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Non-thermal plasma technology for the development of antimicrobial surfaces: a review

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Abstract
Antimicrobial coatings are in high demand in many fields including the biomaterials and healthcare sectors. Within recent progress in nanoscience and engineering at the nanoscale, preparation of nanocomposite films containing metal nanoparticles (such as silver nanoparticles, copper nanoparticles, zinc oxide nanoparticles) is becoming an important step in manufacturing biomaterials with high antimicrobial activity. Controlled release of antibiotic agents and eliminating free nanoparticles are of equal importance for engineering antimicrobial nanocomposite materials. Compared to traditional chemical ‘wet’ methods, plasma deposition and plasma polymerization are promising approaches for the fabrication of nanocomposite films with the advantages of gas phase dry processes, effective use of chemicals and applicability to various substrates. In this article, we present a short overview of state-of-the-art engineering of antimicrobial materials based on the use of non-thermal plasmas at low and atmospheric pressure.

Keywords: plasma deposition, biomaterials, nano-composites, antimicrobial coatings

(Some figures may appear in colour only in the online journal)
designed micro-/nanopatterns [7], whereas the second strategy aims to kill microorganisms that exist on the material surface and in the surrounding biological milieu through the elution of antimicrobial compounds from the materials [8]. A plasma-based strategy for engineering antimicrobial materials is a suitable and versatile approach that can be transferred between a large range of materials including heat sensitive scaffolds and non-woven medical fabrics (wound dressings, abdominal meshes, etc). Plasma deposition of antimicrobial coatings and grafting can be applied to a broad range of polymers, ceramics and metal surfaces in contrast to ‘wet’ chemistry methods which are strongly dependent on substrate properties. In addition the use of chemical solvents is much reduced in plasma-assisted manufacturing processes, making them attractive for large-scale production. Atmospheric pressure plasmas are typically employed for the deposition of thin organic or inorganic films, such as organosilicon thin films. Proper adjustment of the deposition scheme and of the plasma source operational parameters provides the possibility to synthesize more complex hybrid or composite films with advanced properties. Such films are perfect candidates for engineering a new class of biomaterials. Currently, there is a growing interest in the synthesis of nanocomposite films by atmospheric pressure plasma processes for functional materials with antimicrobial properties. To obtain an active antimicrobial activity, an antibiotic needs to be incorporated in the material surface. Molecular antibiotics traditionally used against bacteria face several disadvantages, including the worldwide emergence of antibiotic resistance, difficulty to be incorporated into many materials and sensitivity to harsh environments during many industrial processes [9]. Inorganic nanosized compounds present a strong antimicrobial activity at low concentrations due to their high surface area-to-volume ratio and their unique chemical and physical properties [10]. Currently, incorporation of metal nanoparticles (such as silver nanoparticles, copper nanoparticles, zinc oxide nanoparticles and many others) is gaining prominence as a new strategy to generate antimicrobial activity because of their pronounced biocidal activity and their higher stability under extreme conditions.

In general, two main strategies have been reported to obtain antimicrobial nanocomposite materials based on a plasma method: plasma-assisted surface grafting of antimicrobial components; and plasma polymerization of antimicrobial nanocomposite films.

In the following sections, an overview of the two strategies for the development of antimicrobial nanocomposite materials will be given and some of our recent results will be presented as a demonstration of the success in generating materials with high antimicrobial activities.

2. Plasma-assisted surface grafting of antimicrobial components

Plasma has been widely used as a pre-treatment step to activate or modify material surfaces. Such pre-treatment can improve the efficiency of post-grafting or incorporating antimicrobial components onto the surface. Due to their natural antibacterial/antifungal properties, chitosan and its derivatives have been widely used in biomedical materials. Chang et al used plasma pre-treatment to promote the grafting of chitosan on polyester fabrics to obtain antibacterial activity [11]. In their work, fabrics were firstly pre-treated by an argon/oxygen (Ar/O2) dielectric barrier discharge (DBD) plasma for surface activation, afterwards exposed to the atmosphere for further oxidization and finally immersed in chitosan solvents for chitosan grafting. It was found that the modified fabrics not only exhibited strong antibacterial activity against Bacillus subtilis and Staphylococcus aureus, but also showed good biocompatibility with fibroblasts cells. They also pointed out that the time of plasma pre-treatment was the major factor that determined the antibacterial efficiency of the modified fabrics. In addition, grafting of chitosan onto the surface of woven poly(ethylene terephthalate) (PET) materials and polyethylene (PE) films with air DBD plasma pre-treatment was reported by Theapsak et al [12, 13].

