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Antibiotics application in biomaterials for soft and hard tissue implants

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Abstract: One of the great challenges of modern medicine, pharmacy and chemistry as well is the treatment and replacement of damaged and injured soft and hard tissues, as well as bones and joints. Thus far variety of materials have been investigated and used in treating soft and hard tissues. On the other hand, application of soft or hard tissue implants brings high risk of infection by various microorganisms. Therefore, this paper presents the different possibilities of using soft and hard tissues implants particularly antibiotics-loaded implants with gradually releasing, with the aim to prevent bacterial infection, i.e. treat an infection that appeared as a result of the present microorganisms. The application of different antibiotics, gentamicin, ciprofloxacin, clindamycin, ampicillin, tetracycline, vancomycin for soft tissue implants as well as gentamicin and vancomycin as suitable antibiotics for hard tissue implants are reported.

Keywords: soft tissue implants, hard tissue implants, antibiotics.

1. Introduction

Simultaneously with the development of modern chemistry, pharmacy and medicine, significant increase in treating soft and hard tissues, as well as bones and joints diseases, injuries and wound, has been achieved. However, application of soft and hard tissue implants also brings the risk of infection by various microorganisms.

Traditionally, antibiotics have been used to treat bacterial infections. The most common route for their application is oral administration. In the case of severe infections, antibiotics are usually administered intramuscularly or more often intravenously. The choice of antibacterial agent is an important one, and different antibiotics are often used. However, prolonged systemic application of antibiotics may lead to increase of their side and toxic effects as well as developing resistance. Today, a large area of interest in nanomedicine is the application of antibiotics-loaded soft tissue implants, wound dressings or hard tissue implants. There are many advantages of their application since they can significantly reduce side and toxic effects of incorporated antibiotics or bacteria resistance in comparison to systemic application of antibiotics.

2. Bacterial infections in relation to soft tissue implants

Human skin as a physical barrier, serves as the first line of defence against microbial infection, by secreting sebaceous fluid and fatty acids to inhibit growth of pathogens and by possessing its own normal flora, thus deterring colonization by other pathogenic organisms. However, human skin is environment for many microbes. Infecting microorganisms may cause tissue damage and incite an inflammation processes. Also, different skin and tissue damages breaks this protective barrier and enable activity of pathogens. The organism's characteristic for the skin above the waist are usually Gram-positive species such as *Staphylococcus epidermidis, Corynebacterium species, Staphylococcus aureus* and *Streptococcus pyogenes* while both Gram-positive and Gram-negative species are microorganisms that inhabit at the skin below the waist. The infection agents *Enterobacteriaceae* and *Enterococcus species* (enteric species) mainly colonize this area of the skin and they may originate from the colon and faeces (Eron et al., 2003).

Different factors i.e. chronic diseases, age or trauma and injuries of soft tissue represent specific risk factors that may increase progress of skin and soft tissue infections. Potential risk factors may be divided into two types. There are patient-related factors including critical illness, elderly age, immunocompromised state, liver and kidney disease, and

vascular (especially lymphatic or venous) insufficiency. Certain risk factors (chronic renal or liver failure, immunocompromised state, vascular insufficiency or neuropathy) should be considered in the determination of disease severity. The second category is etiological risk factors. The mechanism of injury (trauma or others) or specific exposures to some infection agents can increases incidence of skin and soft tissue infections. The overlap between risk different factors in this grouping can be noticed (Ki & Rotstein, 2008) A list of different risk factors and their bacterial causes firstly proposed by Eron et al., 2003, were presented by in Table1.

Table 1. Risk elements for soft tissue infections(Ki & Rotstein, 2008)

Risk element	Pathogen
Diabetes mellitus	Staphylococcus aureus, anaerobes, Gram-negative bacilli
Cat or dog bite wounds	Pasteurellamultocida, C canimorsus
Rat bite wounds	Streptobacillusmoniliformis

Regardless of the risk of occurrence, skin and soft tissue infections (SSTIs) are infections with different clinical manifestations and degrees of severity. In Table 2 antibiotic election for different infection agents and degrees of infections are presented.

