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Functionalized hydroxyapatite scaffolds coated with sodium alginate and chitosan for controlled drug delivery

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Abstract. Due to its bioactivity, hydroxyapatite (HAp) is one of the most perspective materials for controlled drug delivery. Biodegradable polymers like chitosan and sodium alginate are used as coating materials for ceramic scaffolds. In this work the application of HAp/sodium alginate or chitosan composite materials which provide controlled lidocaine release is discussed. The polymers and lidocaine hydrochloride were incorporated in porous HAp scaffolds. Release of lidocaine hydrochloride was determined using high-performance liquid chromatography (HPLC). Depending on the polymer type used for coating, the crystal structure of the incorporated drug was determined. Drug/bioceramic scaffold/polymer interactions are discussed. The use of polymer coatings sustained lidocaine release from one day up to four days, compared with lidocaine impregnated but uncoated scaffolds.

Key words: hydroxyapatite, lidocaine hydrochloride, drug delivery, surface modification, alginate, chitosan.

1. INTRODUCTION

The most important goal in drug delivery is to provide certain drug concentrations to specific sites and to ensure a definite drug release profile for a specified period of time. Synthetic calcium phosphates (CaP) have been successfully used as implant materials and drug reservoirs due to their biocompability, osteoconductivity, and surface properties [1]. Among them hydroxyapatite (HAp) is most commonly used in stomatology and orthopaedics for bone replacement and regeneration. The main precondition in bone regeneration strategy is the use of bioceramic scaffolds with optimal architecture in means of porosity, pore size, interconnectivity of pores, etc. [2,3].

Calcium phosphate ceramics are often combined with biocompatible polymers to remodel the natural structure of bone and to ensure controlled drug delivery [4]. There are several studies regarding the preparation of polymer (sodium alginate and/or chitosan) composite scaffolds using HAp of variable crystallinity and amount [5–7]. It has been shown that the porosity of composites decreases by adding the HAp crystalline phase up to 30 wt% to polymer matrices [5,6], whereas higher porosity was obtained by using porous HAp scaffolds. Chitosan is a biodegradable, biocompatible, and non-toxic aminopolysaccharide with unique structure. Containing amino functionality and amino groups, it can be modified to obtain the desired properties [8]. These properties find several biomedical applications in tissue engineering, wound healing, as excipients for drug delivery, and also in gene delivery [9]. Sodium alginate was chosen as scaffold coating material due to its biodegradability and biocompatibility. Due to their suitable rheological properties, alginates have been used not only as vehicles for biologically active molecules, but also in the pharmaceutical industry as colloidal stabilizers and thickening or gelling agents [10–13].

Chitosan coating is used for alginate scaffolds to improve cell attachment [6]. Osteoblast cells seeded on chitosan-alginate scaffolds attach and proliferate better than on pure polymer scaffolds [14]. Scaffolds containing both polymers have significantly improved mech-

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anical strength compared to the pure chitosan or alginate-coated ones [14], therefore in the current research scaffolds were prepared from pure HAp to obtain a better pore structure and then coated with chitosan and/or alginate for better cell attachment and controlled drug delivery. Zhang et al. [15] determined that the release of diclofenac sodium from alginate/HAp nanocomposite beads is 12 h. By comparison, Sivakumar et al. [16] determined the release of gentamicin form coralline HAp/chitosan composite microspheres that was more than 85 h. Pasparakis et al. [17] suggested to use alginate-chitosan mixed beads, but in the first 8 h 90% of verapamil was released.

Lidocaine hydrochloride was used as a model drug. It is a commonly used local anesthetic [18] and lidocaine delivery systems based on porous CaP ceramic scaffolds [19] could be promising not only due to the well-known properties of CaP, but also as painkillers after material implantation *in vivo* [20,21]. The elimination half-life of lidocaine is approximately 90–120 min; by controlling the release it is possible to extend the effect of the drug. The desirable release of lidocaine could be 3–7 days, depending of the type of surgery and other factors [22].