Besides chitosan, many other natural compounds, like nisin peptides, thymol and herbs, have also been grafted onto plasma-treated polymer surfaces to obtain an antibacterial material [14, 15]. Three types of plasma pre-treatment, namely nitrogen (N2) plasma modification, Ar/O2 plasma modification and plasma-induced grafting of acrylic acid (AA), were used to incorporate nisin peptides onto the surface of low density polyethylene (LDPE) films [14]. It was found that nisin adsorption onto the surface was strongly affected by many factors: type of surface, hydrophobic and hydrophilic interactions, surface charge, surface topography, etc. The antibacterial activity of nisin-functionalized films was dominated by the distribution and the amount of nisin on the surface. In general, samples with hydrophilic features, low electrostatic surface charge, and/or granular structures showed a stronger absorption capability of nisin and exhibited stronger antibacterial activities. One has to keep in mind that the general antimicrobial efficiency of a surface should be considered as the combined effect of many factors instead of a single one. Duday et al used plasma polymerized organosilicon coatings as a reactive layer for the immobilization of nisin onto steel surfaces [15]. In addition to an effective bacterial reduction, the organosilicon-based surfaces were also very stable after several cleaning cycles.

Among the different antimicrobial agents, gaining the most interest is the use of metal compounds. In most cases, metal components like silver, copper and zinc occurring in the form of ions, nanoparticles and microparticles are applied because of their pronounced oligodynamic and biocidal activity. Silver-loaded cotton/polyester fabrics with antimicrobial activity were prepared by Kostic et al via a two-step process: the raw fabrics were exposed to an air DBD plasma for surface activation, and then immersed into an aqueous silver nitrate solution for silver sorption [16]. It was demonstrated that both the treatment time and the ageing time strongly affected the silver ion sorption of the fabrics. Maximum silver sorption was found 7 d after the plasma treatment of the fabrics. Antimicrobial activity of the silver-loaded fabrics was determined after one or two washing cycles with laundry detergent. In spite of a slight decrease after the first washing cycle, the antimicrobial activity of the silver-loaded fabrics was stable afterwards. This
method was intended to be used for the preparation of specific textiles like rubber footwear lining aiming at an antimicrobial activity and improved wearing comfort. In addition, a similar method was used to prepare antimicrobial viscose fabrics with the incorporation of silver or copper ions on the surface using a AgNO₃ solution and CuSO₄ solution respectively [17]. It is interesting to note that water sorption of the DBD treated samples exhibited no change after 6 months. Sorption of silver ions increased up to 100% after 7 d ageing, whereas that sorption of copper ions decreased with any further ageing.

