Materials Science and Engineering C 77 (2017) 427–435

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Co-delivery of cisplatin and doxorubicin from calcium phosphate beads/matrix scaffolds for osteosarcoma therapy

Ulrike Hess [a], Shakiba Shahabi [a], Laura Treccani [a,*,1], Philipp Streckbein [b,d], Christian Heiss [c,d], Kurosch Rezwan [a,e]

[a] Advanced Ceramics, University of Bremen, Am Biologischen Garten 2, 28359 Bremen, Germany
[b] Department for Cranio-Maxillofacial and Plastic Surgery, University Hospital of Giessen-Marburg GmbH, Campus Giessen, Klinikstrasse 33, 35392 Giessen, Germany
[c] Department of Trauma, Hand and Reconstructive Surgery, University Hospital of Giessen-Marburg GmbH, Campus Giessen, Rudolf-Buchheim-Strasse 7, 35392 Giessen, Germany
[d] Laboratory of Experimental Surgery, Justus-Liebig-University of Giessen, Kerkrader Strasse 9, 35394 Giessen, Germany
[e] MAPEX Center for Materials and Processes, University of Bremen, Am Fallturm 1, 28359 Bremen, Germany

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form 15 March 2017
Accepted 17 March 2017
Available online 21 March 2017

Keywords:
Co-delivery
Scaffold
Drug carrier
Doxorubicin
Cisplatin

ABSTRACT

Bone substitute materials with a controlled drug release ability can fill cavities caused by the resection of bone tumours and thereby combat any leftover bone cancer cells. The combined release of different cytostatics seems to enhance their toxicity. In this study, calcium phosphate beads and matrix scaffolds are combined for a long-term co-delivery of *cis*-diamminedichloroplatinum (cisplatin, CDDP) and doxorubicin hydrochloride (DOX) as clinical relevant model drugs. Tricalcium phosphate/alginate beads as additional drug carrier are produced by droplet extrusion with ionotropic gelation and incorporated in scaffold matrix by freeze gelation without sintering. CDDP shows a short burst release while DOX has a continuous release measurable over the entire study period of 40 days. Drug release from matrix is decreased by ~30% compared to release from beads. Nevertheless, all formulations follow the Korsmeyer-Peppas release kinetic model and show Fickian diffusion. Cytotoxic activity was conducted on MG-63 osteosarcoma cells after 1, 4, and 7 days with WST-1 cell viability assay. Co-loaded composites enhance activity towards MG-63 cells up to ~75% toxicity while reducing the released drug quantity. The results suggest that co-loaded beads/matrix scaffolds are highly promising for osteosarcoma therapy due to synergistic effects over a long period of more than a month.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Despite improved surgical, radio, and chemotherapeutic techniques, bone cancer still is one of the major causes of severe functional and structural skeletal defects or even death. A major risk for pathologic fractures, severe pain, life-threatening hypercalcaemia, and an overall increased mortality is the local recurrence by residual neoplastic cells remaining due to incomplete marginal resection [1–3].

To control or prevent the risk of local recurrence of bone cancer, local administration by drug carriers can deliver cytostatics in high concentrations with enhanced efficacy to the tumour while minimizing the drug concentrations in the bloodstream or other organs and improving the patient comfort. These carriers include hydrogels [4,5], micro- and nano-particles [5–7], liposomes [8], biodegradable polymers [9,10], or calcium phosphates [6,11]. However, degrading fragments or acidic byproducts, from biodegradable polymer-based drug delivery systems or harsh solvents required for their degradation may adversely affect the drugs to be delivered or the surrounding tissues [12]. For polymer-based systems, often an undesired massive and uncontrolled late stage drug release was observed [13].

In contrast, drug release from calcium phosphates (CaP), usually driven by desorption, is more evenly and can be better controlled [12]. CaP has excellent biocompatibility, bioactivity, and osteoconductivity due to its chemical and physical resemblance to bone mineral [14,15]. Especially apatites has high surface interaction properties and can bind neutral, positively, and negatively charged molecules enabling a delivery of a wide range of pharmaceuticals such as anticancer drugs [12,16]. The localized drug release from CaP-based drug delivery systems can result in tumour inhibition and can minimize high systemic drug concentration to much lower, less-toxic systemic values and can thereby reduce the need for repeated dosing making it more

