Journal of Orthopaedic Translation (2016) 5, 57—68

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/jot

ORIGINAL ARTICLE

Calcium phosphate/thermoresponsive hyaluronan hydrogel composite delivering hydrophilic and hydrophobic drugs

Dalila Petta [a], Garland Fussell [b], Lisa Hughes [b], Douglas D. Buechter [b], Christoph M. Sprecher [a], Mauro Alini [a], David Eglin [a], Matteo D'Este [a,*]

[a] *AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos, Switzerland*
[b] *DePuy Synthes Biomaterials, 1230 Wilson Drive, West Chester, PA 19380, USA*

Received 5 August 2015; received in revised form 11 November 2015; accepted 17 November 2015
Available online 31 December 2015

KEYWORDS
bone graft substitute;
composite;
drug delivery;
thermoresponsive hyaluronan;
β-tricalcium phosphate

Summary *Background/Objective:* Advanced synthetic biomaterials that are able to reduce or replace the need for autologous bone transplantation are still a major clinical need in orthopaedics, dentistry, and trauma. Key requirements for improved bone substitutes are optimal handling properties, ability to fill defects of irregular shape, and capacity for delivering osteoinductive stimuli.
Materials and methods: In this study, we targeted these requirements by preparing a new composite of β-tricalcium phosphate (TCP) and a thermoresponsive hyaluronan (HA) hydrogel. Dissolution properties of the composite as a function of the particle size and polymeric phase molecular weight and concentration were analysed to identify the best compositions.
Results: Owing to its amphiphilic character, the composite was able to provide controlled release of both recombinant human bone morphogenetic protein-2 and dexamethasone, selected as models for a biologic and a small hydrophobic molecule, respectively.
Conclusion: The TCP—thermoresponsive HA hydrogel composite developed in this work can be used for preparing synthetic bone substitutes in the form of injectable or mouldable pastes and can be supplemented with small hydrophobic molecules or biologics for improved osteoinductivity.

* Corresponding author. AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos, Switzerland.
E-mail address: matteo.deste@aofoundation.org (M. D'Este).

http://dx.doi.org/10.1016/j.jot.2015.11.001

Introduction

Biomaterials able to reduce or replace the need for autologous bone transplantation are a compelling need in maxillofacial, orthopaedic, and reconstructive surgery [1]. In the context of an increasingly ageing global population that is keen on maintaining an active lifestyle and expecting improved life quality, this need is particularly urgent. Nowadays, bone grafting is a very common practice, with 2.2 million procedures being performed annually [2]. Examples of grafting procedures include spinal fusions, limb length restoration, bone reconstruction after tumour resection, and maxillary sinus augmentation [3]. In all of these and in similar cases, bone autografts are generally recognized as more effective than off-the-shelf alternatives; however, the autograft harvesting procedure is associated with considerable risks and donor site pain and morbidity, thereby lowering substantially the benefit-to-risk ratio. Therefore, calcium phosphate (CaP)-based synthetic bone graft substitutes are a valid alternative to autografts and have been clinically used for this purpose for many decades [4–6]. Despite the capacity of supporting bone ingrowth from viable bone at the grafting site (osteoconductivity), they generally do not induce bone growth *per se* (osteoinductivity). CaP-based synthetic bone graft substitutes are specifically produced with interconnected porosity, which facilitates body fluids perfusion, cell invasion, and blood vessel ingrowth. In addition, the surface chemistry of CaP is characterized by the presence of charges from calcium and phosphate ions. This feature is exploited in CaP-based drug delivery systems, particularly for genetic material [7], growth factors [8], and antibiotics [9]. For hydrophobic drug species, the ionic nature of the underlying interactions is a limitation to the versatility of CaP materials as drug delivery systems. Therefore, the combination of CaP with matrices improving their versatility as drug delivery systems is highly needed.

