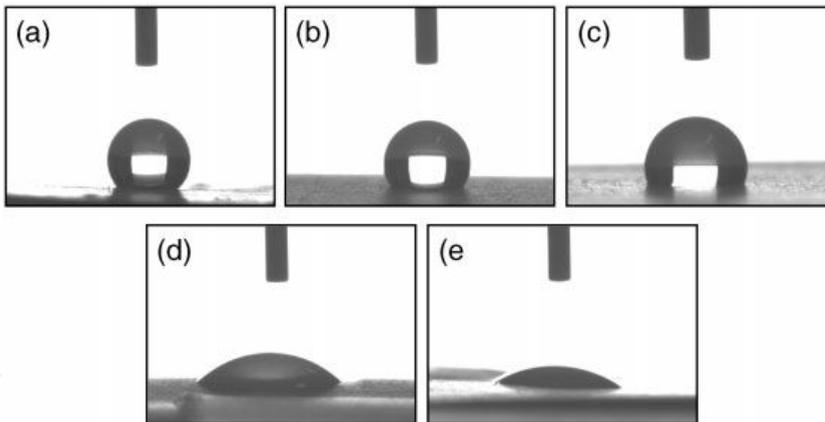
In this specific chapter part, both the enhancement of cell culture applications as well as soft tissue engineering will be discussed together, as they are closely related and in some cases even overlap. This chapter part will therefore be subdivided into a first section dealing with plasma treatment as such and its effect on cell growth, adhesion, proliferation and differentiation and a second section handling plasma grafting and polymerization, talking in more detail about adhesion and homogeneity of the deposited coatings as well as the histological properties.

### 3.3.1. Plasma treatment

The most widely used polymer collection for the production of nonwoven scaffolds for tissue engineering is the biodegradable polymer family. Polylactic acid (PLLA), poly(lactic-glycolic acid) (PLGA), Polycaprolactone (PCL)... are well established biomaterials due to the fact that after implantation they get broken down by the body in harmless end products (ideally  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) that can be secreted by the body, making a second surgery no longer necessary [13, 76]. The mechanical and structural properties of these materials are sufficient for their field of applications. The biocompatibility and bioactivity on the other hand are mediocre at best and often inhibit the migration and differentiation of cells into the textile scaffold structure [77, 78]. The surface treatment of these scaffolds is rather complicated, as the pore size limits the infiltration efficiency of (wet)-chemical treatments and often degrade the structural stability of the delicate nano-fibers. Gas-based treatments such as non-thermal plasmas are promising to penetrate more easily into the electrospun scaffold structure and are at the same time known for the fact that they only alter the surface without affecting the bulk, guaranteeing the mechanical and structural integrity of the modified biomaterial [14, 79].

The contact angle of PCL electrospun scaffolds lies between  $120^\circ$  and  $140^\circ$ , indicating a hydrophobic surface, which is not well liked by most cells. After treatment with either air, Ar,  $\text{NH}_3$  or  $\text{O}_2$  plasmas, all research groups were able to reduce the contact angle to less than  $5^\circ$  [80-85]. The XPS results reveal an increase in the oxygen content with the incorporation of a mixture of C-O, C=O and O-C=O functional groups. Prabhakaran et al. seeded neurolemmocytes (or Schwann) cells onto the nanofibrous scaffolds and found an increase in proliferation rate at all times, with a maximum of 17% compared to the untreated material after 10 days, compared to the untreated scaffolds [80]. They claim that the treatment is as effective as a collagen coating, making it a cost-effective alternative for nerve cell regeneration applications. Yan et al. found that after plasma treatment with  $\text{NH}_3+\text{O}_2$  had no influence on the mechanical properties of the non-woven. After seeding with MC3T3 osteoblasts a 2-3 times increase in cell adhesion was found in the first 24 hours and after 7 days the proliferation was increased by a

