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BIOMATERIALS AND NOVEL TECHNOLOGIES FOR HEALTHCARE

a cura di

Julietta V. Rau, Franca Rossi e Marco Ortenzi

PROCEEDINGS

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3rd biennial International Conference BIOMAH

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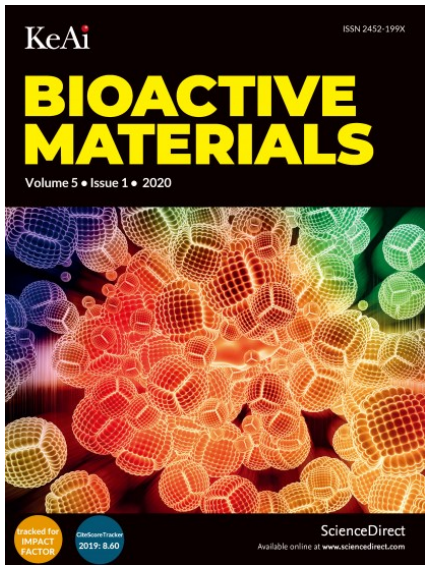

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Bioactive antibiotic-eluting hydroxyapatite-based coatings obtained by electrophoretic deposition on titanium

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Keywords: biomaterial, electrophoretic deposition, antibacterial activity, bioactivity.

Introduction

The osteoarticular implants are meant to enable complete recovery of the lost function and secure successful implant fixation. Inevitably, problems might occur that lead to implant failure due to infection at the implantation site and aseptic loosening. The aging population worldwide is in dire need of these medical devices, but especially vulnerable and prone to infections due to compromised immune systems by the implant presence and subsequently even a few bacteria could easily latch to the solid substrates, and rapidly multiply to form a highly resistant biofilm. These problems could be so severe and potentially life-threatening wih the occurrence of multidrug-resistant bacterial strains. The damage that methicillin-resistant *Staphylococcus aureus* can cause to the human body is sadly evidenced by many medical reports. Therefore, a successful design of an implant should incorporate a potent drug e.g., wide spectrum antibiotic and the composition of the biomaterials that would secure functionality while promoting lasting osteointegration. Since osseointegration implies a direct, firm, and lasting bonding of the periimplant bone to the implant, the process depends on surgical positioning of the device, followed by the cellular response at the bone-implant interface, crucial for the bone healing. Therefore, surface of an implant has to be modified so it would provide osteoinductive and antibacterial properties at the same time.

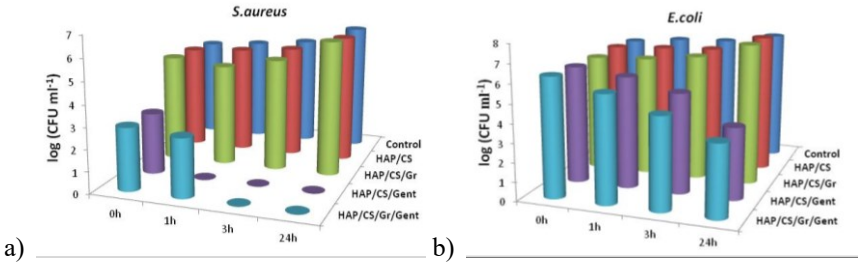
Due to similarity in chemical composition, synthetic hydroxyapatite (HAP) is commonly applied as a metallic implant surfaces' modifier or as a bone filler material since its porous structure could have favorable effect on tissue integration of the implants. Hence, a good combination of HAP biocompatibility and the excellent mechanical properties of metals are considered a promising approach to fabricate more suitable bone implants. However, HAP itself shows poor mechanical properties and is very brittle, so it is necessary to develop HAP-based composites with the addition of certain polymers. The spontaneous formation of passive film is crucial for the long-term stability of metals in human body. The conditions of simulated physiological solutions have been enriched by the additions of synovial fluid, which is the natural environment of joint. The most recent research delt with the problem of drug release and synergistic effect of chitosan and gentamicin showing the effective drug release from the polymeric component of the composite coating assembly on the surface of Ti [1-3]. Chitosan (CS) is a natural polymer, a cationic polysaccharide, obtained by partial deacetylation of chitin. CS shows biocompatibility, biodegradability and antimicrobial action while providing good adhesion and could act as a carrier of antibacterial agents. One of the antibiotics that are often used to treat in-hospital complications is gentamicin. Gentamicin (Gent), a water-soluble aminoglycoside antibiotic, is known to have very potent antibacterial activity for the treatment of wide range of infections, caused by both Gram-negative and Gram-positive bacteria. The reason for gentamicin high potency lies in its mechanism of action since, like most aminoglycosides, the drug irreversibly binds to 30S ribosomes thus inhibiting bacterial protein synthesis. Therefore, gentamicin is indicated for acute serious infections.

The original approach of the presented research is the single-step electrophoretic deposition (EPD) on Ti plates of thus prepared biocomposite that would allow for on-site release of the drug. EPD has numerous advantages comparing to the other techniques, e.g., uniform composition and thickness of the deposited coating, the possibility of deposition on substrates of complex geometries, even inner surfaces and deposition at room temperatures. We successfully obtained hydroxyapatite/chitosan biocomposite coatings with the addition of graphene, and loaded with gentamicin, using EPD and investigated their bioactivity, biocompatibility and antibacterial properties The overall construct on the substrate in such a form would be well-advanced arrangement for future medical device improvement of skeletal implants.