Table 2. Antimicrobial agents for skin and soft tissue infections (Ki & Rotstein, 2008)

Infection	Etiology	Antibiotic(s)
Mild infections (above waist)	Staphylococcus aureus Streptococcus pyogenes	Cloxacillin, cephalexin or clindamycin
Severe infections (above waist)	Staphylococcus aureus Streptococcus pyogenes	Cefazolin, cloxacillin, cephalexin
Mild infections (below waist)	Staphylococcus aureus Streptococcus pyogenes	Cloxacillin or cephalexin, clindamycin or metronidazole (anaerobes), second-generation cephalosporin or fluoroquinolone (if Gram-negative)
Severe infections (below waist)	Escherichia coli, Enterococcus species, Staphylococcus aureus Streptococcus pyogenes	Second-, Third- or fourth-generation cephalosporin, fluoroquinolones or piperacillin-tazobactam (in addition to above Gram-positive coverage)

*± Clindamycin

However, prolonged antibiotic or exposure hospitalization gets risk for infections with resistant organisms. At the first place it can be *Methicillin-resistant Staphylococcus aureus* (MRSA), but also *Pseudomonas aeruginosa*, *Enterococcus species*, *Streptococcus pyogenes* or *Clostridium species*. In such cases guidelines recommend second- or third-generation cephalosporin (mild to moderate), beta-lactam plus a fluoroquinolone or aminoglycoside with addition of vancomycin if MRSA suspected (Cohen & Kurzrock, 2004).

The infections caused by *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria are especially challenging since its resistance to number of usually used drugs. Antibiotics commonly recommended for treatment of MRSA infections are: mupirocin, clindamycin, trimethoprim-sulfamethoxazole, doxycycline, cephalexin, amoxicillin, vancomycin (Rajan, 2012).

In the case of infections caused with different bacteria the usual way of antibiotics administration is systemic: oral, intramuscular or intravenous application. On the other hand, systemic application of antibiotics especially for a long time may increase their side or toxic effects and led to developing of bacteria resistance.

Still, different drugs can be locally administered through the skin due its possibility to absorb by application of adhesive transdermal patch or wound dressings. Application of antibiotics loaded wound dressing can importantly improve soft tissue infections hilling and today takes large area of interest in nanomedicine. The local antibiotics application reduces side, toxic effects or bacteria resistance on applied antibiotics.

Many different polymers have been investigated as wound dressing materials. The applications require the use of biodegradable polymers in order to avoid the need of their removement by additional surgery. Until now, the most extensively used polymers are: polyvinyl alcohol (PVA) and dextran, poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL) and the copolymer poly(lactic-co-glycolic acid) (PLGA), chitosan (Ch), nanofiber mesh (NFM), collagen conjugate, keratose hydrogels. These polyesters are sometimes blended with other synthetic or natural polymers, such as polyethylenoglycol (PEG) or gelatin, in order to regulate the biodegradability and hydrophilic nature of the fibers and to control the drug release kinetics of the encapsulated drug. Also, application of different antibiotics incorporated in wound dressing materials have been also studied, as will be presented in the next section.

3. Antibiotics for soft tissue implants

For soft tissue implants the most frequently used antibiotics according available literature are gentamicin, ciprofloxacin, clindamycin, ampicillin, tetracycline, vancomycin.

3.1. Gentamicin-loaded wound dressing

Hwang et al., 2010 investigated application of gentamicin in wound dressing. For that purpose, polyvinyl alcohol (PVA) and dextran were used for preparation of cross-linked hydrogel films by application of freezing – thawing method. Two wound dressings with different composition were

prepared. The first was composed of PVA (2.5%), dextran (1.13%) with gentamicin (0.1%) addition while no drug was added into the second wound dressings. I*n vitro* protein adsorption test, *in vivo* wound healing test, and histopathology were performed for prepared wound dressings. In aim to investigate *in vivo* wound healing, at the rat dorsum wound spot were made and then covered with hydrogels with gentamicin or without any drug. Each wound was observed for 15 days. Majority of wounds were completely healed after 9 days but for wounds covered with hydrogels with gentamicin reduction size was much greater in comparison to those without gentamicin. The gentamicin-loaded wound dressing enhanced reepithelialization rate, which was much higher for hydrogel with gentamicin (98±2%) then hydrogel without drug (91±2%). Also, hydrogels with gentamicin significantly decreased the possible granulation tissue area. Therefore, gentamicin-loaded wound dressing can be recommended as a potential wound dressing with improved healing effect in wound care (Hwang et al., 2010).

Another material with natural characteristics suitable for applications in wound dressing, chitosan (Ch) and nanofiber mesh (NFM) were proposes by (Monteiro et al., 2015). The gentamicin-loaded liposomes were immobilized at the surface of Ch/NFM. The total concentration of gentamicin was 140mM. The release profile of gentamicin-loaded liposomesimmobilized at the surface of electrospunsurface was investigated by application of dialysis method. Following *in vitro* susceptibility tests were performed to examine whether gentamicin released from the liposomes has activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bactericidal activity was defined as reduction in bacterial number of the initial inoculum. For *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* that reduction represent more than 99.9% for all strains. The bactericidal activity of released gentamicin and developed system has promising performance for wound dressing applications, avoiding infections caused by these common pathogens (Monteiro et al., 2015)..