factor of 6, making the plasma treatment an excellent tool for the introduction of osteo-inductive properties [81, 84]. Martins et al. came to the same conclusions after treating their PCL nano-textile scaffolds both with Ar and O<sub>2</sub> plasma [82]. Seeding 3 different cell lines (L929, ATDC5 and Saos-2) covering a wide variety of cell-types, resulted in a significant increase in both adhesion and proliferation for all cells. Min et al. tested the O<sub>2</sub> plasma treated PCL nano-textile with primary astrocytes and noted an increase in adherence and viability in the first 24 hours [83]. Jeon et al. used a nano-sized template to enhance the nano-roughness introduced by the plasma [85]. After seeding MG63 osteoblasts a dramatic increase in cell adhesion and proliferation were noted as well as an elongated morphology compared to both untreated PCL and PCL treated without the nano-sized template, showing that both the surface chemistry and topography have a significant influence on the histological performance of the PCL electrospun scaffolds. Finally Blackstone et al. used a CF<sub>4</sub> plasma to further increase the contact angle of the non-woven in order to obtain a superhydrophobic scaffold [86]. After seeding a mixture of fibroblasts, keranocytes and MCF-7 cancer cells, they were able to sort out the cancer cells by applying a fixed amount of stress to the textile. The recovered cancer cells did not change in morphology, allowing for post-sorting analysis, making the development of a low cost cancer detection device possible.

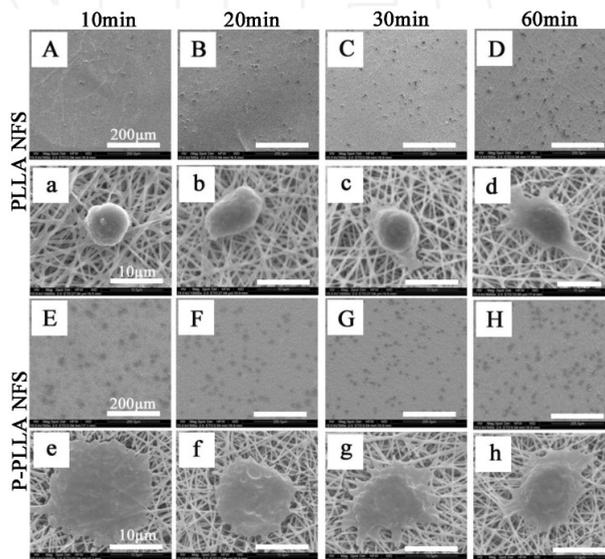


**Figure 3.** Photographs of water droplets taken immediately after contacting (a) non-treated, (b,c) oxygen plasma-treated and (d,e) ammonia plasma-treated PLGA nanofibers. Treatment time was varied from (b,d) 30 to (c,e) 180 s [128].

PLLA and PLGA both exhibit the same hydrophobic properties as PCL non-wovens, giving water contact angles situated between 130° and 150°. After a plasma treatment with the typical discharge gases (O<sub>2</sub>, Ar, NH<sub>3</sub>...) a decrease in contact angle was found to a minimum, ranging between 20° and 45° as depicted in figure 3 [87-89]. XPS reveals that PLLA is less robust compared to PCL as the initial increase in oxygen content is reversed when the substrate is over-treated, resulting in etching/degradation of the polymer structure [88]. Both Park et al. and Dolci et al. seeded fibroblasts (NIH 3T3 and MEF) after NH<sub>3</sub> and air treatments respectively and similar results for the increase in viability and elongated morphology were found [88,

90]. Liu et al. did a study on the adhesion behavior before and after O<sub>2</sub> plasma treatment of pMSC cells in the first hour after seeding [89]. Results revealed that both the adhesion and cell morphology were greatly improved as can be seen from figure 4. These results show that plasma treatment is indeed a valid option for the culturing of stem cells.

As PLLA, PLGA and PCL all show similar histological effects after exposure to a wide variety of plasmas, it is no surprise that blends of PLLA and PCL exhibit similar behavior. Chandasekaran et al. exposed such a co-polymerized fiber mesh to an air plasma treatment and studied the effects on fibroblast growth and proliferation [91]. In both cases a significant increase was noted and stimulation of extra cellular matrix formation was found, opening up the possibility for skin tissue regeneration applications.