Due to their large surface-to-volume ratio and their small size, many metal or metal-based nanoparticles, such as silver nanoparticles, copper nanoparticles, gold nanoparticles (AuNPs) and zinc oxide nanoparticles, have emerged as a new generation of antimicrobial agents for diverse applications [18–20]. Vu et al incorporated silver nanoparticle (AgNP) onto the surfaces of polyamide 6.6 (PA) fabrics by a two-step process: raw fabrics were pre-treated by an air DBD plasma for surface activation, and then immersed into AgNPs dispersions for AgNPs incorporation [18]. It was confirmed that plasma pre-treatment could remarkably increase the content of dispersed AgNPs onto the fabric surface. Dispersions with three sizes of AgNPs (10 nm, 20 nm and 50 nm) were prepared to study the effect of the nanoparticle size on their adsorption in plasma pre-treated PA fabrics. It was found that AgNPs of small sizes exhibited high adsorption in the fibre surfaces. In addition, AgNPs were also incorporated onto cotton textiles with the aid of a low pressure CF₄ plasma pre-treatment [20]. The plasma treatment preserved the colour and the mechanical properties of the cotton textiles and stimulated the adhesion of silver nanoparticles on the fabric surface due to the plasma etching effect. Recently, Taheri et al developed a sophisticated method to obtain AgNP based antimicrobial coatings, in which the AgNPs were capped with mercaptosuccinic acid (MSA) in solvent processes. Then they were grafted onto an allylamine plasma polymerized (AApp) surface [21]. The AgNPs were incorporated into the plasma polymers by electrostatic immobilization of the nanoparticles and were functionalized by carboxyl acid groups to an amine group rich interlayer prepared by plasma deposition. The MSA surfactant could also reduce the oxidation rate of AgNPs and prolong the lifetime of the functional coating. In addition to high antimicrobial activity towards a broad range of bacteria, the films exhibited no toxicity to primary fibroblast cells and there was no significant effect on the innate immune cell function.

An air DBD plasma pre-treatment and air diffuse coplanar surface barrier discharge (DCSBD) plasma pre-treatment were compared by Radic et al to study the effect of different plasma pre-treatments on the incorporation of AuNPs to polypropylene (PP) nonwoven materials [19]. It was found that DCSBD plasma treatment introduced more hydrophilic functional groups, whereas DBD plasma treatment exhibited more pronounced morphology changes of the surface. Compared to grafting of oxygenated functionalities, an increase of the roughness and the ‘porosity’ of the surface, caused by stronger plasma etching was the main reason for a large impregnation of AuNPs on the samples.

3. Plasma polymerization of antimicrobial nanocomposite films

Besides grafting antimicrobial agents on material surfaces, materials with antimicrobial activity can also be engineered by the deposition of antimicrobial nanocomposite films onto the material surface. Nanocomposite films, where antimicrobial nanoparticles are incorporated into a polymerized matrix film, have been successfully deposited by various methods. The method of plasma polymerization has been explored as an effective approach to induce antimicrobial activity on material surfaces. Nanoparticles can be incorporated by a variety of methods including the sputtering of a bulk metal, the on-site reduction of a surface attached metal cation or the direct introduction of nanoparticles into the plasma polymerization zone. In accordance with the state-of-the-art, two types of nanocomposite films have been reported in the literature: films with nanoparticles incorporated in the volume of the matrix; and films with a multilayer structure.

3.1 Antimicrobial nanocomposite films with matrix structure

Nanocomposite films with a matrix structure are synthesized by the simultaneous incorporation of nanoparticles and the growth of the matrix. The deposits typically show a full spread of nanoparticles throughout the bulk structure of the nanocomposite film. Deposition of silver nanocomposite thin films based on an organosilicon matrix has been well studied by associating plasma polymerization and simultaneous silver sputtering in a single step process by Despax et al using hexamethyldisiloxane (HMDSO) as a precursor [22–26]. In their setup as the source of silver nanoparticles, a silver radiofrequent (RF)-power electrode was exposed to an Ar plasma. The balance between silver sputtering and plasma polymerization was monitored through a pulsed HMDSO mass flow rate [24]. Nanocomposite films with silver content ranging from 0 to 32.5 at.% were obtained under different plasma process conditions. It was found that film properties (silver content, nanoparticle size and matrix composition) could be controlled through varying the processing parameters, like HMDSO pressure, plasma dissipated power and bias voltage [25]. Under certain operational conditions, AgNPs were homogeneously distributed inside the bulk matrix [22, 25]. For nanocomposite films with different silver concentrations, the ageing process in a saline solution exhibited two different ageing mechanisms: for coatings with low silver content (7.5 at.%), the silver amount decreased at the surface but the coating thickness was not changed, whereas for the coatings with high silver content (20.3 at.%), matrix erosions were observed together with a reduction of the silver content [26]. Next to organosilicon matrices, different types of matrix compositions were tested. Functional hydrocarbon films were also used as matrix for the incorporation of AgNPs to obtain a nanocomposite coating [27, 28]. Nanocomposite films were deposited by simultaneously sputtering a silver electrode and polymerization of films with CO₂/C₂H₆ or NH₃/C₂H₄ mixtures as gaseous monomers. It was found that the silver content, the
AgNPs morphology and their distribution in the matrix could be controlled by variation of the plasma dissipated power, the gas ratio and the coating thickness [27]. A high CO$_2$/C$_2$H$_4$ ratio results in an increased silver content but small sized particles in the coating. A high power input in the process led to an increase of the incorporated silver content and large particles in the coating. In general when they were immersed in water, the nanocomposite coatings released mostly bound Ag nanoparticles in the coating. Up to a certain extent the type of monomer mixture, CO$_2$/C$_2$H$_4$ or NH$_3$/C$_2$H$_4$, also affected the silver release kinetic properties of the nanocomposite coatings [28].