CaPs are generally supplied as granules of varying sizes. As with most ceramic materials, they are inherently brittle, and their mechanical strength is additionally decreased by the porosity. These are important limitations. In fact, a fundamental desirable characteristic of synthetic bone substitutes is being easily shaped to adapt to an irregular bone defect [10]. Ideally, the construct should therefore be sufficiently soft and plastic. At the same time, the implant should not be dislocated after adjacent tissue compression or body fluids washout, and therefore it should maintain its cohesion after implantation. This is often achieved by combining CaP particles and a polymeric matrix or a hydrogel [11–17].

Hydrogels are hydrated molecular networks well-known for their capability of acting as drug reservoirs and facilitating controlled drug release [18,19]. Hydrogels act as matrices, physically slowing down drug diffusion. Moreover, depending on their composition, they can establish specific chemical interactions with the drug and thereby modulate the drug release kinetics. Chemical affinity is sometimes a limitation, as non-water-soluble drugs can be difficult to incorporate into common hydrogels, which have inherently high water content. In this case, liposomes or micro/nanoparticles composites are generally used [20–22].

To target the needs of osteoconductivity, versatility, improved handling and cohesion properties, and potential drug delivery, we prepared a new combination of β-tricalcium phosphate (TCP) granules with a thermoresponsive hyaluronan (HA) matrix. In 2012, Tian et al [23] reported the use of a thermoresponsive chitosan matrix for demineralized bone matrix delivery. In our study, HA was selected as the main component because it is fully biocompatible, non-immunogenic, and readily available in medical grade. HA is a natural component of the extracellular matrix playing a key role in tissue repair [24,25]. Recently, HA was demonstrated to improve the osteoconductive properties of CaP [26]. In this study, HA was modified with pendant moieties of thermoresponsive poly(*N*-isopropylacrylamide) (pNIPAM) to obtain a co-polymer (HpN) that undergoes a temperature-induced transition to a gel state. Importantly, the transition takes place between room and body temperature without covalent cross-linking and therefore in a bio-orthogonal manner, that is, without interfering with physiological biochemical processes.

In this study, we prepared an array of TCP/HpN composites of varying TCP particle size, TCP concentration, polymer phase concentration, and molecular weight of HA within HpN and assessed their properties as potential synthetic bone grafting materials. Handling properties were determined through rheological and dissolution profiles and composites were characterized via scanning electron microscopy (SEM) imaging and micro-computed tomography (micro-CT). Selected composites were tested for their ability to act as drug delivery vehicles. Release rates from composites of recombinant human bone morphogenetic protein-2 (rhBMP-2) and dexamethasone (DEX), which were selected as models of a hydrophilic biological drug and a small hydrophobic drug, respectively, were determined. Finally, cytotoxicity against telomerase-immortalized human foreskin fibroblasts was evaluated.

Materials and methods

Materials

HA sodium salts from *Streptococcus equi* with weight-average molecular weight of 1506 kDa [high molecular weight (HMW)] and 280 kDa [low molecular weight (LMW)] were purchased from Contipro Biotech s.r.o. (Dolni Dobrouc, Czech Republic). Amino-terminated pNIPAM (number-average molecular weight, 34 kDa) was purchased from Polymer Source, Inc. (Dorval, Canada). ChronOS Beta-TCP granules with different particle sizes were supplied by DePuy Synthes USA Products LLC (West Chester, PA, USA), rhBMP-2 (InductOs; Medtronic BioPharma B.V., Heerlen, Netherlands) was purchased from Alloga AG (Burgdorf, Switzerland); DEX was purchased from TCI Europe N.V. (Zwijndrecht, Belgium); rhBMP-2 enzyme-linked immunosorbent assay (ELISA) kit Duo Set from R&D Systems Inc. (Minneapolis, MN, USA), and Cell Titer-Blue Cell Viability Assay from Promega AG (Dübendorf, Switzerland). All other reagents were purchased from Sigma–Aldrich (Buchs, Switzerland). Chemicals were of analytical grade at least, and were used without further purification.