Study of methodologies for the formation of a layer that inhibits the formation of bacterial strains in cardiac instruments

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Abstract. Implantable cardiovascular electronic devices are now an essential part of clinical practice in cardiology. The presence in a large number of adult population of problems in the electrical conduction system and several indications with multiple comorbidities has led to an increase in use of implantable cardiovascular electronic devices. However, the application benefit of these devices has been affected by the higher incidence of infections, which represent a serious complication that can lead to significant morbidity, mortality and cost for health centers due to antibiotic treatments, device replacement and longer hospitalization times. For this reason, the present analysis is oriented to possibility of reducing the dissemination of Staphylococcus Aureus by evaluating different techniques for adsorption of a vancomycin antibiotic film in implantable cardiovascular electronic devices. Through this analysis it is possible to establish a new alternative prophylactic method to avoid risks to the health of patients who have problems in the electrical conduction system, which are prone to becoming infected by Staphylococcus Aureus strains during the implantation of the device; in this way, infection can be controlled locally without requiring removal of the initial device and thus avoid an intracardiac compromise that leads to major complications such as endocarditis.

1. Introduction

Implantation of electronic cardiovascular devices in patients with heart failure is one of the invasive procedures that are most carried out today and has a fundamental role in clinical practice, despite the fact that its benefit has been affected by growing incidence of infections that are generated during surgical procedure [1]. The insertion or replacement of these implantable cardiovascular electronic device (ICED) represents a high rate of risk for patients who depend on their use. In case of first implant, factors that promote infection both locally and systemically prevail with or without intracardiac involvement, such as previous antibiotic prophylaxis procedures, patient asepsis, patient characteristics and the state of surgical instruments. Likewise, in case of implant replacement, the occurrence of infection increases due to predisposition of the patient as a consequence of previous antimicrobial treatment [1,2].

The onset of the infection is produced locally by a bacteria genus called Staphylococcus Aureus that is commonly found on the skin and that can migrate to the implant area, being treatable initially through targeted antibiotic therapy. However, if the infection spreads systemically due to contamination of the device and the electrodes, it can induce acute endocarditis, which represents a greater risk factor for the
patient’s health, therefore the result of contagion of the ICED implies in all cases a complete removal of the device [3].

Biomaterial from which implantable electronic devices are made has good biocompatibility and corrosion resistance properties, but its surface biological activity is prone to infection from septic agents. Taking this into account, there is evidence that it’s necessary to improve the protection of ICED in order to reduce infections caused by *Staphylococcus Aureus*, avoiding the replacement of the device and in its dissemination to the heart.

2. Background

First permanent pacemaker implants were made in 1954, and the first implantable cardioverter–defibrillators were made in 1980. The use of these cardiac electrostimulation devices (CED) has increased exponentially in the last 10 years due to technological advances expansion of its indications, longer life expectancy of population and survival rate of patients with different electrophysiological heart diseases [4]. According to the Colombian Society of Cardiology and Cardiovascular Surgery, the main microorganism related to this type of implantable device infections is *Staphylococcus Aureus* in 33%, since it is widely distributed throughout the human body [1].

Similarly, a study carried out by the Spanish Journal of Cardiology has indicated that *Staphylococcus Aureus* infection occurs early in 70% of implantations, and it is the most frequent cause for this type of infectious complications [5]. It is important to identify how the proliferation of this microorganism occurs in order to implement techniques that avoid dissemination and contamination of the device. Surgery is the most frequent, followed by an infection due to a lesion close to the pocket, mainly caused by mechanical erosions of the skin that can spread and compromise the device, in addition to a systemic infection of the bacteremia type, although this rarely involves the patient device except for when microorganism involved is *Staphylococcus Aureus* [3].

Local infection is the most common one presented in the ICED pocket, representing about 60% of cases and presenting very recognizable signs and symptoms since they are the same as the ones in a common infection: wound redness, itching, patient hypertermia, localized heat in the wound and sometimes lymphatic fluid leakage [6]. All these symptoms are the first evidence that an infectious process is occurring that can start a week after ICED implantation and, if the symptoms are detected in time, the infection diagnosis will lead to take a blood count for evidence a leukocytosis that indicates the presence of *Staphylococcus Aureus*. In this way, hospitalization and intravenous treatment are initiated to the patient in order to prevent the infection from spreading to the device and subsequently to electrodes [7].

Studies indicate that when pocket infection occurs, in 75% of cases it spreads to the device during the first weeks after implantation, which can lead the patient to a more complicated process such as systemic infection with or without cardiac involvement causing endocarditis [5]. The infection of the device implies that there is a hematogenous spread by the bacteria through the electrodes located in the heart chambers, which according to Garcia, et al. “It seems proven that microorganisms are capable of advancing through the cables even though there is only apparent manifestation at the level of the generator bag” [3], thus causing a bacterial endocarditis that implies in all cases the urgent patient hospitalization and complete removal of the device.