Results and Discussion

Figure 1 depicts the effect of HAP/CS, HAP/CS/Gent, HAP/CS/Gr, and HAP/CS/Gr/Gent coating against *Staphylococcus aureus* TL and *Escherichia coli* ATCC 25922 in phosphate buffer, respectively, using test in suspension, simulating static *in vitro* conditions. Bacteria incubated without any coated sample (control) and bacteria in the presence of HAP/CS and HAP/CS/Gr follow the same growth pattern. Immediately after inoculation HAP/CS/Gent and HAP/CS/Gr/Gent coatings expressed strong bactericidal effect against *S. aureus*, reducing the initial bacteria count by 2 logarithmic units supporting the "burst" release of antibiotic. The complete reduction of bacterial cells was achieved within 1h (HAP/CS/Gent) and 3h (HAP/CS/Gr/Gent) *i.e.,* the effect is highly bactericidal. Retained antibacterial effect of HAP/CS/Gent and HAP/CS/Gr/Gent (Figure 1b) coatings was clearly seen against *E.*

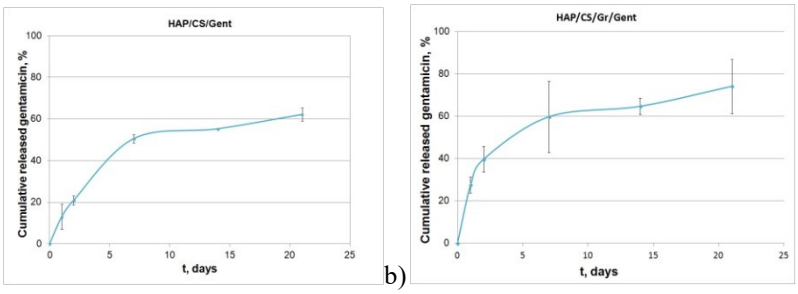
coli. In the initial period after inoculation the release of the drug hardly affected the bacteria, their sensitivity toward antibiotic was low. For the duration of the experiment (24 h post incubation) substantial decline of surviving *E. coli* cells was observed. Comparing the effects of these composite coatings and based on the kinetics testing against *E. coli*, since the reduction in bacterial cells was less than 3 logarithmic units, HAP/CS/Gent and HAP/CS/Gr/Gent composite coatings were classified as bacteriostatic.



a) b)

Figure 1: Reduction of viable cell number of: (a) *Staphylococcus aureus* and (b) *Escherichia coli* after contact with HAP/CS, HAP/CS/Gent, HAP/CS/Gr and HAP/CS/Gr/Gent coatings for 0, 1, 3, and 24 hr in PB compared to the control

The amount of loaded gentamicin in the composite coating, and the corresponding release profiles were obtained by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Gentamicin release from composite HAP/CS/Gent and HAP/CS/Gr/Gent coatings were studied during 21-day immersion in deionized water and measurements were done at predetermined periods of time (24 h, 48 h, 7 days, 14 days, and 21 days). The cumulative release profiles are represented in **Figure 2** as an average of three samples.



a) b)

Figure 2: Cumulative gentamicin release during 21 days in deionized water at 37 °C. (a) HAP/CS/Gent coating (b) HAP/CS/Gr/Gent coating

Gentamicin-release profiles verified the initial burst-release effect of gentamicin from the composite coatings, i.e., more than 50% of the loaded antibiotic was released within 7 days in the case of HAP/CS/Gent coating and at the similar rate for HAP/CS/Gr/Gent coating ~60%. This initial 7-day period was followed by a slower release pattern, only 13% and 14% respectively, of the released drug until day 21. Considering the gentamicin-release profiles, the obtained composite coatings could have potential use as prolonged drug-delivery systems for treating orthopedic infections.

Conclusions and/or Outlook

Titanium surface was modified by applying new composite, biocompatible, and bioactive hydroxyapatite/chitosan coatings with and without gentamicin using the electrophoretic deposition technique (HAP/CS, HAP/CS/Gent, HAP/CS/Gr, and HAP/CS/Gr/Gent). HAP/CS/Gent and HAP/CS/Gr/Gent coatings presented strong activity against both bacterial strains tested and even noticeably more against *S. aureus*, while their counterparts without the antibiotic did not exhibit antibacterial properties. The rapid and strong antibacterial effect monitored during the first 24 h coincides well with the initial burst effect of gentamicin observed on the cumulative release profiles. Presented results are a solid confirmation that bioceramic HAP/CS/Gent and HAP/CS/Gr/Gent composite coatings electrodeposited on Ti are promising biomaterials for further investigation and potential biomedical application as orthopedic and dental implants.

References:

[1] Stevanović M, Djošić M, Janković A, Kojić V, Stojanović J, Grujić S, Matić-Bujagić I, Rhee KY, Mišković-Stanković V; The chitosan-based bioactive composite coating on titanium; J. Mater. Res. Technol.; 15 (2021) 4461-4474;

[2] Stevanović M, Đošić M, Janković A, Kojić V, Vukašinović-Sekulić M, Stojanović J, Odović J, Crevar Sakač M, Rhee KY, Mišković-Stanković V; Gentamicin-Loaded Bioactive Hydroxyapatite/Chitosan Composite Coating Electrodeposited on Titanium; ACS Biomaterials Science & Engineering 4 (12) (2018), 3994-4007;
[3] Djošić M, Janković A, Mišković-Stanković V; Electrophoretic deposition of biocompatible and bioactive hydroxyapatite-based coatings on titanium; Materials 14 (2021) 5391;

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