The biomedical applications of HAp and sodium alginate or/and chitosan porous scaffolds were studied. Hydroxyapatite scaffolds were synthesized by the modified wet chemical method [23]. Hydroxyapatite/polymer/drug composite materials were prepared using vacuum infiltration, which is a simple method and can be carried out at room temperature. The suitability of prepared lidocaine-containing composite materials to act as slow release drug delivery systems was evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Hydroxyapatite was synthesized by the modified wet chemical method – precipitation from an aqueous medium by slow addition of orthophosphoric acid solution to a calcium hydroxide suspension. Porous ceramic scaffolds were sintered at 1150 °C for 1 h. Sodium alginate (alginic acid sodium salt, from brown algae, BCBC6747 BioChemika), chitosan (chitosan from crab shells, WE453770/1, BioChemika), and lidocaine hydrochloride (monohydrate, ≥99%, Cat. No. L5647) were purchased from Sigma. The 3% Na-alginate and 3% chitosan solutions in water were prepared fresh as needed. The concentration of lidocaine hydrochloride solution was 60 mg/mL.

2.2. Preparation of HAp/polymer/drug composites

The dried HAp precipitate was milled to obtain a fine powder which was mixed with an organic additive (polyol) to obtain a highly viscous/plastic mixture, ammonium hydrogen carbonate was added as pore foaming agent, and green bodies were sintered at 1150 °C to obtain porous ceramic scaffolds (disk shape with a diameter of 7 mm and height of 2.5 mm) [24]. Lidocaine hydrochloride and polymer/water solutions were infiltrated in HAp bioceramic scaffolds using the vacuum impregnation technique at 700 mbar pressure for 15 min. More than one impregnation cycle was applied to obtain HAp/polymer/drug composites. The type of polymer and order of coating are shown in Fig. 1.

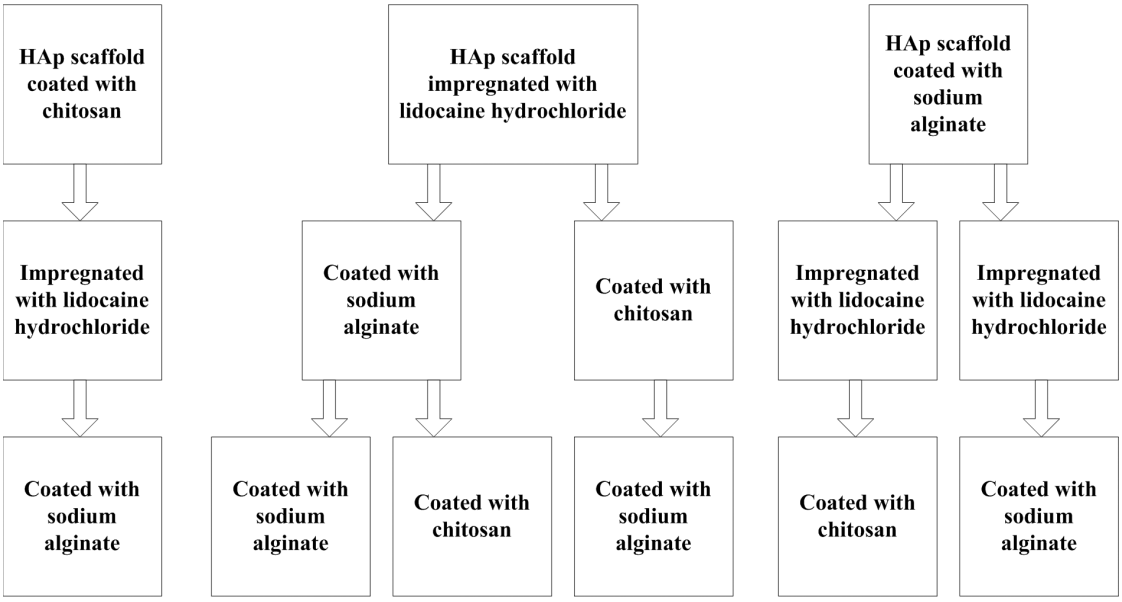

Fig. 1. Preparation of HAp/polymer/drug composites.

2.3. Characterization of HAp/polymer/drug composites

Hydroxyapatite powders and scaffolds where analysed by X-ray diffraction (XRD, PANalytical zX'Pert Pro diffractometer, The Netherlands) using Cu Kα radiation at 40 kV and 30 mA, 2θ range of 5–50°. The obtained pattern was compared with the pattern in previous researches [25].