In Colombia, a study carried out by the Colombian Journal of Cardiology estimates that the incidence of infection in the first implant is 1.9 per 1,000 implanted devices, while in the reimplantation this incidence increases to about 5.32 infected devices per 1,000 with a mortality at 6 months between 18% and 35%. This increase in reimplantation is due to the patient’s predisposition to a new procedure, which leads to hospitalization and treatment of the initial infection that can be acquired again in a nosocomial way [8].

World Health Organization in its practical guide Prevention of nosocomial infections defines a nosocomial infection as “an infection that occurs in a patient admitted to a hospital or other health care establishment in whom the infection had not been manifested and was not in the incubation period at the time of hospitalization” [9]. Likewise, in a study carried out by the Spanish Journal of Cardiology
Some risk factors that predispose the patient to developing infection of the device have been evidenced, such as: the experience of the implanter, the formation of hematomas in the generator pocket, previous reviews by part of unskilled personnel, abandonment of previous cables and insertion of transient catheters [5]. For this reason, pre–surgical prevention techniques have been adopted in the insertion of ICEDs in order to avoid infection associated with the implant, which can lead the patient from hospitalization to complete removal of the device. Some of these prophylactic techniques include: recommendations described to the patient, pre–surgical antibiotic prophylaxis with a time prescribed by the institution before entering the procedure and correct asepsis and antisepsis of the medical and paramedical personnel who will perform and/or support the procedure [8, 10].

On the other hand, consideration should be given to reducing the high costs for health sector that generates the patient hospitalization and therefore a new implant due to the infection of the device, preventing other users from accessing the different health services [11]. In addition to this, there are also side effects to which the patient is predisposed in hospitalization such as psychological trauma due to a new intervention and the nephrotoxic risk that increases for patients with chronic kidney disease (CKD). Studies carried out in patients with ICED infection have shown that 50% of them had chronic kidney disease or acute kidney failure, which adds an additional risk to the patient hospitalization [8]. The use of intravenous vancomycin as an antibiotic for device infections treatment is the most efficient in the hospital setting, but this drug has high renal toxicity so when using vancomycin as an antibiotic for treatment of Staphylococcus Aureus, the activity of the patient's kidney should be monitored as well as other risk factors that may be associated with nephrotoxicity. Elimination of vancomycin is carried out almost completely through glomerular filtration, so adequate kidney function is crucial for complete elimination of the drug.

It is important to note that drug elimination in an adult with normal kidney activity occurs approximately 4 hours to 6 hours after being administered, but in an adult with renal failure, drug elimination can increase considerably up to 7.5 days. Therefore, it is important to control and monitor serum creatinine levels before, during and at the end of treatment with this drug in order to monitor the evolution of renal function and detect possible nephrotoxicity. In a study carried out by the Ibero Latin American Journal of Health System Pharmacy, 46 patients required for the vancomycin concentration in management were studied, from different services of a hospital, where the incidence of nephrotoxicity was 22 [12].

Therefore, antibiotic films are used as a new alternative prophylactic method to avoid health risks of patients who have problems in the electrical conduction system, which are prone to being infected by these bacteria during device implantation, so infection can be controlled locally without requiring removal of the device and thus avoid intracardiac compromise leading to endocarditis [13]. Therefore, different techniques are analyzed to deposit an antibiotic film on a surface of an implantable cardiovascular electronic device.

3. Discussions
Layer-by-layer (LbL) assembly, which corresponds to sequential deposition of species that interact on a substrate, emerged as a versatile, simple, efficient, reproducible and flexible bottom–up technique that has become one of the most used techniques for coating various types of substrates, including flat surfaces, spherical objects, porous matrices and highly curved surfaces [14]. LbL assembly is one of the most promising ways to make multilayer thin films and coatings with precisely controlled composition, thickness and architecture at nano-scale [15].

LbL assembly has several advantages such as: low cost and easy process implementation, incorporation of different materials through different solutions (polyelectrolytes, nanoparticles or polymers with similar charges, among others) and formation of uniform coatings on flat and curved surfaces [15]. However, there are different generalized variables that affect the final coating such as: intermediate rinsing, layering process, compatibility between solutions, compatibility of substrate and solutions and preparation prior to application, among others [16]. In addition to this, generally the process of layer accumulation is carried out through electrostatic attraction phenomena as shown in
Figure 1, where the mechanism is mainly given by interaction between species with opposite charge (in addition to hydrogen and covalent bonding) and is affected by variables such as: pH, temperature, solvent, ionic strength and type and properties of each polyelectrolyte [14, 15].

To carry out the LbL assembly it is possible to use different methods such as: dip coating, spraying, centrifugation and electromagnetism, among others, the first three being the most common [16]. Dip coating consists of submerging a substrate alternately in aqueous polycation and polyanion solutions in a cyclical manner, employing a rinsing step between the deposited layers to remove unbound material and avoid contamination of the subsequent solution. This method is a simple technique that allows to cover substrates of almost any shape and size, where solutions concentration and rinse time are taken into account. However, another interest parameter is the immersion time required for a polymer adsorption step, which is between 15 minutes and 20 minutes and makes it a relatively slow process [14].