The new hydrogels with gentamicin addition for infected wound treatment was proposed by (Păunică-Panea et al., 2015). Two proteins, fibrous, type I collagen and globular, albumin, were the main components of proposed hydrogels. The proposed hydrogels were prepared by continuous stirring of collagen 1% (at pH value 7.3), with 1M sodium hydroxide, gentamicin and albumin of different concentrations (10, 20 and 30%). The content of added gentamicin were 0.2% (w/v). Following, obtained gels were crosslinked with specific amount of glutaraldehyde (0.5% to collagen dry substance). The properties of the proposed hydrogels were investigated by rheological measurements and water up-take capacity. The hydrogels provided good properties for maximum of 20% albumin and 0.2% gentamicin indicating that proposed hydrogels may also provide good antimicrobial properties (Păunică-Panea et al., 2015).

(Nnamani, 2013) produced improved topical hydrogels using three polymeric agents with addition of gentamicin in aim to achieve predictable permeation of gentamicin in skin. The applied agents were: poloxamer407 and two polyacrylic acids (Cabopols ® 971P and 974P). The hydrogels were prepared with three concentrations of gentamicin which were varied (0.03, 0.06 and .09 w/w). For determination of total concentration of antibiotic in hydrogels antibiotic loaded hydrogel was dissolved in distilled water, centrifuged, filtered and after derivatization with o-phthaldialdehydeconcentration of gentamicin determined spectrophotometrically. The *in vitro* test for permeation of gentamicin in skin were done on male rats which were sacrificed with prolonged anesthesia and their abdominal skin was excised and prepared for experiments. The skin permeation studies were performed by using a Franz diffusion cell method. The prepared rat abdominal skin was mounted between the donor and receptor compartment of the diffusion cell and the gentamicin-loaded hydrogel was placed in the donor compartment containing 5 ml of PBS. The gentamicin-loaded hydrogels with higher percentage of drug offered better permeation and possible treatment of skin infections caused by gentamicin-susceptible bacteria (Nnamani, 2013).

(Lukáč et al., 2019) proposed a novel collagen wound dressing sponge prepared from freshwater fish (Cyprinus Carpio) skin collagen type I. Half of the sponges were cross-linked with carbodiimide. Both cross-linked and non-cross-linked collagen sponges were impregnated with gentamicin. Following, the impregnated sponges were frozen and lyophilized. The sponges were tested through a rat model for activity on *Pseudomonas aeruginosa* infected wound and compared with a reference commercial product. The examination of the rats on the first day after Pseudomonas aeruginosa inoculation showed that the rats in group treated with collagen sponge with gentamicin and group treated with intramuscularly administered gentamicin exhibited similar body temperature and hilling degree. However, rats in group treated with collagen sponge without gentamicin or no gentamicin intramuscularly showed importantly higher signs of generalized infection. The obtained results indicate that gentamicin released from the sponges, had good clinical properties and was active against investigated infective agents (Lukáč et al., 2019).

Recently,Coimbra et al., 2019 investigated the electrospunfiber mats composed of poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL) and the copolymer poly(D,L,lactic-co-glycolide) (PLGA, L/G 50:50, ester terminated) as materials for immobilization of the antibiotic gentamicin sulfate (in concentration of 10% of the polymer weight). The four different formulations were prepared using the polymers PCL, PLA, PLGA and a mixture composed of 70% PLA and 30% PLGA (w/w): PCL, PLA, PLGA and by a PLA/PLGA which were blend using an suspension (S) (S-PCL, S-PLGA, S-PLA, S-PLA/PGA) electrospinning and emulsion (E) (E-PCL, E-PLGA, E-PLA, E-PLA/PLGA) electrospinning method. The antibacterial activity of the gentamicin sulfate-loaded fiload was examined against Staphylococcus aureus, the most common strain of bacteria associated with bone infections, by a disc diffusion assay. The inhibitory effect was observed and evaluated by measuring the diameter of the inhibition zones (Fig 1). As

can be seen all membranes produced inhibition zones (diameters between 2.4 and 3.6 cm), proving the antibacterial activity of the immobilized drug (Coimbra et al., 2019).