* Corresponding author at: Petroceramics SpA c/o Kilometro Rosso Science & Technology Park, Viale Europa 2, 24040 Stezzano, BG, Italy.
E-mail addresses: uhess@uni-bremen.de (U. Hess), shahabi@uni-bremen.de (S. Shahabi), treccanilaura@gmail.com, treccani@petroceramics.com (L. Treccani), philipp.streckbein@uniklinikum-giessen.de (P. Streckbein), christian.heiss@chiru.med.uni-giessen.de (C. Heiss), krezwan@uni-bremen.de (K. Rezwan).
[1] Present address: Petroceramics SpA c/o Kilometro Rosso Science & Technology Park, Viale Europa 2, 24040 Stezzano, BG, Italy.

http://dx.doi.org/10.1016/j.msec.2017.03.164
0928-4931/© 2017 Elsevier B.V. All rights reserved.

comfortable for the patient [12,16]. Consequently, CaP ceramic is an ideal candidate for the dual role as principal filling material for bone defects and for drug-carrying.

In this study we present a CaP beads/matrix composite as open-porous, resorbable scaffold co-loaded for osteosarcoma therapy with two model but clinically relevant cytostatics, namely *cis*-diamminedichloroplatinum (cisplatin, CDDP, *cis*-$[PtCl_2(NH_3)_2]$) [17,18] and doxorubicin hydrochloride (DOX, $C_{27}H_{29}NO_{11}\cdot HCl$) [19]. While the toxicity of CDDP is based on binding to DNA which leads to apoptosis [20,21], DOX is an anthracycline antibiotic which prevents cell replication by intercalating in DNA [22].

Combination chemotherapy using two or more drugs has been proven to be effective and clinically successful [23,24]. Using several drugs can enhance the overall cytotoxicity of each drug at reduced doses, maximizing therapeutic efficacy and overcoming drug resistance [8, 25]. The multicomponent drug treatment may lead to additive, synergistic, or antagonistic effects. This applies also to the combination of CDDP and DOX which may yield strong synergy in the efficacy and may show an increased response rate [26,27].

β-Tricalcium phosphate beads were prepared by droplet extrusion coupled with ionotropic gelation, an established method to produce ceramic beads with tuneable properties [28,29]. They were incorporated in a hydroxyapatite matrix scaffold fabricated via freeze gelation. The possibility to employ these scaffolds as cytostatic depot either by loading matrix and/or beads on cytostatic release was assessed and toxicity of released solutions was evaluated via WST-1 assay on MG-63 osteosarcoma cells.

2. Materials and methods

2.1. Materials

β-Tricalcium phosphate (TCP, specific surface area of 1.1 m^2/g, lot. BCBB7609) powder, hydroxyapatite (HAP, specific surface area of 65 m^2/g, lot. A3420) powder, anhydrous citric acid (lot. BCBB7128), concentrated ammonium hydroxide solution (≥25%, lot. SZBA1400), tris(hydroxymethyl)aminomethane (Tris, lot. MKBD9221V), *n*,*n*-dimethylformamide (DMF, biotech. grade ≥ 99.9%, lot. SHBD8911V), *o*-phenylenediamine (peroxidase substrate ≥ 98.0%, lot. SLBC9002V), 0.1 M sodium phosphate monobasic monohydrate (ACS reagent, 98.0–102.0%, lot. BCBG8983V), 0.1 M sodium phosphate dibasic (≥99%, cell culture tested, lot. BCBD8825V), foetal calf serum (FCS, lot. 010M3395), and *cis*-diamineplatinum(II)dichloride (cisplatin, CDDP, ≥ 99.9%, trace metals basis, lot. MKBR7630V) were purchased from Sigma-Aldrich (Germany). Calcium chloride dihydrate (lot. BCBK7809V) and hydrochloric acid solution (1 M, lot. SZBB2900V) were supplied from Fluka (Germany). Doxorubicin hydrochloride CRS (DOX, code D2975000, batch 6.1, European Pharmacopoeia Reference Standard, France), tri-sodium citrate dihydrate (1 M, lot. 3Z003926, AppliChem, Germany), Dulbecco/Vogt modified Eagle's minimal essential medium (DMEM, high glucose, lot. 1206393, Invitrogen, Germany), antibiotic-antimycotic (lot. 1209917, Invitrogen, Germany), sodium alginate (viscosity of 350–550 mPas, lot. 9O008361, BioChemica, Germany), ammonia stabilised silica sol (SiO_2 content of 30 vol%, particle size of 5–8 nm, surface area of 230–360 m^2/g, BINDZIL® 30NH3/220, lot. 0590b, Eka Chemicals, Germany), and absolute ethanol (≥99.8%, lot. 14G080506, VWR, France) were obtained from different suppliers as specified and used without further purification. Cell culture tests were carried with human osteosarcoma cells (MG-63, passage 98, lot. 2006399, ATCC, Germany). Double deionised water (ddH_2O) with a conductivity of 0.05 μS/cm obtained from an ultra-pure water system (Synergy system, Millipore, Germany) was used for all studies.