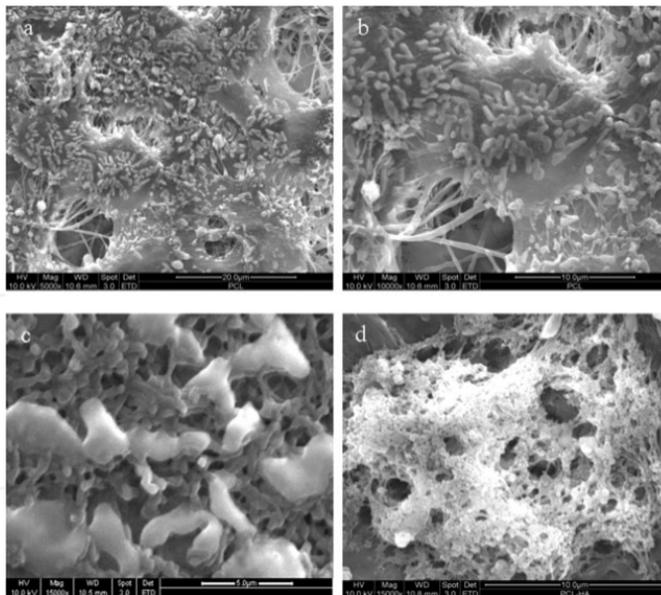


**Figure 4.** SEM images of pMSCs on Plasma treated PLLA nanofibers (NFS) and pristine PLLA NFS. (A–D) pMSCs on PLLA NFS after cultured for 10 min, 20 min, 30 min, 60 min, respectively; (E–H) pMSCs on P-PLLA NFS after cultured for 10 min, 20 min, 30 min, 60 min, respectively; (a–h) higher magnification for (A–H) [129].

The biodegradability of the textile scaffold material is not required in every case and sometimes has to be avoided all together (tissue culture ‘plates’, vascular grafts...) as the loss of the mechanical framework would result in the permanent failure of the implant. A polystyrene electrospun scaffold was treated by Baker et al. with a low pressure Ar plasma, followed by the seeding of smooth muscle cells [92]. The in-vitro tests revealed a significant increase in cells and an alignment with the electrospun material. The excellent results show that the polystyrene electrospun scaffold could be a valid alternative for 2D tissue culture plates. Zandén et al. treated a PU fiber mesh with an oxygen plasma in an attempt to improve the interaction with red blood cells [93]. SEM images showed that prolonged exposure to the plasma resulted in a reduction of the fiber diameter and finally the degradation of the structure. As was the case

with PLLA and PCL, a significant increase in hydrophilicity was noted due to the incorporation of polar functional groups. The in-vitro tests revealed that there was no significant difference between the adhesion of red blood cells before and after treatment. This shows that not all plasma treatments have a positive effect on biocompatibility and that the treatment gas and operation parameters should be carefully selected.

The final paragraph of this chapter part on plasma treatment for tissue engineering will go over the possibilities to use non-thermal plasmas to stimulate the formation of apatite on flexible scaffold structures. Yang et al. immersed an Ar plasma treated PCL fiber structure in an SBF 10 solution for a period of 7 days [94]. In the first 24 hours, already a CaP coating had grown on the fibers, consisting of nano-apatite and dicalcium phosphate dehydrate. After 7 days a structure closely resembling bioapatite was found. Luo et al. performed the same analysis on air plasma treated PEEK (reinforced with carbon fiber) and whereas the untreated PEEK resulted in no apatite formation, a fully grown apatite layer could be found on the treated nonwoven scaffold [95]. Other research groups made a solution of PCL mixed with hydroxyapatite/ $\text{CaCO}_3$  which was electrospun, resulting in a composite nano-textile which was followed by an oxygen/air plasma treatment [96, 97]. After seeding hFOB osteoblasts, a significant proliferation rate was noted, as well as the first signs of mineralization similar to human bone as depicted in figure 5. These papers show that plasma activation of polymeric nonwoven scaffolds is an excellent tool for the promotion of apatite growth.



**Figure 5.** Mineralization of hFOB on PCL/HA-P nanofibrous scaffolds at different magnifications: (a) mineral deposition 5000 $\times$  (6 days), (b) mineral deposition 10000 $\times$  (6 days), (c) mineral deposition 15000 $\times$  (6 days), (d) apatite-like morphology of natural bone 15000 $\times$  (10 days) [130].

