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Diseño nanotecnológico de superficies con propiedades antibacterianas: el grafeno

Nanosurface design with antibacterial properties: graphene

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Abstract

In recent years, nanotechnology has emerged as an excellent tool for choosing materials for the architectural design of spaces that are sensitive to bacteria, such as hospitals, clinics, etc. This article deals with the main finishes. Graphene provides incomparable possibilities thanks to its properties. Its high bactericidal activity allows improving finishes.

Keywords: *Nanotechnology, graphene, oligodynamic effect, graphene oxide, reduced graphene oxide*

Resumen

La nanotecnología se está convirtiendo en los últimos años en una inmejorable herramienta en la elección de materiales para el diseño arquitectónico de espacios sensibles a las bacterias, como hospitales, clínicas, etc. El presente artículo hace un recorrido sobre las principales superficies antibacterianas. Siendo el grafeno un material que ofrece unas posibilidades inigualables gracias a sus propiedades, permitiéndonos mejorar los acabados superficiales por su alta actividad bactericida.

Palabras clave: Nanotecnología, grafeno, efecto oligodinámico, óxido de grafeno, óxido de grafeno reducido

1. Introduction

Every now and then, scientific transformations obtained through nanotechnology radically change the possibilities of solving the problems that people are facing. The nanoscale becomes a common space for any discipline and its applicability is evident in a large number of research fields. Currently, there are countless studies developing the opportunities that these new open fronts offer us (Serena, 2013).

The architectural design has been favored by nanotechnology, since it is a wonderful improvement for designs used on certain finishes, such as operating rooms and spaces that are sensitive to the possible transmission of pathogens affecting the users' health, both patients and the clinical staff.

The purpose of this paper is to study different surface areas with antibacterial properties and what the latest developments of nanotechnology has to offer in the finishing of surfaces that are sensitive to the presence of microbial life.

2. Antibacterial surface areas

The initial approaches concerning the election of materials to be used can provide very important tools to control infections, but it should be considered that all surfaces that come into contact with the users are an important focus of contamination; therefore, adequate cleaning and disinfection are necessary, together with an extreme care when it comes to choosing the materials that prevent the formation of bacterial colonies on their surface.

Consequently, surfaces with antibacterial characteristics must prevent the fixing of microorganisms (bacteriostatic effect) and if this occurs, they should be eliminated (bactericidal effect). Whether it works or not depends on several factors: type of substrate used, concentration of the active principle in the substrate, type of microorganism to be fought, type of existing light, room temperature, relative humidity, pH and oxygen.

Historically, lime has been used as the sole surface finish of vertical parameters with fungicide and disinfection properties, since lime is a highly alkaline substance, which is very effective against microorganisms, because it affects the outer membrane of bacteria. However, its mechanical and solubility properties make it incompatible with the cleaning and disinfection processes performed on any clinical surface.

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Additionally, in the natural world there are surfaces that protect themselves against the attack of bacteria though their morphology; specifically, three types of insects have been studied (Psaltoda claripennis; Diplacodes bipunctata; Gryllus firmus), where the surface of their wings have protuberances with the shape of micropillars, which despite the fact that they do not prevent bacteria from depositing on them, they do have a bactericidal effect (Pogodin et al., 2013; Ivanova et al., 2013).

Currently, there is a large number of finish typologies with antimicrobial benefits.

- ***Metal Surface Areas***

  Metal surfaces have certain properties that make them very interesting as antibacterial finishes, because they are uniform, with high physical-chemical resistance, low or zero toxicity and easy to clean; furthermore, some metals show an oligodynamic effect on microorganisms.

  Some metals have an antibacterial capacity in their pure state, while others need to be combined with other elements in order to obtain this quality. In other words, metals forming part of a matrix give the whole the oligodynamic property.

  Many of these metals are micronutrients, but in high concentrations they are toxic, since they can inhibit the cellular activity by changing the enzyme structure and damaging the integrity of the membrane, acting on the DNA or generating oxygen reactants.

  The most important metal ions that affect microorganisms are:

  Silver: it is one of the most toxic metals, its action mechanism can reveal itself mainly in three ways: as silver metal, which affects the surface of bacteria by inhibiting the breathing process, it also acts on the molecules' absorption and release mechanisms that are essential for bacteria; as silver nanoparticles (AgNPs), by releasing a large amount of silver ions; and as silver ion, it is the most used active principle of many surfaces, degrading the DNA of bacteria, inhibiting their multiplication and modifying the protein synthesis, which results in the death of bacteria.

