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Influence of different polymeric gels on the ectopic bone forming ability of an osteoinductive biphasic calcium phosphate ceramic

Davide Barbieri [a,*], Huipin Yuan [a,b], Florence de Groot [a], William R. Walsh [c], Joost D. de Bruijn [a,d]

[a] Xpand Biotechnology BV, Prof. Bronkhorstlaan 10, Building 48, 3723 MB Bilthoven, The Netherlands
[b] MIRA Institute for Biomedical Technology and Technical Medicine, Department of Tissue Regeneration, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
[c] Surgical and Orthopaedic Research Laboratories, Prince of Wales Clinical School, University of New South Wales, Randwick, New South Wales 2031, Australia
[d] School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS, UK

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ABSTRACT

To evaluate moldable osteoinductive putties for bone repair we combined microstructured biphasic calcium phosphate (BCP) particles with five different polymeric gels, carboxymethyl cellulose (CMC), Pluronic® F-127 (PLU), polyvinyl alcohol (PVA), chitosan (CHI) and alginate (ALG). In vitro gel dissolution showed that CMC, PLU and ALG gels dissolved rapidly (within hours), while the CHI gel took several days and the PVA gel did not dissolve within 2 weeks. Implanting the putty formulations into sheep muscle for 12 weeks demonstrated ectopic bone formation in the control BCP group as well as the putties prepared with dissolving gels (CMC, PLU, ALG and CHI). Bone was not seen in the putty comprising PVA. Quantitative data showed that the CMC and PLU gels did not significantly affect the osteoinductivity of BCP granules, while the ALG and CHI gels showed a significant decrease in bone formation. These results suggest that the dissolvability and chemistry of the gels may be factors affecting the osteoinduction of the putties.

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1. Introduction

Due to the many disadvantages of the gold standard, autologous bone grafting (i.e. additional surgical procedures, pain and limited availability), there is a need to develop alternative (synthetic) bone graft materials that can stimulate bone healing. Commercially available bone growth factors such as rhBMP-2 have a strong osteoinductive potential but can result in undesired ectopic bone growth [1], making them sub-optimal to replace autografts in the long term. Inorganic, synthetic materials such as polymers and calcium phosphate ceramics, on the other hand, generally do not have bone-inducing potential and can therefore only be used to repair small bone defects in an osteoconductive fashion.

In the past 20 years calcium phosphate ceramics with microstructured surfaces have been reported to have the unique ability to induce bone growth when intramuscularly implanted in large animals (e.g. dog, sheep and goat) [2–10]. Although the process underlying bone induction by these materials has not yet been fully elucidated [11], surface microstructural features such as grain and micropore size have been shown to play a crucial role in several studies. Yamasaki and Saki [4] and Yuan et al. [12] implanted hydroxyapatite (HA) with and without a microstructured surface into the muscles of dogs and reported that only those materials possessing a microstructure could induce ectopic bone formation [4,12]. Similar results were shown in studies by Habibovic et al. [13], who compared HA and biphasic calcium phosphate (BCP) ceramics with various microporosities. It has furthermore been shown that microstructured, osteoinductive calcium phosphate ceramics are superior to non-osteoinductive calcium phosphate ceramics for the repair of critical sized bone defects (e.g. 17 mm diameter in goats) [14,15]. Recently, Yuan et al. [11] showed that these materials are a promising synthetic alternative to autologous bone grafting.

Since most calcium phosphate ceramics are prepared in block or particulate form, their handling properties are not optimal. Filling bone voids with individual particles may lead to incomplete filling of the defect or granule dispersion and loss during surgery. These disadvantages might be overcome by developing injectable pastes or putties in which a binder or gel is added to the calcium phosphate ceramic granules [16–21]. This would allow moldability of the material, i.e. its ability to be shaped and then placed to fit a defect of irregular geometry, as are encountered during surgery, and to be in direct contact with the bone surrounding the defect. Currently, several commercial injectable or moldable bone grafts are available [22], but none of them contain osteoinductive ceramics. Adding a polymer binder to osteoinductive calcium phosphate

* Corresponding author. Tel./fax: +31 0 30 2297299.
E-mail addresses: davide.barbieri@progentix.com, davide.barbieri@xpand-biotech.com (D. Barbieri).

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ceramic would appear the method of choice, but this may be detrimental to its osteoinductive potential. It has been suggested that the mechanism of material-directed osteoinduction involves the attachment of progenitor cells to the material surface, followed by osteogenic differentiation and bone deposition [11], thus covering the microstructured material surface with a polymer binder or gel could inhibit or delay osteoinduction.

We hypothesise that, besides an inert chemistry, rapid dissolution and clearance of the polymer binder surrounding microstructured calcium phosphate granules is needed to allow osteoinduction to occur. To test this, we investigated the influence of five polymeric gels with different chemistries and dissolution rates on the osteoinductivity of surface microstructured biphasic calcium phosphate particles. The effect of the polymeric gels on the bone forming ability of the putties was ectopically evaluated using an intramuscular implantation model.