3.2. Ciprofloxacin-loaded wound dressing

Puoci et al., 2012proposed application of ciprofloxacin-collagen conjugate (CFX-T1C) for successful wound healing with reducing side effects usually recorded in systematic patient therapy. The antibacterial activities of CFX-T1C with addition of gentamicin in concentrations varied from 1,000 to 0.5 µg/mL, were determined through the testing against *Staphylococcus aureus* and *Escherichia coli*. The various concentrations of gentamicin were prepared with sterile, double-deionized water (autoclaved) in 96-well microtiter plates. The test organisms were added to each well. The microtiter plates were incubated at 37 °C for 24 h in a shaker. Following a small part of each mixture was spread on agar plates and incubated at 37 °C. The growth of bacterial cells was observed on agar plates after 48 h incubation. The antibacterial test was repeated at least four times in plates as negative and positive controls, respectively. The growth of the test bacterium was observed in all wells with positive controls while no growth was observed in the wells with theciprofloxacin-collagen conjugate (Puoci et al., 2012).

Sripriya et al., 2007 investigated the efficacy of bilayer dressing with drug ciprofloxacin in comparison with bilayer dressing without drug. The bilayer dressing was prepared from succinylated collagen and with incorporated ciprofloxacin-HCl in final concentration 0.2 mg/cm^2. Ciprofloxacin represents a class of quinolone antibiotics. Ciprofloxacin was used in presented experimental design because it has been proven as a very potent agent in eradicating *Pseudomonas aeruginosa* on biomedical device. Number of tests for collagen sponge and bilayer dressing with cipeofloxacin were performed. *In vitro* and *in vivo*tests of prepared bilayer dressings were made. *In vitro,*the release of ciprofloxacin from the bilayer dressing was investigated in Franz-type diffusion cells with HPLC determination of released antibiotic. Following, *in vitro* investigation of ciprofloxacin-incorporating dressings antimicrobial properties made on agar plates inoculated with a mixed culture of *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed important zone of inhibition. *In vivo* investigations were performed on 90 intraperitonealy anaesthetized animals. The hair from the dorsal region was removed and wound (2 × 2 cm^2) was excised on the back of rats. The mixed culture of *Pseudomonas aeruginosa and Staphylococcus aureus* was injected. Following, wounds develop infection for 24 h. The release of drug gradually increased and 24.45 ± 3.2% of release was observed on day 1, which was continued on days 2 and 3 (31.45 ± 3.45% and 35.36 ± 3.54%, respectively). The bilayer dressing containing ciprofloxacin showed a clear zone of inhibition (33 ± 3 mm) against the mixed culture (*Staphylococcus aureus and Pseudomonas aeruginosa*) using the agar diffusion method and the zone was maintained for more than 3 days, but no zone was observed around the collagen dressing without drug. Also *in vivo* investigations of prepared ciprofloxacin-loaded wound dressing showed significant higher wound closure and confirmed possibility of its application in proper wound healing (Sripriya et al., 2007).

Roy et al., 2015investigated ciprofloxacin-loadedkeratose hydrogels using a porcine wound model, infected with *Pseudomonas aeruginosa*. Prepared hydrogels contained from 0 to 20mg/mL of ciprofloxacin. *In vitro* investigations were performed to determine if ciprofloxacin-loaded keratose hydrogels inhibit *Pseudomonas aeruginosa.* The keratose hydrogels loaded with 2 mg/mL ciprofloxacin completely inhibited bacteria growth for 9 days in vitro, while keratose hydrogels without ciprofloxacin do not exhibit any antimicrobial activity. Following, the examination of ciprofloxacin release was performed in period of nine days during which keratose hydrogels were placed in tubes overlaid with phosphate-buffered saline (PBS). The concentration of ciprofloxacin in the collected samples was quantified using the inherent fluorescence of ciprofloxacin. All keratose hydrogels released from 50% to 63% of their total ciprofloxacin amount within the first 5 days, independent of the initial ciprofloxacin concentration. Finally, ciprofloxacin-loaded keratose hydrogels were investigated *in vivo* in pigson skin wounds infected with *Pseudomonas aeruginosa* and treated on days 1 and 3 postinjury with keratose hydrogels with or without ciprofloxacin. Treatment with keratose hydrogels loaded with 5 mg/mL ciprofloxacin or 10 mg/mL ciprofloxacin significantly reduced the amount of *Pseudomonas aeruginosa* in the wound by > 99.9% compared to keratose hydrogel without ciprofloxacin which did not possess any antimicrobial activity against *Pseudomonas aeruginosa*. Ciprofloxacin-loaded keratose hydrogels displayed decreased wound contraction and reepithelialisation (Roy et al., 2015).