  Copper: as in the case of silver, its bactericidal power has been known for thousands of years. The US EPA considers it the first antibacterial material. Given its malleability, it offers a great number of possibilities in clinical design, thereby constituting the basis of highly-contaminant elements due to their constant use, such as door knobs, plumbing accessories (water taps, shut-off-valves, etc.), bed rails, switch-on mechanisms (power switches), and so on. The copper activity produces changes in the metabolism of bacteria, by affecting their cell membrane and fragmenting the DNA (Lalueza, 2013; Yasuyuki et al., 2010; Sreekumari et al., 2005; Grass et al., 2011).

- ***Glass***

  The antibacterial action of glass is based on the addition of silver ion, which eliminates microorganisms as soon as they come into contact with the surface of the glass. Accelerated aging tests demonstrate that the effectiveness does not decrease over time.

  The possibilities of glass are not limited to its use in windows and doors; they can be extended to the antibacterial coating of wall faces (Cuoghi et al., 2012; Lalueza, 2013).

- ***Wood***

  In the market there is a compound of melanin and paper that uses the silver ion as an active principle, which allows making wood surfaces coated by this compound to prevent the growth of microorganisms (Li et al., 2010). There are also wood finishes that use a coating of zeolites doped with silver ion, with the same result (Lalueza, 2013).

- ***Plastic***

  The use of plastic as a surface finish, in addition to being used to contain or receive materials (packaging, containers), can also be employed as an architectural surface finish in flooring, wall faces, furniture, etc.

  There are two processes by which plastic becomes an antibacterial surface: by using inorganic doping agents, such as silver ion, and its slow release of ions, used in very sensitive spaces due to its elevated cost; or through the use of an organic doping agent, such as dichlorodiphenyl ether (triclosan), which is highly effective, but deteriorates when exceeding 260°C during processing (Hasan, 2013).

- ***Paints***

  The action mechanism of antibacterial paints is based mainly on their presence of silver ion in its composition, using vegetal oils as oxidative drying mechanism instead of the usual reducing or stabilization agents in paints (Kumar et al., 2008).

  There are paint doping agents, such as titanium oxide, copper oxide and zinc oxide, which also have antibacterial properties (Mateus, 2014).

- ***Fabrics***

  Fabrics with antibacterial behavior can be created by coating their fibers with silver nanoparticles, which have a high specific surface area, thereby having a large active front of ions (Maneerung et al., 2008; Khalil-Abad and Yazdanshenas, 2010). Fabrics with antimicrobial power are also produced, whose composition includes Ag-Zeolite, which is a crystalline aluminosilicate with silver ions that are gradually released (Matsuura et al., 1997).

- ***Ceramics***

  Among the antimicrobial ceramic surfaces there is the application of nanoparticles of titanium oxide; due to its photocatalytic effect, the presence of UV radiation is necessary, therefore, it can only be used in exteriors or places that are very much exposed to the sun (Cuoghi et al., 2012). Doping agents of metal oxides are also used with bactericidal purposes, both in the composition of *the ceramic matrix and that of glaze (Verné et al., 2009; Sultana et al., 2013).*

Currently, vitroceramic materials that, besides reducing the growth of bacteria, also inhibit their adhesion to the surfaces and prevent the formation of biofilms are also being developed (Cabal et al., 2014).

- ***Colorants***

  Some researchers have obtained colorants, to be applied in surface finishes, which prevent the proliferation of bacteria. These studies suggest the possibility of using gold nanoparticles and application technologies that would make the final product cheaper.

  The experiments were applied on silicone surfaces similar to those used in operating room instruments, and they showed a high bactericidal effect during the first 3-6 hours. The silicone did not modify their properties, kept its water-repellent characteristic and preserved the properties when being subsequently cleaned with alcohol (Wang et al., 2011).

Figure 1. *Tests on antibacterial paints doped with graphene at the Construction Science Institute Eduardo Torroja*

3. Graphene

It is made of single-atom carbon sheets, whose atoms are bonded together according to a hexagonal structure. It was first isolated and identified at the University of Manchester in 2004 (Novoselov and Geim, 2004), where researchers discovered that its properties make graphene a unique material.

