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Full Length Article

# Lincomycin–embedded PANI–based coatings for biomedical applications

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ABSTRACT

We report on the successful laser transfer of biocompatible composite coatings based on polyaniline (PANI) embedded with magnetite (PANI-$Fe_3O_4$) and Lincomycin hydrochloride (PANI-Lincomycin) or Lincomycin-functionalized magnetite (PANI-$Fe_3O_4$@Lincomycin) by matrix assisted pulsed laser evaporation (MAPLE) technique.

The physico-chemical investigations revealed relevant data regarding the stoichiometric deposition, morphology and topography of the as-deposited coatings. Regarding the MAPLE coatings, the FTIR studies evidenced the vibrational bands characteristic to pristine PANI material, while the SEM investigations unveiled a preferential particulate morphology (with aggregates shape and size depending on the deposited material). Additionally, the AFM measurements indicated variations of RMS value, following the Lincomycin and magnetite incorporation. The wettability measurements displayed a hydrophilic behavior of the synthesized coatings, while the electrochemical studies emphasized an enhanced resistance against corrosion in simulated body fluid when compared with bare Ti.

Cellular viability, immunofluorescence and SEM results proved that the MAPLE coatings were suitable materials for beneficial adhesion, spreading and proliferation of osteoblast-like cells (MG-63). Moreover, an increased efficiency was evidenced against *Staphylococcus aureus* biofilm development.

The multifunctional properties of the laser processed composite coatings – confirmed by cumulative biocompatible, antimicrobial and anticorrosive behaviors – recommend them as promising solutions for biomedical applications.

## 1. Introduction

Current medical and healthcare background and the specialized clinical studies focus more and more on the improvement of patient life quality and to increase patients' lifetime. Such a particular request is strongly transposed into an intensive research activity that aims to reduce or eliminate the failure risks related to the performed medical act.

On this line, for a long time, a significant part of the medical research was oriented towards the development and improvement of implantable medical devices (IMDs), which are clinically used in a wide variety of applications and whose performances are considered to be critical for the patient's health status and quality of life [1–3]. When selecting a medical device, the healthcare specialists must consider several key aspects, such as treatment efficiency and cost, as well as biocompatibility and risk of infections, which could lead to severe complications [4–6]. The main factors incriminated in IMD-associated infections is the formation of microbial biofilms, which represent an alarming concern due to the actual increasing resistance and tolerance of such attached microbial communities to conventional antimicrobial strategies [4,7]. The clinical relevance of microbial biofilms is mainly related to the complex composition and pathophysiological evolution of embedded microorganisms, but also to the wide variety of microbial pathogens which may form such particular communities. Such microbial agents responsible for the biofilm related-infections include Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus viridans*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*) or yeasts (*Malassezia* sp. and *Candida* sp.) [6,8,9]. In

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order to overcome this problem, novel nanotechnology-based approaches (such as surface modification or protective coverage of the IMDs) are currently developed in order to prevent the microbial biofilm formation [4,10,11].

The coatings applied for the improvement of IMDs surfaces by using restorative materials are the cumulative result of the tremendous development and progress reported in medical industry and materials science, which significantly changed the conventional strategies implied in substituting or improving any part of the body function [12]. Furthermore, such multifunctional coatings can provide enhanced surface properties such as anti-corrosion behavior, wear resistance and suitable support for cell and tissue growth [12–14].

In the last two decades, the interest was focused on a new generation of "smart" biomaterials, the so-called electroactive biomaterials, which allow the direct distribution of electrical, electrochemical and electromechanical incitement to cells [15–17]. Conductive polymers (CPs), known as the "fourth generation of polymeric materials", display no conductive properties in the neutral state and possess the great advantage of easy-tunable multifunctionality, by specifically tailoring their chemical, electrical and physical properties [15,18,19]. In particular, polyaniline (PANI), the second most studied CP after polypyrrole, can occur in various forms depending on its oxidation level: pernigraniline (fully oxidized), emeraldine (half-oxidized) and leucoemeraldine base (fully reduced) [15,20,21]. The most stable and conductive form of PANI proved to be the emaraldine base, which is non-toxic in nature, as well. [15,20,22]. Taking into account the product conductivity and stability, the most common dopants for protonation of PANI are hydrochloric and sulfuric acids [23]. Furthermore, there are also reports which support the *in vitro* and *in vivo* biocompatibility of PANI coatings with potential applications in tissue engineering, biosensors research or even for electrical stimulation of nerve cells [24–26].

Nowadays, composite materials based on CPs and magnetic nanoparticles have attracted the worldwide scientific attention, thanks to their promising potential for various technological applications, such as electromagnetic shielding devices [27,28] and protective coatings for IMDs [29–31]. De Araújo et al. reported that $Fe_3O_4$ magnetic nanoparticles serve as an oxidant agent for PANI under UV irradiation and the obtained nanocomposite PANI-$Fe_3O_4$ is a magnetic and conducting promising material [29].