3.3. Clindamycin-loaded wound dressing

The polyvinyl alcohol and sodium alginate were used to develop clindamycin wound dressing. The hydrogel films were prepared using freeze-thawing method from solution which were including different proportions of polyvinyl alcohol and sodium alginate and 3%w/v clindamycin. The healing effect of prepared hydrogels were investigated on male rats. Male rats (from 250 to 300 g) were used to evaluate wound healing characteristics of hydrogels. The dorsal hair of rats was shaved and two full skin wounds of 1.5 cm x 1.5 cm area were prepared. Their wounds infected with *Pseudomonas aeruginosa* were covered with PVA/SA wound dressings containing antibiotic clindamycin while control group of wounds were covered with dressings which contained no drug. It was found that application of hydrogels with clindamycin showed a significant effect on infected wound. For control group of animals after application of hydrogel without clindamycin more inflammatory cells were found in wounds and granulation tissue formation were also

observed. On the other side application of hydrogel with clindamycin led to significant decrease of inflammation and inflammatory cells in wound. At fifteen day, the defect area was almost completely hilled. The obtained results could recommend application of clindamycin-loaded wound dressing for efficient hilling of wounds infected with *Pseudomonas aeruginosa* (Kim et al., 2008).

The polymeric nanofiber patch for topical disease treatment were prepared using several different concentrations of polyvinyl alcohol and tamarind seed gum. It was loaded with clindamycin (in concentration 1.0% to 2.5%) as antibacterial agent. The antimicrobial activity of the drug-loaded polyvinyl alcohol/gum polymeric nanofiber patch was evaluated and compared to that of a commercially available clindamycin gel for acne treatment. A disk-diffusion method was used to investigate drug-loaded wound dressing antibacterial activity against test strains (*Staphylococcus aureus or Propionibacterium*) on Müller–Hinton agar plates. To prepare the plates, 0.1 ml of Staphylococcus aureus or Propionibacterium acnes was transferred and then inoculated at 37 °C for 18–24 h. For comparison antibacterial efficiency of the polymeric nanofiber patch with commercial 1% clindamycin gel, beside clindamycin loaded polymeric nanofiber patch a standard paper disk was impregnated with 10 μl of 0.1% clindamycin solution. The zone of strain inhibition obtained with clindamycin loaded polymeric nanofiber patch was recorded and compared with inhibition obtained with commercial 1% clindamycin gel as well as with erythromycin disk and a blank disk which were used as the positive and negative controls, respectively after 16–18 h of incubation at 35 °C. The antibacterial activities of prepared wound dressing clindamycin showed excellent antibacterial activity which were in relation with clindamycin content. The antibacterial activities of the clindamycin-loaded wound dressing were significantly higher in comparison to commercial 1% clindamycin gel. However, the wound dressing prepared without drug did not exhibit any bactericidal activity indicating that the investigated clindamycin-loaded wound dressing has good antibacterial activity and can be applied as wound dressings in acne healing (Sangnim et al., 2018).

3.4. Ampicillin-loaded wound dressing

Ampicillin-loaded wound dressing were prepared from hydrophobic polyurethane and hydrophilic ampicillin which were mixed together in the same solvent and electrospun into a fibrous scaffold. The various ratios of ampicillin: polyurethane, were prepared (1:10 wt%, 1.5:10 wt%, 2:10 wt%). In vitro study of cytotoxicity of the examined nanofibrous scaffolds was performed with human keratinocyte showing that prepared scaffolds can be used as an appropriate biocompatible wound dressing material. The antibacterial property of prepared ampicillin loaded wound dressings were determined on Muller and Hinton Agar using disk diffusion method. Test pathogens, Staphylococcus aureus and Klebsiella pneumonia, were spread on Muller Hinton agar plates and antibacterial activity of polyurethane nanofiber with ampicillin were evaluated on the bases of zone of inhibition against Staphylococcus aureus and Klebsiella pneumonia. Ampicillin polyurethane fibers exhibited good zone of inhibition against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumonia*. Also, increasing the ampicillin content in electrospun improve its antibacterial effect (Sabitha & Rajiv, 2015).

3.5. Tetracycline-loaded wound dressing

For preparation tetracycline-loaded wound dressing poly(vinyl alcohol) and chitosan were used with addition of Tetracycline hydrochloride to the polymer solution before the electrospinning process. The 5 mg/mL of tetracycline were mixed to the polymer solution, which correspond to 5% of the polymer weight in the nanofibers.