Generally, initially a positively charged solid substrate is immersed in a solution composed of an anionic polyelectrolyte, allowing adsorption and the electrostatic force generated together to build a first layer. Subsequently, an intermediate rinse is carried out with water that does not represent any layer but allow to keep the surface active to continue with the process without reducing the attraction between the positive or negative charges of the deposited layer [16]. Finally, the substrate is immersed in a cationic polyelectrolyte solution, restoring the original surface charge. Through repetition of these cycles it is possible to achieve a desired number of layers in curved planes, controlling the thickness of the multilayer throughout the substrate [15].

Spraying technique is a simple method that consists of sequentially spraying polycation and polyanion solutions onto a solid substrate (which may or may not be flat) to assemble multiple layers of coating. In this technique the thickness of the deposited film is influenced by concentration of the polymer solutions, the spraying flow rate, spraying time (a few seconds per coat) and waiting time in cases where intermediate rinses are carried out [14].

It should be noted that, compared to dip coating, it is possible for spraying to omit the rinsing step, leading to thicker films without altering their quality and further reducing the deposition time. In addition to this, the assembly of this process is fast and suitable for automation and expansion at an industrial level. Finally, the properties of the film such as morphology, uniformity and chemical composition can be adapted to be similar to those prepared by dip coating [14].

Finally, LbL assembly by centrifugation is based on rotation of the solid substrate to facilitate the polymers deposition. In this case, it is possible to apply the polycation and polyanion solutions on a rotating substrate or on a static substrate that is rotated once the polymers have been deposited [14]. Its main advantage is the deposition time, which is less than 30 seconds per layer. However, the great limitation of this technique is that it is not possible to use it to deposit uniform films on surfaces that are not flat or that have complex shapes, and additionally it is not possible to coat surfaces of large areas.

According to the background analysis, LbL assembly techniques for manufacture of multilayer films offer adequate results and economy in its manufacturing process. The most relevant characteristics of the three main LbL assembly techniques are compiled in Table 1, where it is possible to easily compare them in order to select the most suitable technique for a specific application.

Additionally, selection of coating materials for LbL assembly is limited to low molecular weight polyelectrolytes, less than 65 kDa, due to the restricted space for adsorption of the polymer chain [17]. An example of this process is the study carried out by Wang et al., where alginate (polyanion) and chitosan (polycation) were used to form a film on a metallic substrate in order to generate a protective barrier against corrosion. In this case, chitosan and alginate were used to encapsulate Artemisinin with an encapsulation efficiency of 96%, while for Docetaxel the efficiency was between 94% and 97% [18].

These polymers, and in general polymers of natural origin, are of great interest in the biomedical field because they present multiple characteristics such as: biodegradability, since they do not present adverse effects for environment or human beings; biocompatibility and non–toxicity, since almost all of these materials are carbohydrates in nature and are composed of repeating monosaccharide units;
biosafety, since they do not have side effects while synthetic polymers can produce side effects; low cost compared to synthetic polymers; and availability because they occur naturally in large quantities.

These characteristics cause natural polymers to generate increasing attention in conjunction with the LbL assembly, since this technique can be used to design and generate systems for biomedical applications where their biocompatibility is a priority. Additionally, an innovation is generated in application types where research field is very large, from devices design to their evaluation under in vitro and in vivo physiological conditions [14].

**Figure 1.** Sequential deposition of polycations and polyanions during LbL assembly.

**Table 1.** Main characteristics of LbL assembly techniques.

| Variable                          | Dip coating      | Spraying          | Centrifugation |
|-----------------------------------|------------------|-------------------|----------------|
| Deposition time                   | Long (minutes)   | Short (seconds)   |                |
| Film size                         | Large surfaces   | Large surfaces    | Up to 10 cm    |
| Deposition surface                | Flat, rough, complex shape, three–dimensional | Flat, rough, complex shape | Flat |
| Parameters that influence the film thickness | Immersion time, solution concentration and rinsing time | Solution concentration, spray flow, spray time, evaporation time and rinsing time | Spin speed, solution concentration and rinsing time |
| Visual appearance                 | Opaque films     | Transparent films | Transparent films |

4. Conclusions

Within the background, relevant works were found that use methodologies to impregnate drugs in devices or structures that are implanted inside the body in order to keep it sterile against bacterial activity. Analysis of these studies demonstrated high efficiency in controlling inhibition against infectious agents. Taking into account the performance of each of the variables studied in the present analysis of the background, it is determined that the layer-by-layer assembly technique by dip coating offers greater versatility in the elaboration of multilayer films and can be used for the deposition of the antibiotic film on the surface of an implantable cardiovascular electronic device.

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