Thanks to the rapid progress in the synthetization of graphene and its derivatives, a great number of researches have been carried out in many scientific fields, such as electronic devices, aeronautics, textile industry, food industry, sports, etc.

In addition to all these great qualities, graphene is a biocompatible material and does not allow the growth of bacteria on its surface, therefore making it a material with an important development potential in architectural surfaces that are sensitive to infections.

3.1 Antibacterial graphene

Most of the researches regarding its antimicrobial activity deal with graphene oxide and reduced graphene oxide, and not with pure graphene, since these compounds are much more active on the viability of bacteria.

Since the research by Dai et al. in 2008, concerning the use of graphene oxide as an efficient nanotransporter for the administration of drugs, several publications have concentrated on graphene as a key element of clinical researches, which range from the administration of drugs, biosensors, creation of biocompatible elements, ophthalmological devices to antibacterial materials (Shen et al., 2012).

Recent studies on the possibilities of graphene and its bactericidal effect are classified in papers that expose graphene derivatives directly to bacteria, and papers that study graphene in combination with a substrate or by doping it with another material.

Graphene – Bactericidal

In 2010, graphene paper was manufactured through the suspension of derivatives thereof and its subsequent vacuum filtration, where very interesting physical properties were obtained that combine mechanical flexibility and stiffness, and also have antibacterial properties. Moreover, it was demonstrated that both GO (graphene oxide) and rGO (reduced graphene oxide) nanosheets show a strong bacteriostatic and bactericidal effect, with mortality rates higher than 90% in only two hours of exposure with the proper concentrations, and equally affecting Gram-positive and Gram-negative bacteria. When bacteria come into contact with the graphene derivatives, they are affected on the exterior, deteriorating due to a strong oxidative stress, which causes the loss of the cytoplasm and their death. The activity of the rGO nanosheets was slightly lower than that of GO (Hu et al., 2010).

In 2011, the team formed by Liu et al. made a comparative study between four types of materials based on graphene: graphite (Gt), graphite oxide (GtO), GO and rGO. Therefore, Escherichia coli was used under similar concentration and incubation conditions, where the GO dispersion presented the highest antibacterial activity, sequentially followed by rGO, Gt and GtO. It was demonstrated that direct contacts between the outer part of bacteria and the graphene nanosheets severely affect their integrity, in a similar way as carbon nanotubes (CNTs), which also act by a synergy between physical and chemical principles, causing the release of intracellular contents in addition to interrupting with specific microbial processes, with the subsequent death of bacteria. The variables emphasizing these properties are solubility, dispersion, time of exposure, and the size of graphene nanosheets (Liu et al., 2011).

In 2012, studies addressed the interaction between chemically exfoliated GO nanosheets and the Escherichia coli bacteria, which demonstrated that as the time of exposure increases, functional groups containing oxygen were significantly reduced by the metabolic action of the surviving bacteria, through their glycolysis process; the surface of the material presented two fronts, the areas where the GO had not been reduced and those where it had been reduced, being the latter the areas with greater inhibition of bacterial growth (Akhavan and Ghaderi, 2012).

The research by Krishnamoorthy et al. in 2012 compares the effect of GO nanosheets on colonies of Escherichia coli and Streptococcus iniae. The GO inhibitory affects growth of both bacterial species for concentrations of 38 μg/ml and 29 μg/ml, respectively. From the results obtained, it was estimated that the GO antibacterial activity is based on the production of hydroxyl radicals, which attack the cellular walls of bacteria, causing their death. That is, reactive oxygen species are generated during the process, which lead to an oxidative stress exceeding the antioxidant defense capacity of the cells, causing damages in lipids, proteins and the DNA. It was demonstrated that with lower GO concentration, results were obtained in the Gram-positive before than in the Gram-negative, although the situation was inverse when bactericidal results were searched for, since the necessary concentrations were 100 μg/ml and 125 μg/ml (Krishnamoorthy et al., 2012).

Studies have also been undertaken regarding the Pseudomonas aeruginosa type of bacteria, subjecting them to the action of GO and rGO sheets, using beta-mercaptoethanol as a reduction agent to obtain rGO, where the GO antibacterial activity rate is higher than that which is produced with the rGO contact. Different concentrations were analyzed during two hours at 37°C, in the presence of P. aeruginosa cells, and as the concentration was increased, the activity did so too, although it was more active in the GO samples than in the rGO samples. It was observed that as of 150 μg/ml (peak of the maximum antibacterial activity) the viability was almost zero. Then, experiments were performed with the same concentration (75 μg/ml), but with different exposure times (1, 2 and 4 h), causing, in both cases, an oxidative stress and the fragmentation of the DNA, which entails a loss of cellular viability (Gurunathan et al., 2012).

