Acta Biomaterialia xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Novel bone wax based on poly(ethylene glycol)–calcium phosphate cement mixtures

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ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form 15 January 2016
Accepted 19 January 2016
Available online xxxx

Keywords:
Bone wax
Poly(ethylene glycol)
Tetracalcium phosphate
Cement

ABSTRACT

Classic bone wax is associated with drawbacks such as the risk of infection, inflammation and hindered osteogenesis. Here, we developed a novel self-setting bone wax on the basis of hydrophilic poly(ethylene glycol) (PEG) and hydroxyapatite (HA) forming calcium phosphate cement (CPC), to overcome the problems that are linked to the use of conventional beeswax systems. Amounts of up to 10 wt.% of pregelatinized starch were additionally supplemented as hemostatic agent. After exposure to a humid environment, the PEG phase dissolved and was exchanged by penetrating water that interacted with the HA precursor (tetracalcium phosphate (TTCP)/monetite) to form highly porous, nanocrystalline HA via a dissolution/precipitation reaction. Simultaneously, pregelatinized starch could gel and supply the bone wax with liquid sealing features. The novel bone wax formulation was found to be cohesive, malleable and after hardening under aqueous conditions, it had a mechanical performance (~2.5 MPa compressive strength) that is comparable to that of cancellous bone. It withstood systolic blood pressure conditions for several days and showed antibacterial properties for almost one week, even though 60% of the incorporated drug vancomycin hydrochloride was already released after 8 h of deposition by diffusion controlled processes.

Statement of Significance

The study investigated the development of alternative bone waxes on the basis of a hydroxyapatite (HA) forming calcium phosphate cement (CPC) system. Conventional bone waxes are composed of non-biodegradable beeswax/vaseline mixtures that are often linked to infection, inflammation and hindered osteogenesis. We combined the usage of bioresorbable polymers, the supplementation with hemostatic agents and the incorporation of a mineral component to overcome those drawbacks. Self-setting CPC precursors (tetracalcium phosphate (TTCP), monetite) were embedded in a resorbable matrix of poly(ethylene glycol) (PEG) and supplemented with pregelatinized starch. This formulation was found to be malleable and cohesive underwater. While immersion in an aqueous environment, CPC precursors formed highly porous, nanocrystalline HA via dissolution/precipitation reaction as water penetrated the novel wax formulation and PEG molecules simultaneously dissolved. The bone wax further withstood blood pressure conditions. After hardening, mechanical performance was comparable to that of cancellous bone and we also successfully provided the bone wax with antibacterial properties.
In our opinion, the described bone wax formulation outmatches conventional bone waxes, as it circumvents the detriments being associated with the term "bone wax". Our wax has a novel composition and would broaden the application of CPC and besides, the general interest in bone waxes will increase, as they were long considered as a "first-line treatment" to avoid.

1. Introduction

Since its introduction in 1886 by V. Horsley [1,2] bone wax is used as mechanical hemostatic agent in the case of bone injury without having intrinsic hemostatic properties [3]. From historical

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http://dx.doi.org/10.1016/j.actbio.2016.01.021
1742-7061/

point of view, bone wax was a mixture of beeswax, salicylic acid and almond oil [2]. Nowadays, sterilized [3] mixtures of beeswax, isopropyl palmitate and softening agents are used [1,2]. Bone wax is getting soft and malleable when warmed [1] and can be pressed onto an osseous wound to create a physical barrier (tamponade) to bleeding [3]. It is easy to handle and cost-effective [3], but its suitability as bone sealant should be considered critically. Beside allergic reactions [1], several clinical case reports recovered foreign body reactions with fistula [4], abscess [5] or granuloma [6] formation, serous discharge [7] and inflamed fibrous tissue [8] after treatment of cranial defect [4], mastoid [5], iliac crest [7] or sternum [6,8] with bone wax. Furthermore the risk of infection increases when bone wax is used as sealant agent as it serves as nidus for bacteria [1] such as *Staphylococcus aureus,* that may promote diseases like osteomyelitis [9]. Damages of the surrounding tissue are also reported in terms of inferior alveolar nerve morbidity [10] and cord compression [11]. Bone wax further hinders osteogenesis as it is not resorbable under physiological conditions [1,2]. These adverse effects on bone regeneration could be seen in animal models with defects at iliac crest [12], tibia [13] and sternum [14,15] associated with inflammation and reduced mechanical integrity [15]. For those reasons, bone wax is only recommended as a first-line treatment [3] in non-infected defect sites [1] and a reconsideration of the classical bone wax composition should be performed urgently.

Some efforts were made to improve the properties of bone wax formulations. The use of resorbable polymers as for example alkylene oxide copolymers [14,16], poly(ethylene glycol) (PEG) [17], PEG/microfibrillar collagen composites [18], PEG-poly(propylene glycol)-PEG copolymers [19,20] or chitosan [21] may improve osteogenesis. As they dissolve in the body, no interferences with bone healing take place and infection rates may be reduced [14,16,20]. The supplementation with hemostatic agents, as for example pregelatinized starch [22], is an approach to promote liquid sealing ability of bone wax as it absorbs water and low molecular weight components from blood. Starch is a natural polysaccharide [19] being composed of two glucose polymers [23] amylopectin and amylose [19,23,24] with varying amylose contents between 0 and 80% [24]. Gelatinization of native starch with subsequent dehydration leads to the formation of pregelatinized starch [23,24], which is known to form gels in cold water whereas native starch is water-insoluble [23]. Pregelatinized starch is known to be biodegradable [25], non-toxic, non-irritating and cost-effective [19] and was found to be suitable for controlled release matrices [23,24] and tissue engineering scaffolds [26,27].