The antibacterial efficacy of prepared tetracycline-loaded wound dressing was evaluated and compared with wound dressings where tetracycline was not included. Their activity was investigated against the Gram-negative *Escherichia coli* as well as Gram-positive *Staphylococci epidermidis* and *Staphylococcus aureus*, using disc diffusion test (zone of inhibition). The bacterial suspension was seeded and then grown in Mueller Hinton broth at 37 °C for 24 h. Rectangular pieces of 20 mm × 10 mm were cut from the tetracycline-loaded and also unloaded wound dressing and they were placed on the agar plates. The wound dressing prepared without tetracycline were used as controls. After 24 h incubation at 37°C, the area of inhibition zones was measured. Examination showed no bacteria growth at the area of inhibition zones for the tetracycline-loaded wound dressing. Also, the results showed that the tetracycline loaded electrospun PVA and chitosan scaffolds are not deleterious for cell activity and may be safe for their use as wound dressing and soft tissue repair (Alavarse et al., 2017).

3.6. Vancomycin-loaded wound dressing

Kurczewska et al., 2015 used vancomycin as a model drug, very potent against gram-positive bacteria, for a new antibacterial wound dressing. Vancomycin-loaded wound dressing were prepared by application of alginate or gelatine/alginate hydrogel with addition of vancomycin in concentration which should remain 10mg per gram of the dried gel.

The *in vitro* release study of vancomycin from prepared wound dressing were performed. For this study samples of vancomycin-loaded wound dressing were immersed in phosphate-buffered saline solution and analysed using UV-vis Spectrophotometer. According obtained results after 24h about 95% of vancomycin was released from alginate and 75% from gelatine/alginate hydrogel.

The activity of vancomycin-loaded wound dressing was studied using disc diffusion method with Mueller-Hinton agar and zone of inhibition bacteria growth (various Staphylococcus and Streptococcus bacteria) were determined. The inhibition zone was determined by measurement of the appropriate diameter (mm). The discs used for the study had 9-11mm and 10-15mm diameter for vancomycin loaded alginate and gelatin/alginate hydrogels respectively. The

strong antimicrobial activity against investigated bacteria were obtained. The inhibition zones obtained for vancomycin loaded alginate hydrogel were bigger (20-32nm depending on infection agent) in comparison to vancomycin loaded gelatine/alginate hydrogel (16-27nm). These differences can be consequence of differences in release rate of antibiotic from the hydrogel (Kurczewska et al., 2015).

4. Bacterial infections in relation to hard tissue implants

The application of bone cement dates from the mid-nineteenth century when it was introduced for total knee prosthesis fixation by Themistocles Gluck and reported in historic text, The Classic: Report on the positive results obtained by the modern surgical experiment regarding the suture and replacement of defects of superior tissue, as well as the utilization of re-absorbable and living tamponade in surgery (Gluck, 2011).

The firstly applied bone cement was consisted primarily of plaster and colophony. Almost whole century later, in 1960, Sir John Charnley introduced polymethylmethacrylate (PMMA) as a new modern type of bone cement in total hip replacement surgery and since this introduction, PMMA was for years used as golden standard in fixation of cemented total joint arthroplasty (Charnley, 1960).

Following, different materials were investigated and used as bone replacement. A number of biomaterials for hard tissue implants such as hips, knees, ankle, shoulder, elbow joints and their application as drugs delivery systems were investigate lately. During last 60 years, three different generations seem to be clearly marked: bioinert materials (first generation), bioactive and biodegradable materials (second generation), and materials designed to stimulate specific cellular responses at the molecular level (third generation). These applications require the use of biodegradable and/or bioerodible polymers in order to enable the permanent persistent of implanted drug carrier. The only requirement was for the first generation materials was their minimal toxic effect. To the first generation meterials belongs metallic stainless steel and cobalt–chrome-molibdene based, titanium based, nickel-titanium based), ceramic materials high alumina ceramics, bioglasses, biopex, glass–ceramics, calcium phosphate cements) and polimers (polymethacrylic acid (PMMA), polyethylene (PE), polydimethylsulphoxide (PDMS), polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL), polydioxanone (PDS)) (Ghalme et al., 2016; Navarro et al., 2008).

However, one of the most important problems in orthopaedic surgery are bacterial infections especially infections after total joints replacement. The infection after fracture fixation can be classified according time of appearance into three groups: infections with early appearance (less than two weeks), delayed (between two and ten weeks) and late (more than ten weeks) (Metsemakers et al., 2018).