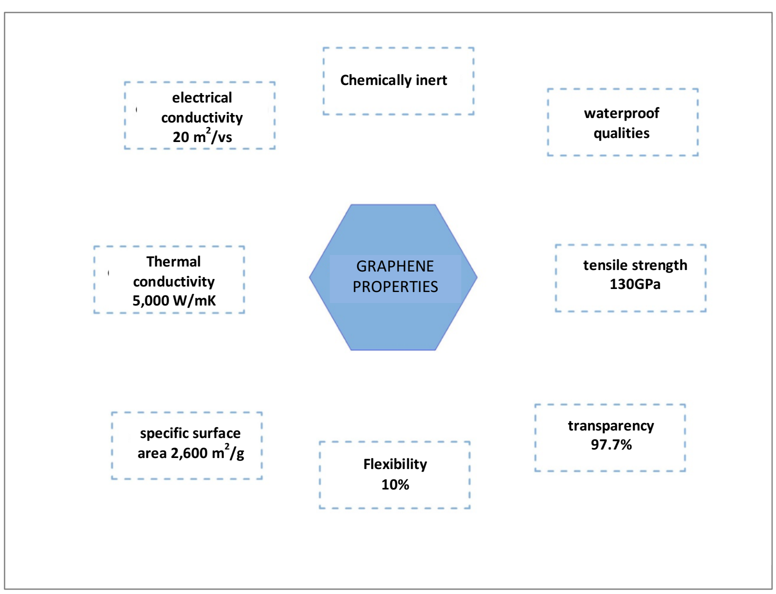

Figure 2. *Diagram of graphene properties*

Graphene – composite material

The large number of researches performed have started to delimit the aspects that have an influence on this activity, for example, the type of graphene used (G; GO; rGO), its concentration, exposure time, size of the nanosheets, but also the properties of the substrate used or the material used to combine it.

In 2010, the team formed by Akhavan et al. studied the bacterial toxicity of graphene nanosheets deposited on stainless steel substrates, both for Gram-positives and Gram-negatives in the form of nanowalls.

As we saw in the insects' wings, where the surface morphology was in charge of giving the surface a bactericidal effect, something similar occurs with the graphene nanowalls: when deposited on a stainless steel substrate an oxidative stress action is combined with a mechanical action, due to the effect produced by the nanowalls' sharp edges on the bacteria, since when they are deposited, they cause irreparable damage in the cell membranes.

Cell membranes have a slight negative charge that interacts with the nanowalls' edges, which are good electron acceptors, therefore they are attracted and the cell wall is damaged. The nanowalls' combined action makes graphene an excellent material, since it turns into a homogenous and efficient surface that interacts with microorganisms, due to a very good relationship between the edge length and its coating thickness.

It was demonstrated that the Gram-negatives having an outer membrane were more resistant than the Gram-positives. It was also observed that nanowalls obtained by GO reduction were more toxic than the non-reduced GO nanowalls (Akhawan and Ghaderi, 2010).

The University of Zhengzhou, in China, has developed a composite material based on a direct growth of silver nanoparticles on halloysite nanotubes (HNTs) and rGO nanosheets, improving the activity of silver nanoparticles when they act individually. The combined action of silver and graphene strengthens the antibacterial properties, both in Gram-negative and Gram-positive bacterial strains. It was demonstrated that this composite material (Ag/HNTs/rGO) acts much better than any of the parts separately (Yu et al., 2014).

Another research combining nanoparticles of silver and GO is being undertaken in the Science Faculty of Beirut, where the team of Sheet et al., 2013 are developing filters for purifying water (Sheet et al., 2013).

Other researchers have studied the behavior of rGO with biogenic silver nanoparticles (Bio-AgNPs), produced by the Fusarium oxysporum fungus, causing an adsorption of Bio-AgNPs on the GO sheets.