In general it can be concluded that non-thermal plasma treatments, both at lower and elevated pressures, are excellent tools for the stimulation of the histological properties of a wide variety of cells seeded onto flexible scaffolds for tissue culture and tissue engineering applications.

### 3.4. Plasma grafting, polymerization and immobilization

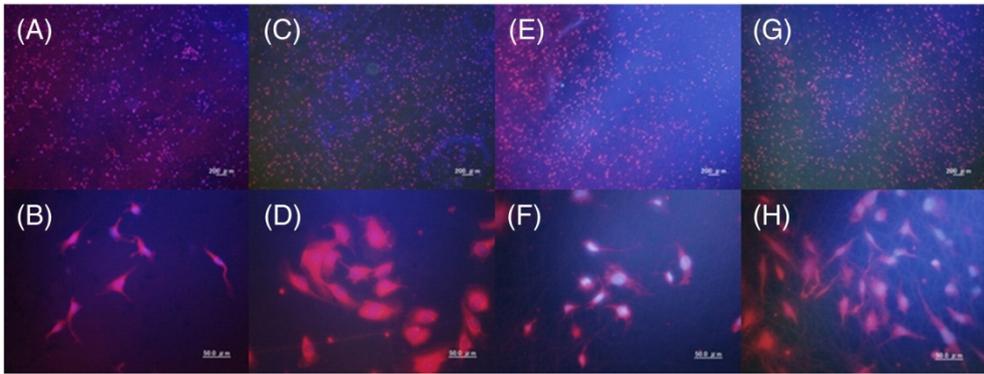
The tissue engineering nanofibrous scaffold materials subjected to plasma grafting and plasma polymerization are, in the majority of the papers reviewed, more or less the same ones used for plasma activation, PCL being the most popular one. A minority of papers investigated less obvious material choices that are inherently not (sufficiently) biocompatible, but once coated exhibit sufficient bioactive properties, as will be discussed in the last paragraph of this chapter part.

Guex et al. coated a PCL mesh using a combination of ethylene and CO<sub>2</sub> in an Ar discharge in order to restore the functionality of damaged myocardium [98]. After seeding extracted mesenchymal stem cells onto the modified electrospun scaffold, it was implanted in a rodent model. Post-mortem analysis revealed a stabilized cardiac functionality as well as an attenuated dilation. Zander et al. covalently bonded lamin proteins onto an air plasma treated PCL scaffold [99]. PC12 neuron-like cells were seeded onto the modified substrate and analysis showed a positive correlation between the neuron outgrowth and the concentration of the immobilized proteins. Xie et al followed a similar strategy, immobilizing dopamine [100]. The dopamine coating itself was then used to immobilize fibronectin, which significantly stimulated the attachment, spreading and cytoskeletal development of NIH 3T3 cells. Furthermore it was proven that the coatings could be used for the controlled release of active substances. Ma et al. used a combination of air plasma and wet carboimide chemistry to graft gelatin onto both random and aligned PCL fiber meshes [101]. The spreading and proliferation of endothelial cells was greatly enhanced and the cells aligned themselves along the fibers, which was not the case for the untreated material. Finally Hegeman et al. performed a degradation study of PCL nanofibrous scaffolds coated with amine containing polymers and showed that the incorporation of oligomers in the deposited films can leach out, causing cell death [102]. Storage of the coated nano-textiles in liquid media removed the low molecular weight residue, solving the problem. Overall, the literature shows that PCL nonwoven scaffolds, either activated or coated, can be used for a wide variety of tissue engineering applications, exhibiting excellent bioactive properties. It is essential though to use the right set of parameters and avoid the inclusion of unreacted products as this can have a detrimental effect on the histological performance of the 3D electrospun scaffold.

Several research teams used non-thermal plasmas to immobilize bioactive macromolecules (collagen, cRGD peptides and heparin respectively) onto a PLLA nano-textile scaffold, after which they were seeded with either BOECs, hMSC or endothelial cells [78, 103-106]. In all cases a positive influence was found on the scaffold's histological properties. Park et al. also obtained an increase in adhesion and proliferation after seeding NIH 3T3 fibroblasts onto PLLA nano-textile scaffolds that were grafted with an acrylic acid coating using a low pressure O<sub>2</sub> plasma [107]. He et al and Chan et al. performed a similar procedure compared to Feng et al. to immobilize collagen onto PLLA-PCL electrospun scaffolds [108, 109]. The first group success-

fully seeded hCAEC's, showing the possibilities for vascular grafts (see figure 6), while the Chan et al. studied the enhancement of the adsorption properties of MSC cells in the first hour after seeding, proving that coated nonwoven scaffolds are more efficient than plasma treated samples, which in turn are more effective than untreated samples.