Scanning electron microscopy (SEM) was used to evaluate the surface morphology and inner structure of the prepared composites. Samples were sputter coated with gold using a fine coat ion sputter device, and observed using a Tescan Mira/LMU scanning electron microscope. Fourier transform infrared spectroscopy (FT-IR, VarianScimitar 800 in the wavenumber range 4000–400 cm^{-1}) was used to evaluate the drug/bioceramic scaffold/polymer interaction. The FT-IR spectra of blank Na-alginate, chitosan, Hap, and lidocaine hydrochloride were obtained and used as references. Scaffolds were pulverized and analysed as KBr pellets.

2.4. Determination of lidocaine hydrochloride release kinetics

The prepared drug/bioceramic scaffold/polymer composites were placed in 50 mL of phosphate buffered saline pH = 7.4 (PBS) [26,27] and incubated at 37 °C and 50 rpm. The rate of lidocaine release from composites was determined by taking 100 µL aliquots of PBS after 0.5 h, 1 h, 2 h, 4 h, 8 h, 24 h and once every following day for a period of 148 h.

Lidocaine hydrochloride content was determined using high-performance liquid chromatography (HPLC, Waters 2695 Alliance Separations Module with Waters 2487 Dual λ Absorbance Detector) with UV detection (λ = 210 nm) and C18 (YMC-Pack Hydrosphere C18 (5 µm), 12 nm, 150 × 3.0 mm, YMC Separation Technology) column. For data acquisition and processing the software Empower 2 was used. The mobile phase was 85% of 0.1 mol/L potassium hydrogen phosphate buffer (pH 3.2) and 15% of acetonitrile.

2.5. Statistics

The results are represented as the mean value ±SD (standard deviation) of three experiments. The statistical analysis was done on release data with unpaired Student's *t*-test and $p < 0.05$ was used as a limit to indicate statistical significance.

3. RESULTS

X-ray diffraction analysis was used to determine the quality of synthesized HAp. The XRD pattern of synthesized HAp corresponds to the HAp pattern without impurities (ICDD No. 09-432). Pure HAp, chitosan, and alginate XRD patterns are shown in Fig. 2. The XRD patterns for composite materials corresponded to the HAp pattern (see Fig. 3), indicating that the amount of drug and polymer against HAp is too low for quantitative analysis of composite materials using XRD.

By applying the impregnation cycle twice it was possible to increase the amount of polymer introduced in samples. It was not possible to introduce the necessary amount of polymers to coat the entire surface in one cycle due to their low solubility in water at pH = 7. After two impregnation cycles with polymer solutions the sample mass increased by 1.5–3%. The thickness of the alginate layer was up to 1.2 µm after the first impregnation cycle and up to 2.5 µm after the second impregnation cycle, in the case of chitosan,

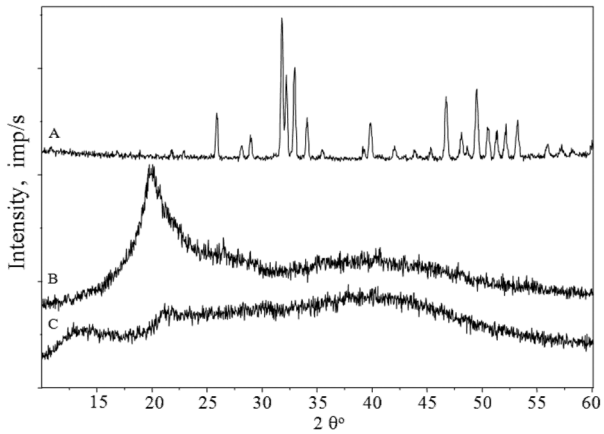

Fig. 2. XRD patterns of: A – HAp, B – chitosan, C – sodium alginate.

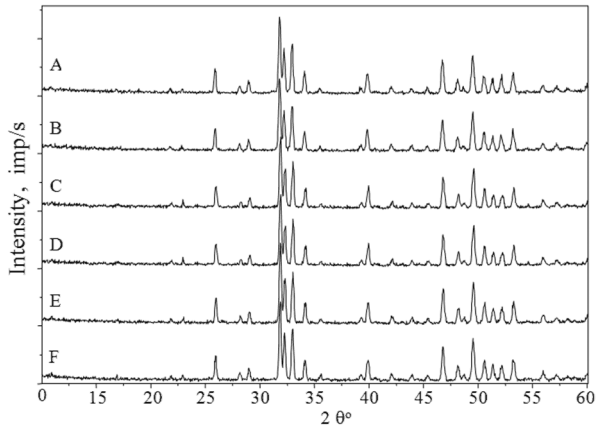

Fig. 3. XRD patterns of composite scaffolds: A – HAp/chitosan/lidocaine/alginate, B – HAp/lidocaine/alginate/alginate, C – HAp/lidocaine/alginate/chitosan, D – HAp/lidocaine/chitosan/alginate, E – HAp/alginate/lidocaine/chitosan, F – HAp/alginate/lidocaine/alginate.