Different bacteria may cause these infections such as Gram-negative species, *Escherichia coli*, *Pseudomonas* including *Enterobacteriaceae* or Gram-positive species such as *Staphylococcus aureus*, *Corynebacterium species and Streptococcus pyogene*s including *Methicillin-resistant Staphylococcus aureus* (MRSA).

MRSA infection is caused by a type of staph bacteria that's become resistant to many of the antibiotics used to treat ordinary staph infections. In many different studies, number of antibiotics (gentamicin, vancomycin, tobramicin, clindamicin, erythromycin, ciprofloxacin, cephalosporins, tetracycline and rifampicin) were investigated for application in antibiotic-loaded implants.

Antibiotics application are in relation not only with infective agents but also with implants consisting. Polymethylmethacrylate (PMMA) also referred as (acrylic) bone cement was tested for local delivery of number different antibiotics in orthopaedic treatment of bones or joints. The obtained results showed that gentamicin and vancomycin can cover broad spectrum of pathogens including MRSA (van Vugt et al., 2019).

5. Antibiotics for hard tissue implants

According available literature the most frequently used antibiotics for hard tissue implants are gentamicin and vancomycin what is in accordance with severity of bacterial infection that can occur during orthopaedic surgery for hips, knees, ankle replacement.

5.1. Gentamicin application

The chitosan, chitosan/bioglass and chitosan/bioglass/ gentamicin coatings were prepared to investigate antibacterial activity of incorporated antibiotic. Solutions of chitosan in acetic acid in water were prepared by magnetic stirring following with dispersion of bioglass particles and addition of gentamicin sulfate in suspension in concentration of 2 mg/mL.

The biological behavior of the prepared coatings and their in vitro bioactivity were studied using antibacterial tests. In order to quantify the amount of gentamicin incorporated in the coatings, coatings were scraped o ff the substrate and immersed in deionized water with borate buffer (pH 10.4) while in vitro release of gentamicin was studied by incubating coated samples in phosphate buffered saline. The amounts of loaded and released antibiotic were determined by HPLC UV method. It was found that during five days 40% of the loaded gentamicin in phosphate buffered saline were released. The antibacterial activity of released antibiotic were investigated against the *Staphylococcus aureus*, using disc diffusion test (zone of inhibition). The bacterial suspension was seeded and then grown in Mueller Hinton broth at 37 °C for 24 h.

The antimicrobial disc susceptibility test indicated that the CS/BG/GS coatings subjected to immersion in PBS developed a zone of inhibition of about 13 mm up to 2 days while PBS control samples, and chitosan/bioglass coatings,

did not develop any zone of inhibition against *Staphylococcus aureus* growth. The cellular metabolic activity was measured by alamar. Blue assay and the percentage of proliferated cell number was estimated. The proposed coatings exhibit significantly smaller number in comparison to previously prepared positive control (Pishbin et al., 2014).

The ceramic material containing of hydroxyapatite and calcium sulphate as bone substitute was developed with addition of gentamicin to prevent the occurrence of infection while enhancing the bone healing. The gentamicin concentration in synthetic bone substitute was 175mg per 10 mL. The gentamicin release examination was made by determination of its concentration in serum and in urine of number of patients for at least 30 days, to give evidence for local antibacterial activity and systemic toxicity. The gentamicin concentrations in the serum were always well below the maximum recommended level of 12 mg/L. For the week urine concentrations of gentamicin were approximately 100 times higher than in serum. The peak levels were around 100 mg/L while after one week it was around 1 mg/L, indicating high local gentamicin concentrations at the implantation sites. Following, the gentamicin concentration in the urine decreased below 1mg/L for the rest of the sampling time. At approximately28 days, all gentamicin was eluted from the implants reducing risk of developing bacterial resistance on gentamicin loaded to ceramic material (Stravinskas et al., 2018).

Composite coating on titanium substrate, containing natural polymer chitosan (CS) and hydroxyapatite (HAP) with addition of antibiotic gentamicin (Gent) (1mg/mL) was developed to avoid the incidence of postoperative infection in bone healing. Determination of gentamicin incorporated into coating was made by scrapping off coating surfaces and resuspending powder in distilled water. Gentamicin in the solution was determined by HPLC/UV method after its derivatization with o-phtalaldehyde. It was obtained that the average of gentamicin loaded on 1 cm^2 area of hydroxyapatite / chitosan /gentamicin coating was around 7.3μg. The antibacterial activity of gentamicin loaded coatings in comparison with coatings without gentamicin was tested by agar diffusion method against gram positive pathogenic bacterial strain *Staphylococcus aureus* and gram-negative *Escherichia coli*. The coating without antibiotic, hydroxyapatite / chitosan, did not exhibit antibacterial activity against applied bacterial species. However, hydroxyapatite / chitosan /gentamicin coating expressed strong bactericidal activity after inoculation, reducing initial count by 2 logarithmic units. The complete reduction of bacterial cells was achieved within 1h for *Staphylococcus aureus.* But, antibacterial activity of hydroxyapatite / chitosan /gentamicin coating against *Escherichia coli* was much less expressed, and noticeable reduction of bacterial cells was achieved within 24 h post incubation.