PANI-$Fe_3O_4$ composite material is an interesting combination which can be successfully implied in various biomedical applications, including biosensors, substrates for neural prostheses and drug delivery systems, by electrically modulated properties [32–34]. One attractive and required application could be the deposition of PANI-$Fe_3O_4$ composite as thin coatings for IMDs protection. Moreover, the coating functionality could be improved by the addition of a drug in the composition, in order to provide IMDs prevention and protection against external stimuli or biofilm formation. The external modulation of the electric or magnetic field is a parameter that could adjust the quantity of drug releasing, enhancing the therapeutic effect and decreasing the toxic effects [33,35,36].

Lincomycin, a member of the lincosamide group of antibiotics, has been used in the treatment of diseases caused by Gram-positive bacteria, for the last four decades [37–39]. The complex material PANI-$Fe_3O_4$-antibiotic is of real interest for the IMDs research field, but the question regarding its deposition as thin coatings is still active. In this regard, a valuable laser technique, namely the matrix assisted pulsed laser evaporation (MAPLE) method, which is a non-contact strategy suitable for laser processing of both inorganic and organic materials with promising applications in biosensors, drug delivery systems and IMDs research fields, proved to be ideal for the deposition of such coatings [40–42].

This work reports on the synthesis of composite coatings based on polyaniline and magnetite nanostructures in which was embedded Lincomycin hydrochloride and their evaluation as potential innovative systems for controlled drug release in external-activated electric or magnetic field. In order to emphasize the multifunctionality of the synthesized coatings, the biological performances were evaluated. To achieve this goal, the biocompatibility and the antimicrobial activity were assessed, along with their physico-chemical characterization.

## 2. Materials and methods

### 2.1. Materials

The anhydrous ferric chloride ($FeCl_3$), heptahydrate ferrous sulfate ($FeSO_4{\cdot}7H_2O$), ammonia solution ($NH_3$, 25%), polyaniline (emeraldine base) ($M_w \sim 50000$) and Lincomycin hydrochloride were purchased from Sigma-Aldrich (Germany). The solvent used for the preparation of MAPLE targets was dimethyl sulfoxide (DMSO) that was acquired from Merck.

The products (paraformaldehyde, hexamethyldisilazane (HMDS) and non-essential amino-acids (NEAA)) required for the biological investigations were purchased from Sigma-Aldrich. Other products, such as Minimum Essential Medium (MEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin mixture (P/S) were procured from Biochrom Ltd. (Merck Milipore, Germany). The MTS kit used for the cellular viability evaluation (CellTiter96® Aqueous One Solution Cell Proliferation Assay) was provided from Promega (USA). AlexaFluor® Phalloidin solution and Hoechst® 33,342 stains used for the immunofluorescence evaluation were procured from Invitrogen (Thermo Fisher Scientific, USA). American Type Culture Collection (ATCC®, UK) was our provider for the human-derived osteoblasts-like cells (MG-63) and for the microbial pathogens, namely *Staphylococcus aureus* (*S. aureus,* ATCC 25923), *Escherichia coli* (*E. coli,* ATCC 25922) and *Candida albicans* (*C. albicans,* ATCC 10231).

The pristine and composite coatings were deposited onto transparent infrared (IR) (1 0 0) silicon, glass slides ($10 \times 10\,mm^2$) and commercial titanium disks, grade 4 purity (12 mm in diameter and 0.2 mm thickness) for physico-chemical characterization, antimicrobial evaluation and biological assays, respectively.

### 2.2. Synthesis of magnetite and $Fe_3O_4$@Lincomycin nanoparticles

The simple and antibiotic-functionalized magnetite nanoparticles ($Fe_3O_4$ and $Fe_3O_4$@Lincomycin, respectively) were synthesized by means of wet chemical co-precipitation strategy, using, in this respect, an adjusted protocol described in Refs. [43] and [44]. Both metallic precursors ($FeCl_3$ and $FeSO_4{\cdot}7H_2O$) were dissolved in ultrapure water and the resulted solution was dropwisely added into a $NH_3$-containg aqueous solution, under continuous stirring. Once the formation of the metallic particles occurred, the color of the resulted mixture gradually changed to black. The collected precipitate was subjected to a washing treatment with ultrapure water and dried at room temperature condition. For the antibiotic-functionalized particles, we used the same synthesis recipe, but the Lincomycin hydrochloride was suspended into the ammonia-containing solution.

### 2.3. Synthesis of MAPLE coatings

The MAPLE experiments were conducted in a stainless steel reaction chamber at 1 Pa pressure by using a KrF* excimer laser, model CompexPRO 205 from Coherent ($\lambda = 248$ nm, $\tau_{FWHM} \approx 25$ ns). Within our experiments, the deposition parameters were maintained constant: the target-substrate distance (5 cm), the laser fluence ($300\,mJ/cm^2$) and the repetition rate (20 Hz). The applied number of pulses was of 70,000 (for PANI and PANI-$Fe_3O_4$ samples) and 100,000 (for PANI-Lincomycin and PANI-$Fe_3O_4$@Lincomycin samples), respectively. Furthermore, the laser energy distribution into the laser spot was accurately controlled using a laser beam homogenizer.