respectively, 0.6 and 1.5 μm. Loss of chitosan in HAp scaffolds was caused by impregnation cycles with lidocaine hydrochloride because chitosan is more soluble in weakly acidic solutions than in water; due to that, coatings of chitosan were affected by acidic drug solution. Each impregnation cycle influenced not only the sample mass but also the polymer/lidocaine ratio.

To establish whether the introduced polymer or lidocaine reacts with HAp scaffolds, FT-IR spectra were taken (see Fig. 4). Figure 4 shows the FT-IR spectra of sodium alginate (A), lidocaine hydrochloride (B), HAp (C), and HAp/lidocaine/alginate/alginate composite material (D). In the spectra of (C) and (D) samples the absorption bands at 3570 and 630 cm^{-1} are attributed to the O–H stretch vibration. Characteristic absorption bands of HAp are 1050 and 1090 cm^{-1} and are ascribed to the P–O symmetric stretch vibration. In composite material these bands are observed as well. The N–H stretch vibration absorption bands in lidocaine are at 1654 and 1672 cm^{-1}. The absorption bands at 1625 cm^{-1} (Fig. 4A) are ascribed to stretching vibrations of C–C. In the FT-IR absorption spectrum of the HAp/lidocaine/alginate/alginate composite (see Fig. 4D), characteristic peaks of both HAp and lidocaine, as well as of the alginate absorption spectrum appear, indicating that no chemical reaction between HAp, lidocaine hydrochloride, and sodium alginate occurs during the impregnation cycles. The same observation can be made for HAp/chitosan/lidocaine scaffolds – all characteristic peaks of the raw material appear in the FT-IR absorption spectrum. During the impregnation cycles no chemical reactions between polymer, drug, and HAp were observed by FT-IR.

The SEM micrographs (see Fig. 5) show that the crystalline structure of lidocaine varies, depending on the order of polymer and lidocaine impregnation. The HAp scaffold coated with chitosan and impregnated with lidocaine contains acicular lidocaine crystal morphology (Fig. 5a), which is positioned mostly in HAp scaffold pores. No changes in the crystalline structure of lidocaine after sodium alginate coating were observed for HAp/chitosan/lidocaine composite material (see Fig. 5c). An alternative morphology is acquired if the HAp scaffold is coated with sodium alginate and impregnated with lidocaine. The composite material HAp/alginate/lidocaine has rectangular lidocaine crystal morphology (see Fig. 5b) that is located on the entire surface. The HAp/alginate/lidocaine composite scaffolds were coated either with alginate or chitosan. Lidocaine crystal morphology depends on the second coating as well. If the HAp/alginate/lidocaine scaffold is coated with alginate, the lidocaine crystals are rounded and uniformly dispersed on the composite surface (see Fig. 5d); on the contrary if the HAp/alginate/lidocaine scaffold is coated with chitosan, it contains needle-like lidocaine crystals (see Fig. 5e). Needle-like lidocaine crystals are also observed for the composites that are first impregnated with lidocaine hydrochloride solution and then coated with polymers (see Fig. 5f–h). Four crystal morphologies are observed in the samples, indicating that polymers affect the morphology of lidocaine crystals.

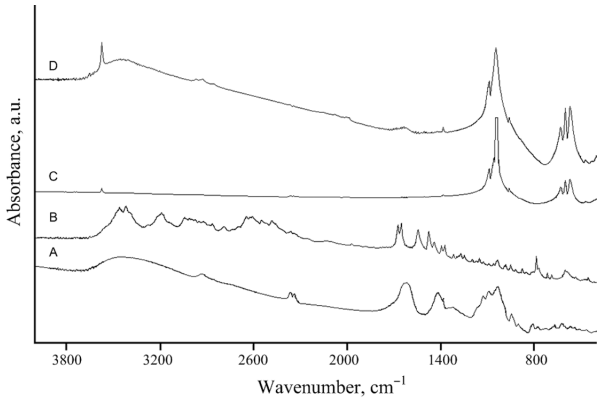

Fig. 4. FT-IR analysis of sodium alginate (A), lidocaine hydrochloride (B), HAp (C), and HAp/lidocaine/alginate/alginate composite material (D).