Besides silver, other metals like copper and platinum were also used as sputtering electrodes for the deposition of nanocomposite films [29]. Daniel et al synthesized an antimicrobial nanocomposite, consisting of a copper containing organosilicon thin film, on stainless steel [29]. It was found that antimicrobial activity was strongly dependent on the content of incorporated copper in the nanocomposite coatings.

Electrode sputtering and plasma polymerization can also be spatially separated for the deposition of nanocomposite films as suggested by Peter et al [30]. In their device, silver clusters were generated in a gas aggregation cluster source (GAS) and directed as a focused beam into the downstream plasma region where they were incorporated into nanocomposite films. The main advantage of this method is the avoidance of complex interference between sputtering (for generation of AgNPs) and plasma polymerization (for the matrix formation). Therefore, the two processes can be more precisely controlled as independent steps of the whole procedure. The authors also pointed out that this system can provide a high deposition rate of composite materials within a wide range of metal clusters concentrations.

Most of the studied plasma-assisted methods for manufacturing antimicrobial materials are based on the use of low pressure discharges. Operating the plasma source at low pressure provides a way to generate a large-scale uniform discharge but has certain drawbacks mainly related to the need for pumping units and low pressure reaction chambers. Another approach tested by a number of groups is the use of atmospheric pressure plasmas. Atmospheric pressure plasma jet has been used for the deposition of antimicrobial nanocomposite coatings, as reported by Beier et al [31]. Silver nanocomposite films were directly deposited on glass surfaces using an open air plasma jet, in which HMDSO was fed as an organosilicon precursor and a silver nitrate solution was sprayed above the substrate. AgNPs were in situ generated in the plasma region and incorporated into the downstream films. It was suggested that, instead of silver oxide, mainly pure silver nanoparticles were created by the plasma. AgNPs, with various sizes up to about 100 nm, were homogeneously incorporated into the plasma polymers. In washability tests, the nanocomposite films showed a significant removal of loose particles at the first few washing cycles and then demonstrated a diffusion process of silver ions at later washing cycles. The diffusion of silver ions was suggested to contribute to the long-term antimicrobial activity. Recently, this method was used for three types of textiles to obtain antimicrobial wound dressing materials [31].