They act better on Gram-negative bacteria, especially against the Salmonella typhimurium strain, with a concentration of 2 μg/ml, inhibiting the formation of the biofilm in 100% of the cases after an exposure of 1 hour (Fonseca de Faira et al., 2014).

In 2014, in the Ceramics Institute of Shanghai, experiments were carried out with different types of substrates on which graphene was deposited to determine their antibacterial capacity. Researchers considered that the bactericidal efficiency might be affected depending on the substrate's conductivity; therefore, the idea was to make some samples with conduction, semiconductor and isolation materials. For the first sample, a copper substrate (conduction material) was used on which a continuous graphene film was deposited; a sample of germanium was used as a semiconductor material, on which a graphene film was also deposited. In both cases, the method of chemical vapor deposition at atmospheric pressure was used. Finally, for the isolation material sample, a SiO_2 substrate was used. The latter demonstrated that large amounts of viable bacteria were kept, but not so in the samples with copper and germanium, which evidenced that bacteria membranes suffered serious alterations with the subsequent loss of cytoplasm; the antibacterial activity was greater in the samples with copper substrate, so it was considered that cooper ions might have participated in the bactericidal action. In order to determine the possible combined action of copper ions, a mass spectrometry was carried out, which determined that there were no ions on the graphene sheet, so the bactericidal action was due solely to the contact between the graphene surface and the cell membranes. In fact, the graphene sheet on the copper substrate prevents its oxidation, acting as an efficient dissemination barrier.

It is believed that the conductivity of the substrate can affect the microbial response, when interfering with the breathing process of the bacteria, since they require acceptors of extracellular electrons and graphene is an excellent acceptor of electrons, so the microbial membranes can lose electrons constantly, causing alterations and the resulting death of bacteria (Li et al., 2014).

Consequently, GO and rGO improve their antibacterial capacity when using metal substrates or combined with doping agents such as silver, generating a synergy in the qualities of the material obtained.

ANTIBACTERIAL PRODUCT	Active Principle	Bacteriostatic	Method
Saltona claripennis	Natural		Surface morphology
Diplacodes bipunctata			Surface morphology
Gryllus firmus			Surface morphology
Metal surfaces			
Ag	Synthetic	X	Ag ion; AgNPs
Cu	Synthetic	X	Cu ion
Ti	Synthetic		TiO_2
Cd	Synthetic		Cd ion
Ni	Synthetic		Ni ion
Al	Synthetic		Al ion
Glass	Synthetic		Ag ion
Wood	Synthetic		Ag ion; Zeolites/Ag ion
Plastic	Synthetic		Ag ion; triclosan
Paint	Synthetic		Ag ion; TiO_2
Fabric	Synthetic		AgNPs; Zeolites/Ag ion
Ceramics	Synthetic	X	Metal oxides
Colorants	Synthetic	X	Ag ion
Black silicon	Synthetic		Surface morphology
Graphene	Synthetic	X	G film; GO; rGO; nanowalls; Ag/HNTs/rGO

Figure 3. *Table of the active principle of different materials in the presence of bacteria*

4. Conclusion

Microorganisms are the most ancient life form in our planet, consequently they have generated very versatile adaptation mechanisms for the colonization of surface areas over the years. With the antibacterial use of nanotechnology, the problem is approached from another perspective, since it fights microorganisms from different fronts, physical and chemical, making its implantation in the environment almost impossible.

The main lesson that nanotechnology teaches us is that, at a nanometric scale, the materials properties change drastically due to the quantum mechanics phenomena. Nanomaterials present a closer relationship between their surface and their volume, so boundary atoms grow in relation to the inner ones, thereby modifying the behavior.

Graphene is a nanomaterial with an incredible potential in the world of architecture, because it is capable of developing highly efficient solutions, both as surface finishes and as part of the composition of the products themselves, by generating new composite materials. As indicated above, a large number of publications are analyzing graphene with bactericidal purposes, but each method to obtain graphene sheets, each derivative obtained from it and each composite material confer different properties to the result, therefore it is important to systematize and classify each one of these materials.

Despite the fact that they have been under research for only ten years, graphene applications have grown exponentially. The constant progress made with the aim of obtaining a cheap and efficient synthetization will result, within a short time, in the entry of construction products to the market, whose main active principle will be graphene.

Graphene has a great development potential as a new tool in the fight against hospital-acquired infections, used as a new antibacterial surface and applied to the design of materials employed in sensitive places for patients and users.

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