In order to evaluate the lidocaine release kinetics, composites were placed in PBS for 148 h. The released amount of lidocaine was plotted against the incubation time (see Fig. 6). For composites coated with chitosan in the first hour the initial burst release up to 70% was observed. Scaffolds coated with alginate showed the initial burst release between 16% and 30%. In the incubation period the slowest lidocaine release was for HAp/alginate/lidocaine/alginate composites where lidocaine release was sustained even up to 60 h, while from the HAp/chitosan/lidocaine scaffold complete lidocaine release was observed already in the first 6 h. Lidocaine release from chitosan-coated scaffolds was faster than from alginate-coated HAp scaffolds, due to chitosan solubility in weakly acidic solutions, which could be provided by the influence of drug [28]. The prepared composites showed drug release up to 60 h, which is ~50 h longer than for alginate/HAp nanocomposite beads [15] and alginate-chitosan mixed beads [17], but ~24 h faster than for coralline HAp/chitosan composite microspheres [16].

4. CONCLUSIONS

Hydroxyapatite scaffolds containing different biodegradable polymer coatings and lidocaine were prepared. No chemical reactions between HAp, lidocaine, alginate, and chitosan were observed by FT-IR. It was detected that each polymer coating affected the crystalline morphology of lidocaine. If alginate is used as the coating material, rectangular and uniformly dis-