A rather large number of publications can be found on a variety of other biomaterials that are not always biodegradable such as PDMS, PU, PET, silk fibroin, cellulose, PHBV... Most of these textile scaffolds get coated with well-known bioactive macromolecules such as collagen, galactose, laminin, peptides, or polymer films containing functional groups that are well-liked by cells, such as primary amines or carboxylic acids [92, 110-118]. A whole spectrum of cells is seeded on the coated textile scaffolds, ranging from osteoblasts and fibroblasts to endothelial cells, nerve cells and even stem cells. Discussing all of them again would lead to far, especially as the effects are similar to the histological performance of the coated PCL and PLLA textiles. Therefore the results have been summarized in table 1.



**Figure 6.** Fluorescent micrographs of HUVECs cultured on PU (A and B), plasma treated PU (P-PU) (C and D), P-PU/PLGA (E and F), and plasma treated (P-PU/PLGA) films for 3 s (G and H). Cells were stained with Texas-Red Maleimide C2 for cell membrane and nuclei were stained with Hoechst33258. Images are 40× (A, C, E, and G) and 400× (B, D, F, and H) magnified [131].

Scaffold material	Plasma treatment	Deposited coating	Cell line	Histological Effects	Authors	Reference
Silk fibroin	RF Ar low pressure	Heparin	L929 EVC	Better proliferation	Wang et al.	[110]
PDMS	RF Ar low pressure	pNIPAm	aoSMC	Smooth muscle cell formation	Rayatpishesh et al.	[111]
PU	RF O <sub>2</sub> low pressure	Galactose	HepG2/C3A	Improved albumin secretion	Chien et al.	[112]

Scaffold material	Plasma treatment	Deposited coating	Cell line	Histological Effects	Authors	Reference
PCL + starch	RF O <sub>2</sub> low pressure	Vinyl sulphonic & phosphonic acid	Fibronectin Vitronectin Saos-2	Enhanced protein adsorption & better cell growth and proliferation	López-Pérez et al.	[113]
PET	RF C <sub>2</sub> H <sub>4</sub> low pressure	NH <sub>3</sub>	HUVEC	Better growth and proliferation	Savoji et al.	[114]
PES	MW O <sub>2</sub> low pressure	Collagen	USSC	Excellent infiltration	Shabani et al.	[115]
PS	Ar	Lamelin	Smooth muscle cells	Enhanced differentiated phenotype	Baker et al.	[92]
PSU	RF air cleaner	Methacrylic acid + F3GA	BSA	Fast purification small scale proteins	Ma et al.	[116]
Cellulose	APPJ Ar	f-Cyclodextrines	Fatty acids	Excellent inclusion & no cytotoxicity	Nada et al.	[117]
PC-PU	RF O <sub>2</sub> low pressure	PDMS	L929	Cytocompatibility	Arjun et al.	[118]

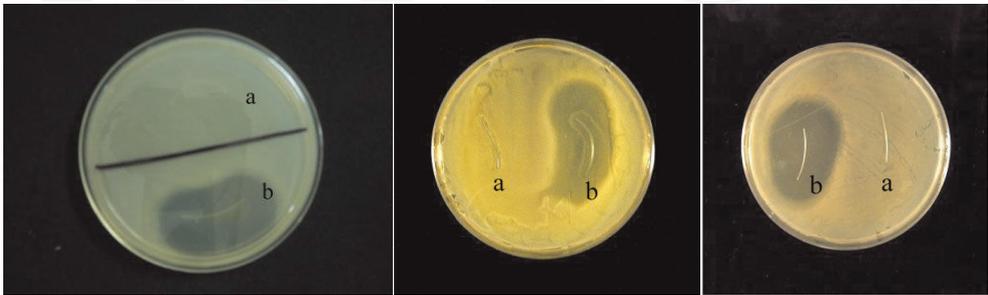
**Table 1.** Overview of papers on the histological performance of plasma coated 3D electrospun scaffolds (no PCL and PLLA)