In our research group, Deng et al developed a single step dry process for the deposition of nanocomposite thin films with a high concentration of AgNPs using an atmospheric pressure plasma jet [32]. In that work, AgNPs of 100 nm size were introduced by passing a nitrogen gas flow through a feeding module into the direct current (DC) plasma jet sustained in nitrogen. A very high deposition rate of 350 nm min$^{-1}$ was achieved in these experiments which is almost an order of magnitude higher than common rates obtained in low pressure plasma sources. A series of SEM images in figure 1 shows the morphology of the nanoparticles in the films. It was shown that control of the morphology of AgNPs in the films can be achieved by the variation of the AgNPs feeding rate. Silver content in the films can be controlled from a few percent to more than 30%. Particle growth from nanosize to big clusters in the deposited films is explained by the aggregation of AgNPs during the deposition of the organosilicon matrix. It was found by x-ray photoelectron spectroscopy (XPS) measurements that the AgNPs are partially oxidized during the deposition process and they are also coated by a 50–150 nm layer of the matrix material. Antimicrobial assays of films performed by a macrodilution method using *Escherichia coli* and *S. aureus* strains demonstrated the antimicrobial activity of the deposited coatings. The described approach to engineer antimicrobial biomaterials based on the incorporation of nanoparticles in a coating matrix is highly attractive due to its simplicity, fast deposition and high bonding of both the nanoparticles and the matrix. Unfortunately, an associated drawback is the poor control over the release of antimicrobial agents, limiting the widespread use of the method. Obviously, operating parameters during deposition and the plasma source operating parameters affect the incorporation of nanoparticles into the matrix and so also the release of ions (i.e. active agents) from the coating structure. Recently, in order to overcome this bottleneck associated with a matrix approach, nanocomposite films with a multilayer structure were proposed.

### 3.2. Antimicrobial nanocomposite films with multilayer structure

The second approach to obtain nanocomposite antimicrobial films is based on the engineering of multilayer coatings, mostly with three layers in a sandwich structure. In such a structure, antibiotic compounds are enclosed between two polymer layers. Vasilev et al developed a tuneable antimicrobial triple-layer coating based on amine plasma polymerized films loaded with silver nanoparticles [33]. A 100 nm thick n-heptylamine (HA) plasma polymerized film was firstly deposited on a substrate, then loaded with AgNPs, which were synthesized by a local reduction of silver ions through solvent reactions, and finally covered by another thin layer of HA plasma polymer [33]. The amount of AgNPs loaded was determined by the immersion time in a silver nitrate solution,
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The release of silver ions from the nanocomposite films can be controlled by the thickness of the second HA plasma polymerized layer. With an appropriate thickness below 100 nm, the nanocomposite films could maintain efficient antimicrobial activity and support the growth of mammalian cells.

Other polymers, such as polytetrafluoroethylene (PTFE) and organosilicon coatings of a more complex composition have also been used to obtain multilayer nanocomposites [34, 35]. Alissawi et al deposited two layers of PTFE films to immobilize an AgNPs layer, which was deposited on the first PTFE layer by thermal evaporation from an alumina crucible [34]. They found that the strong dependence of silver ion release on the particle size leads to a significant redistribution of the composite morphology and a suppression of silver ion release with time. Due to the high polymer hydrophobicity, the second layer (also known as barrier layer) of the PTFE film exhibited insufficient water uptake which made high Ag ion release difficult. Later on, the same group tested plasma polymerized HMDSO films to replace the second layer of PTFE in the coatings [35]. They suggested that the water uptake behaviour of the nanocomposite films was strongly dependent on the chemistry and the thickness of the HMDSO coatings. In general, a high Ag ion release occurred in films with a high oxygen content and small thickness.

Airoudj et al reported on a type of mechanically responsive bioactive biomaterial based on multilayer plasma polymers [36]. Silver nanoparticles, as antimicrobial agent reservoirs, were trapped between two maleic anhydride plasma-polymer (MAPP) films. Silver ions were released through diffusion processes over the barrier layer. It was proposed that tensile strength generated cracks within the plasma polymer overlayers which could be used as diffusive channels for silver ion species. A tailored release of silver ions was achieved by mechanically reversible fragmentation in the plasma deposited polymer top-layer. Release of silver ions was reduced when the material returned to its initial length due to the closure of cracks. Additionally, they reported interesting effects...
of ageing and sterilization methods on these multilayer coatings [36]. It was found that the autoclave sterilization method significantly influenced the surface structure, but exhibited no influence on the chemical characteristics, whereas an electron beam sterilization method decreased the O/C elemental concentration ratio in the films, and there was no influence observed on topographic changes. A better antimicrobial efficiency against planktonic bacteria was observed for the materials after electron beam irradiation. The proposed approach has been tested on flat substrates whereas in practical applications in the health and hygiene fields, materials with complex 3D structures, like non-woven polyethylene terephthalate (PET) fabrics, are widely used. In our team, Deng et al developed PET non-woven fabrics with antimicrobial properties by firmly immobilizing silver nanoparticles via a double layer of plasma deposited organic films [37]. Nanosilver non-woven PET fabrics were prepared using a three step procedure as shown in figure 2.