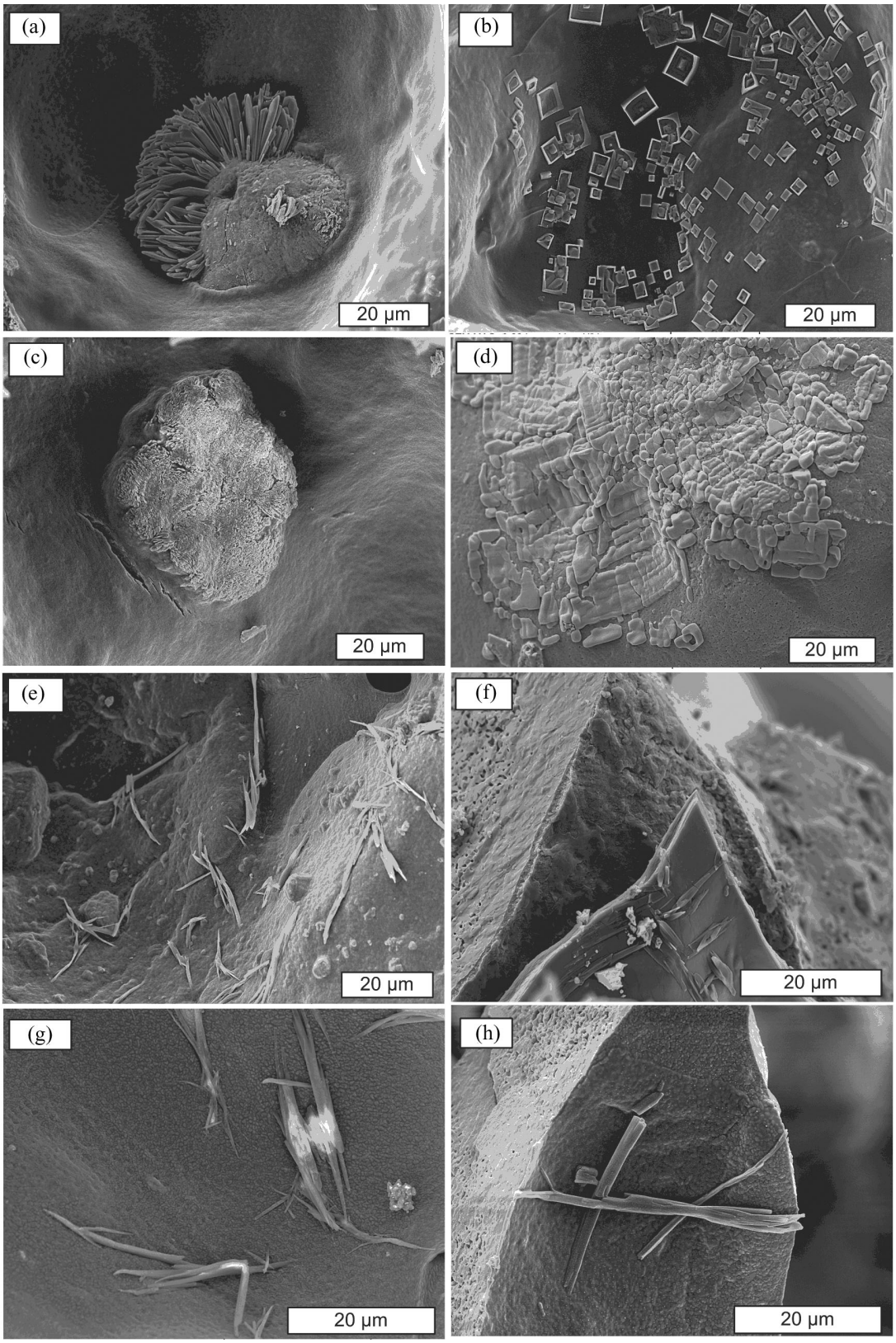

Fig. 5. SEM micrographs of scaffolds: (a) HAp/chitosan/lidocaine, (b) HAp/alginate/lidocaine, (c) HAp/chitosan/lidocaine/alginate, (d) HAp/alginate/lidocaine/alginate, (e) HAp/alginate/lidocaine/chitosan, (f) HAp/lidocaine/chitosan/alginate, (g) HAp/lidocaine/alginate/chitosan, (h) HAp/lidocaine/alginate/alginate.

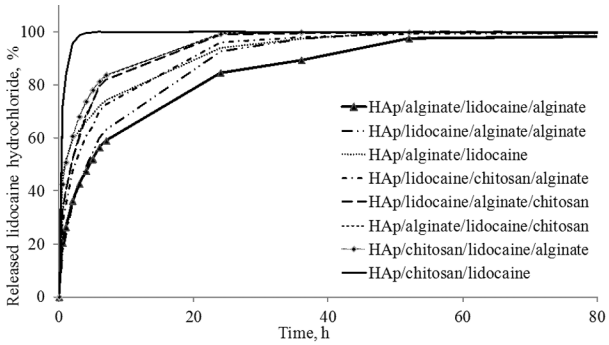

Fig. 6. Lidocaine hydrochloride release kinetics from HAp/polymer/drug composites.

persed lidocaine crystals can be obtained. Needle-like lidocaine crystal morphology can be ensured if chitosan is used as coating material.

Lidocaine release profiles show that sodium alginate-coated HAp scaffolds are more promising materials for drug delivery, and lidocaine release could be sustained for 60 h.

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Naatriumalginaadi ja kitosaaniga kaetud modifitseeritud hüdroksüülapatiidi karkasside kasutamine kontrollitud kiirusega ravimite manustamiseks

Arita Dubnika, Dagnija Loca ja Liga Berzina-Cimdina

Oma bioaktiivsete omaduste tõttu on hüdroksüülapatiit (HAp) kontrollitud kiirusega ravimite manustamiseks üks perspektiivsemaid materjale. Biolagunevaid polümeere, nagu kitosaan ja naatriumalginaat, kasutatakse kattematerjalina keraamilistel alustel (karkassidel). Käesolevas töös uuriti lidokaiini järkjärgulist vabanemist poorsest HAp-st, mida oli immutatud naatriumalginaadi ja/või kitosaaniga. Lidokaiinhüdrokloriidi vabanemist määrati kõrgrõhuvedelikkromatograafia (HPLC) abil. Näidati apatiiti imbunud ravimi kristallstruktuuri sõltuvust kasutatud polümeeri tüübist. On vaadeldud ravimi/biokeraamilise karkassi/polümeeri vastasmõjusid. Immutatud, kuid polümeeriga katmata karkassiga võrreldes eraldub lidokaiin polümeeriga kaetud alusest (karkassist) pidevalt ühe kuni nelja päeva jooksul.