2.2. Bead preparation

TCP beads were obtained by ionotropic gelation via droplet extrusion using a protocol adapted from Klein et al. [28]. Briefly, a TCP/alginate suspension was prepared by adding TCP (15.4 wt%) stepwise under stirring (1000 r/min, Dispermat LC-2, VMA-Getzmann, Germany) to a water-based suspension containing sodium alginate (0.7 wt%), silica sol (30.3 wt%) and sodium citrate (0.2 wt%) (Fig. 1). To remove possible agglomerates, the suspension was homogenized for 15 min with an ultrasound horn (Sonifier 450, Branson, Germany; power: 150 W, pulse rate: 0.5 s). Subsequently, the suspension was dropped with a syringe (5 ml Injekt® Luer Solo; needle diameter: 0.55 mm) in a cross-linking solution of ddH_2O and ethanol (ddH_2O/ethanol ratio: 80/20 v/v) and 0.1 mol/l calcium chloride. Beads were left in the cross-linking solution at room temperature for 18 h. Afterwards they were washed three times with ddH_2O to remove excessive calcium ions from their surface. The beads were frozen for 30 min at −150 °C (Ultra-Low Temperature Freezer MDF-1155, Sanyo Electric Biomedical, Japan) and subsequently freeze dried at −20 °C (P8K-E-80-4-80 °C, Piatkowski, Germany) for ~5 days. The beads were used without any further treatment or sintering.

2.3. Bead loading

For loading the TCP beads with cytostatics, concentrated solutions with 60 μg/ml CDDP or 60 μg/ml DOX (Fig. 2,B) dissolved in ddH_2O were used (Fig. 1). 0.5 ml cytostatic stock solution was added to 0.055 g beads and incubated at 37 °C under continuous shaking at 100 r/min (Unimax 1010 with Inkubator 1000, Heidolph Instruments, Germany) to guarantee a uniform drug load. After DOX and CDDP supernatants were removed after 60 min and 48 h, respectively, they were centrifuged for 15 min at 14500 r/min (Heraeus Fresco 21, Thermo Scientific, Germany) to separate and remove any particulates.

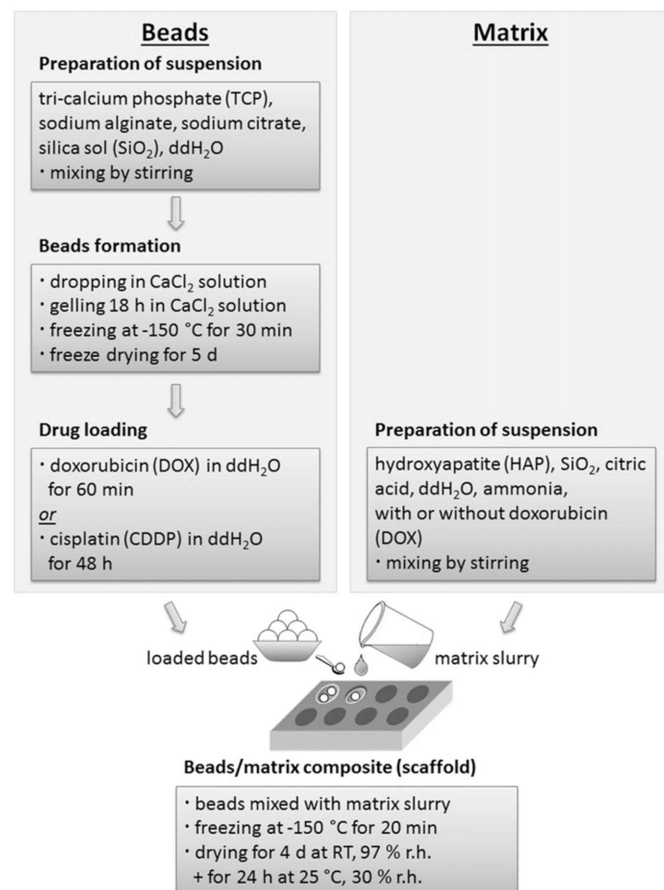

Fig. 1. Schematic representation of non-sintered tricalcium phosphate (TCP) beads production, drug loading and beads/matrix composite preparation.

Download English Version:

https://daneshyari.com/en/article/5434541

Download Persian Version:

https://daneshyari.com/article/5434541

Daneshyari.com