In vitro cytotoxicity of prepared coating was evaluated by standardized colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The coatings of hydroxyapatite and chitosan with or without addition of antibacterial agent gentamicin developed on titanium substrate were compared by MTT tests. According obtained results no toxicity for HAP/CS sample was found. However, HAP/CS/gent-coated Titanium samples have shown slightly increased toxicity which can be considered as consequence of the gentamicin presence. However, prepared gentamicin loaded composite coating can be classified as noncytotoxic (Stevanović et al., 2018).

5.2. Vancomycin application

Cabrejos-Azama et al., 2016 developed new hard tissue implant, calcium phosphate cement with magnesium addition, loaded with antibiotic vancomycin in aim to obtain new vancomycin carrier system which will be active against *Staphylococcus aureus*, the most relevant pathogen related with bone infection in postoperative implant surgery.

Two methods to load the cements with vancomycin were applied, adsorption from a vancomycin solution (with concentration 5 g/mL) or incorporation of vancomycin (2g) into the solid phase (40g) during the cement production. Vancomycin release from the cement was investigated by immersion in phosphate-buffered saline (pH 7.4) at 37 ± 1 °C. The amount of drug released was measured using UV spectroscopy. The cumulative release profiles shown indicate fully release of drug from the cements during 6 days. However, cement prepared with 26.67% Mg presents faster release of vancomycin. The 50% of the antibiotic was released after 3 h and 98% after 72 h. The *in vitro* antimicrobial examination of vancomycin-loaded cement was performed in order to determine its antibacterial effect on pathogen *Staphylococcus aureus*. The antibiotic activity of released vancomycin was investigated by *in vitro* tests using pathogen *Staphylococcus aureus*. The antimicrobial assay consisted in the measuring inhibition zone of the antibiotic in a standard gel inoculated with the bacterium strain. The presence of clear zones of inhibition surrounding the cement tablets loaded with vancomycin confirms the inhibition of *Staphylococcus aureus* growth. However significantly bigger zone of inhibition was observed for cement prepared with 26.67% Mg what is in accordance with its faster release of vancomycin (Cabrejos-Azama et al., 2016).

Gentamicin and vancomycin are antibiotics that exhibits bactericidal activity against a broad spectrum of microorganisms, such as *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* but also MRSA bacteria since bacterial resistance to gentamicin and Vancomycin are lower than to other group of antibiotics. The mixture of these antibiotics was prepared as bioabsorbable beads and used in operative revision of infected hip replacement in dog. The vancomycin and gentamicin, were both put into the femoral canal before closure the implant. There has been no evidence of postoperative reinfection during next five years. Also, no additional antibacterial drugs were necessary during recovery period and demonstrating that antibiotic-impregnated bioabsorbable calcium sulphate beads can be very useful in veterinary orthopaedics (Guthrie & Fitzpatrick, 2019).

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Conflicts of Interest

Authors declare that there is no conflict of interest.

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Primena antibiotika u biomaterijalima za implantate mekih i tvrdih tkiva

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Abstrakt: Jedan od velikih izazova savremene medicine, farmacije i hemije je lečenje i zamena oštećenih i povređenih mekih i tvrdih tkiva, kao i kostiju i zglobova. Do sada su istraženi različiti materijali koji se koriste u njihovom lečenju. Sa druge strane, primena implantata mekog ili tvrdog tkiva nosi visok rizik od infekcije različitim mikroorganizmima. Zbog toga su u ovom radu prikazane različite mogućnosti primene implantata mekih i tvrdih tkiva, posebno implantata sa antibiotikom sa postepenim otpuštanjem, u cilju prevencije bakterijske infekcije, odnosno lečenja infekcije koja je nastala kao posledica prisutnih mikroorganizama. Prikazana je primena različitih antibiotika, gentamicina, ciprofloksacina, klindamicina, ampicilina, tetraciklina, vankomicina za implantate mekog tkiva, kao i gentamicina i vankomicina kao odgovarajućih antibiotika za implantate tvrdog tkiva.

Ključne reči: implantati mekog tkiva, implantati tvrdih tkiva, antibiotici