### 3.5. Sutures

Of all the textiles for biomedical applications, sutures are probably the most low-tech. The amount of research conducted to improve the performance of surgical sutures is therefore not as extensive. Traditionally sutures were either non-biodegradable, requiring removal afterwards or biodegradable, but lacking the necessary mechanical strength and flexibility [49]. Eventually glycolide and lactide polymers such as PLLA and PLGA found their way into the suture market, introducing the required mechanical properties combined with biodegradability. Yet, as has been discussed earlier in the chapter, these biodegradable polymers do not always exhibit the wanted bioactive surfaces. A small number of research groups have investigated if non-thermal plasma technology can help to further improve the performance of medical sutures, of which a brief overview will be given.

Loh et al. performed a study, using both activation and deposition, analyzing the hydrolytic degradation rate of commercially available synthetic absorbable sutures [119, 120]. Dexon (PGA), Vicryl (PGLA), PDS11 (PpDO) and Maxon were either coated with parylene or treated by a number of different plasma gasses. Using the right set of plasma treatment parameters significantly increased the degradation rate of Vicryl and PDS11, while for Dexon and Maxon

only marginal differences were found. The plasma coating process, using parylene, resulted in an increase in tensile strength, most likely due to the hydrophobic character of the coating. Saxena et al. published 3 papers on plasma grafting of PP sutures [121-123]. In all the articles an RF O<sub>2</sub> plasma was used to activate the monofilament, followed by the immersion in an acrylic acid solution. The introduced carboxylic acid groups were then used to successfully immobilize chitosan. While the first 2 articles elaborates on the preservation of the mechanical properties and the surface chemical characterization, the second article focusses more on the antimicrobial, in-vitro and in-vivo properties. The viability of both *E. Coli* and *S. Aureus* were reduced with more than 90%. The in-vitro studies revealed excellent adhesion and proliferation of MC3T3 cells and the in-vivo use in a rodent resulted in a better quality of tissue integration and a minimal inflammatory response. The grafting of acrylic acid onto a monofilament for improved antimicrobial functionality was inspired by Gupta et al. who grafted acrylic acid onto PET monofilaments, obtaining similar antimicrobial results [124, 125].



**Figure 7.** Left: Zone of inhibition against *E. Coli* (a) control PP suture and (b) drug-loaded PP suture (degree of grafting, 5%). Middle: Zone of inhibition against *K. Pneumonia* (a) control PP suture and (b) drug-loaded PP suture (degree of grafting, 5%). Right: Zone of inhibition against *S. Aureus* (a) control PP suture and (b) drug-loaded PP suture (degree of grafting, 5%) [132].

Albeit being a rather low-tech application, plasma technology is still able to improve the performance of medical sutures. If the results found for tissue engineering applications would be applied for monofilament applications, it is beyond doubt that the biomedical properties could be further enhanced.

#### 4. Conclusion

In this chapter a broad range of applications has been reviewed where non-thermal plasma technology could play a beneficial role in the biomedical performance of technical textiles. Albeit being more limited in the number of functional groups that can be incorporated and the limited stability over time, plasma activation still leads to improvements in cell viability, adhesion, proliferation and differentiation as well as better adsorption and chemical bonding of bioactive and bactericidal macromolecules. Plasma grafting and polymerization is equally able to do all of the above, while having access to a wider variety of functional groups and

results in more stable surfaces. The incorporation of low molecular weight species into the coatings has to be avoided at all costs as they can have a detrimental effect on the cells viability. Up to now, the low pressure systems are by far the most used treatment systems, as the physics behind the process are well understood and multiple systems are commercially available. In the last decade there has been a growing interest in atmospheric pressure systems as they are more low-cost and can be more easily incorporated in textile production systems and it is our personal believe that atmospheric pressure systems such as the plasma jets will become the most prominently used set-ups. All in all it can be concluded that non-thermal plasma technology has earned its place in the (bio)medical textile market and will continue to do so in the future.

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