Incorporation of osteoconductive particles into the above mentioned bone wax like systems is rather described in literature. Chen et al. [28] analyzed bioactive glass/chitosan/carboxymethyl cellulose composites. Hoffmann et al. [21] used starch and chitosan modified hydroxyapatite particles to form a water-based slurry that could be suitable as substitute for classic bone wax. Here, we exchanged these osteoconductive, but non-reactive calcium phosphate particles by reactive calcium phosphate cement (CPC) precursors, which were directly incorporated into the resorbable PEG matrix. To our knowledge, such a system has not been presented so far.

CPC were first described by Brown and Chow in the 1980s as seen in Eq. (1). In an aqueous environment, one (or more) calcium phosphate (CaP) phases dissolve and a CaP with a lower solubility precipitates [29].

$$\underset{\text{tetracalcium phosphate}}{Ca_4(PO_4)_2O} + \underset{\text{monetite}}{CaHPO_4} \rightarrow \underset{\text{hydroxyapatite}}{Ca_5(PO_4)_3OH} \quad (1)$$

Mechanical rigidity of the resulting specimen is provided by crystal growth and entanglement [29]. CPC can be classified into precipitated hydroxyapatite (pH > 4.2; $Ca_5(PO_4)_3OH$; HA) and brushite (pH < 4.2; $CaHPO_4 \cdot 2H_2O$) forming cement systems [30]. HA forming cements are widely used in clinics for non-load-bearing applications [29] as they are known to be self-setting, biocompatible, bioactive, osteoconductive [30] and stoichiometrically similar to the mineral bone component [31]. The implementation of HA forming precursor powders into a water-soluble polymer matrix prevents a setting reaction of the cement before contact with physiological fluids. Cement systems based on this principle are known from literature as ready-to-use [32,33] respectively premixed [34–39] cement pastes, but have lower viscosity for minimal invasive application and are not suitable for non-cavity-like bone defects.

The present study evaluated a novel bone wax system based on HA forming precursor powders tetracalcium phosphate (TTCP)/monetite that were implemented into a plastic PEG matrix. To improve its liquid sealing ability up to 10 wt.% pregelatinized starch from corn were supplemented and the cohesion, compressive strength, mass loss, phase composition, morphology, PEG and ion release, porosity and water sealing duration were analyzed. As infection with *S. aureus* is a well-known problem with classic bone wax, antibiotic release and antibacterial properties were also tested via vancomycin hydrochloride incorporation.

2. Materials and methods

2.1. Raw powder preparation

TTCP raw powder was synthesized by sintering a 1.05:1 M mixture of $CaHPO_4$ (monetite, J.T. Baker, Griesheim, Germany) and $CaCO_3$ (Merck KGAa, Darmstadt, Germany) for 5 h at 1500 °C in a sintering furnace (Oyten Thermotechnic, Oyten, Germany). The sintering cake was crushed and sieved <125 μm and milled for 10 min at 200 rpm in a planetary ball mill (PM400 Retsch, Haan, Germany). Monetite was milled in ethanol for 24 h at 250 rpm followed by drying in vacuum. The cement powder was prepared by mixing 30.0 g monetite and 74.2 g TTCP for 20 min at 100 rpm.

2.2. Bone wax preparation and release study

5 g of starch from corn (food grade, Frießinger Mühle GmbH, Bad Wimpfen, Germany) was gelatinized in 30 mL hot water, mixed with 30 mL ethanol, filtered and dried. The resulting pregelatinized starch, just named starch in the following sequences, was crushed with a pestle and mortar and sieved <125 μm.

4 g of the TTCP/monetite raw powder were additionally mixed with 1 g NaH_2PO_4 and dispersed in 3 g of a molten PEG mixture consisting of PEG 1,500 and PEG 400 in a 4:1 weight ratio. Starch amounts of 1.0 wt.% and 10 wt.% were previously added to the solid phase of the bone wax. After hardening, the bone wax was softened and kneaded until a homogeneous texture was reached. Cuboidal samples with 6 × 6 × 12 mm dimensions were kneaded and deposited in 5 mL phosphate buffered saline (PBS; 8.0 g/L NaCl, 1.1 g/L Na_2HPO_4, 0.2 g/L KCl, 1.1 g/L KH_2PO_4; both potassium salts purchased from Merck KGaA, Darmstadt, Germany) per cuboid for up to 24 d at 37 °C with a change of PBS every second day. Before, variant compositions were tested to find a suitable composition for bone wax. Thus differently composed cuboids were placed in petri dishes with 5 mL water and their cohesiveness was evaluated subjectively. Additionally their wet compressive strength was measured after 24 h setting in water. All chemicals, if not else described, were purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany.

Please cite this article in press as: T. Brückner et al., Novel bone wax based on poly(ethylene glycol)–calcium phosphate cement mixtures, Acta Biomater. (2016), http://dx.doi.org/10.1016/j.actbio.2016.01.021