2. Materials and methods

2.1. Polymeric gels

Five polymeric gels with a history of medical or pharmacological use were prepared using raw polymeric particles. The polymers were (1) high viscosity carboxymethyl cellulose sodium salt (CMC) (VWR, Lutterworth, UK), (2) Pluronic® F-127 (PLU) (Sigma–Aldrich, Steinheim, Germany), (3) polyvinyl alcohol (PVA) (Merck, Darmstadt, Germany), (4) native chitosan (CHI) (Marinard Biotech, Rivière-au-Renard, Canada) and (5) native sodium alginate (ALG) (Sigma–Aldrich). The technical details and clinical applications of the polymers are summarised in Table 1 [23–30]. Depending on the polymer used, different concentrations of polymeric gel were prepared by combining raw polymer particles with an aqueous solvent (Table 2). The final concentrations of the gels were set according to the ability of the gel to retain calcium phosphate granules in a putty (described in detail in Section 2.2). The solutions were stirred until complete dissolution and the pH of the chitosan solution was adjusted with an aqueous solution of 48% w/v glycerol phosphate disodium salt (Sigma–Aldrich) to 7 at 37 ± 2 °C. The pH of the other solutions was around the physiological range and was therefore not adjusted (Table 2). The gels were then placed in syringes (BD Plastipak™, 50 cc, BD, Temse, Belgium), sealed with plastic syringe caps and stored till use. Depending on the kind of gel, the storage could be at room temperature (polymer gels prepared with synthetic origin raw powders, i.e. PLU and PVA) or in a refrigerator at 4 °C to prevent possible formation of mildew in the case of natural/animal origin gels, i.e. CMC, ALG and CHI (Table 2).

2.2. Preparation of putties

Irregularly shaped particles of osteoinductive surface microstructured biphasic calcium phosphate ceramic (BCP) (composition 20 ± 5% β-tricalcium phosphate (TCP) and 80 ± 5% hydroxyapatite) HA, porosity 60 ± 5%, particle size 1–2 mm (Xpand Biotechnology BV, Bilthoven, The Netherlands) were used. The chemistry of the ceramic particles was confirmed by X-ray diffraction (XRD) (MiniFlex I, Rigaku, Tokyo, Japan), while the surface morphology and grain size were evaluated by environmental scanning electron microscopy (ESEM) (XL 30 ESEM-FEG, Philips, Eindhoven, The Netherlands). The microporosity of the ceramic was evaluated with mercury intrusion (Micromeritics, Norcross, GA). For each putty 1 cc of ceramic particles was mixed with the respective gel to obtain as low as possible a gel/BCP volume ratio, but high enough to guarantee moldability of the material. The final compositions (gel/BCP volume ratio) of the putties are summarised in Table 2. As may be observed, the gel/BCP ratios of the putties are not the same because of the different gels used. Once the putties were ready they were placed in plastic syringes (Braun Injekt™, 2 cc, Braun Medical, Sheffield, UK) and sealed with plastic syringe caps. The materials were then sterilized using γ-irradiation (irradiation dose 25 kGy,

Table 1
Details on the polymers used in this study.

Polymer	Uses
Carboxymethyl cellulose sodium salt (viscosity in 1% water at 20 °C 1500–2500 mPa)	Several fields, including the preparation of putties and injectable pastes for tissue engineering [16,23,24]
Pluronic® F-127 (M_w 12,, molar ratio PEO:PPO:PEO 98:68:98)	Pharmaceutical applications as drug delivery material [23,25]
Polyvinyl alcohol (Merck) (M_w 22,000, degree of hydrolysis ⩾98%)	Excellent for medical applications such as long-term implants [23,26] and soft contact lenses [27]
Chitosan (degree of deacetylation 94%)	Recently approved for clinical use as wound bandages due to its biocompatibility and antibacterial properties [23,28]
Sodium alginate (native, low guluronic content)	Clinically used in the dental (e.g. dental impression materials and denture fixatives) and drug fields (controlled release tablets and encapsulation), but has also been studied as an attractive material for bone tissue engineering [23,29,30]

Table 2
Details of the preparation of the putties.

		Gel				Putty
Polymer used	Solvent	Concentration (% wt)	Preparation temperature	Final pH	Storage conditions	Gel/BCP (ml/ml)
Carboxymethyl cellulose sodium salt	Distilled water	5	60 ± 5 °C	7.3–7.8	Refrigerator	1
Pluronic® F-127	Distilled water	38	40 ± 5 °C	6.8–7.5	Room temperature	0.85
Polyvinyl alcohol	Distilled water	25	80 ± 5 °C	7.2–7.6	Room temperature	0.75
Chitosan	1.2% acetic acid	1.6	37 ± 2 °C	6.9–7.1 (adjusted)	Refrigerator	0.95
Sodium alginate	Distilled water	5.25	60 ± 5 °C	7.1–7.8	Refrigerator	1

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