At first, an organosilicon thin film was deposited on the surface of the fabrics using the plasma jet deposition system. This first layer of 70–350 nm is used as a reservation layer for the silver immobilization and to control silver nanoparticle adhesion to the PET fibres. In a second step the samples with the plasma-polymerized layer on top were immersed into a suspension of AgNPs in ethanol and hanged up for drying. In the final step, a second layer of organosilicon film was deposited using the plasma jet system. This second layer had a thickness 5–50 nm and was used as a barrier to prevent detachment of AgNPs from the substrate.

Samples with two different thicknesses of barrier layer were tested. The durability of silver nanoparticle bonding in the matrix was confirmed with the stability of the antimicrobial effect even after 10 washing cycles. Antimicrobial tests against *Pseudomonas aeruginosa*, *S. aureus* and *Candida albicans* revealed that samples with a 10 nm barrier layer have a stronger activity than those with a 50 nm barrier layer (figure 3(b)).

It is found that for the samples with barrier layers the antimicrobial activity is maintained on a constant level and this observation agrees with the XPS results (see figure 3(a)), confirming the stability of the AgNP bonding to the fibres and the positive effect of a barrier layer. This work reveals that a double layer immobilization of AgNPs in non-woven fabrics using an organosilicon matrix can provide a good way to control the release of silver ions with a high and lasting antimicrobial activity. Additional control of the antimicrobial activity of PET fabrics has been obtained by a variation of the immobilized silver concentration in double layer coatings [38]. PET samples were prepared under four different concentrations of AgNP dispersions, i.e. 1 mg ml⁻¹, 2 mg ml⁻¹,
5 mg ml\(^{-1}\) and 10 mg ml\(^{-1}\). The effect of silver concentration on key microbial growth kinetic parameters, such as lag time, reduction rate and final cell concentration, has been studied through bacterial growth curves as presented in figure 4.

Recently, Nikiforov et al. explored the validity of the plasma-assisted deposition of antimicrobial coatings containing different types of nanoparticles (AgNPs, CuNP, ZnONP) on non-woven PET fabrics [39]. Figure 5 represents the surface element composition of the treated fabrics.

The appearance of Ag, Cu and Zn peaks in the XPS spectra for the samples after nanoparticle incorporation reveals that the corresponding nanoparticles were successfully merged into the materials. It was confirmed that the samples with nanoparticles incorporated exhibit an effective antimicrobial activity against \(E.\ coli\) and \(S.\ aureus\). ZnONPs containing coatings possess a lower efficiency: a bacterial reduction of about 85\% was observed compared to a 97\% reduction for materials with AgNPs or CuNPs embedded. The latter behaviour was attributed to the difference in mechanisms involved in the antimicrobial action of the specific nanoparticles [40–43].

4. Conclusions

Plasma-assisted deposition of antimicrobial nanocomposite coatings is extensively investigated in view of new biomedical applications and continues to show promise. Different strategies including plasma-assisted surface grafting of antimicrobial components and plasma polymerization of antimicrobial nanocomposite films have been developed in the past decade. Although significant progress has been made in engineering such antimicrobial nanocomposite coatings by plasma-assisted methods, further research is required to fully understand the influence of surface morphology, the influence of the chemical composition of the deposited films and the influence of the nanoparticle properties (size, shape and type) on the bactericidal activity of the deposited coatings. Therefore, future interdisciplinary research and collaboration between scientists with different backgrounds and expertise ranging from physics, chemistry, nanomaterials and microbiology are necessary for the final success of the use of functional nanocomposite films for ‘real life’ applications. This approach is the only way that could lead to engineering a new class of biomaterials with high antimicrobial efficiency.

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