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Osteoinduction by calcium phosphate biomaterials

HUIPIN YUAN*, ZONGJIAN YANG, YUBAO LI, XINGDONG ZHANG
Institute of Materials Sciences and Technology, Sichuan Union University, Chengdu, 610064, People's Republic of China
E-mail: Huipin.Yuan@Isotis.com

J.D. DE BRUIJN, K. DE GROOT
Biomaterials Research Group, Leiden University, and IsoTis BV, Professor Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands

Different materials were implanted in muscles of dogs to study the osteoinduction of calcium phosphate biomaterials. Bone formation was only seen in calcium phosphate biomaterials with micropores, and could be found in hydroxyapatite (HA) ceramic, tricalcium phosphate/hydroxyapatite ceramic (BCP), β-TCP ceramic and calcium phosphate cement. The osteoinductive potential was different in different materials. The results indicate that osteoinduction can be a property of calcium phosphate biomaterials when they exhibit specific chemical and structural characteristics. © *1998 Kluwer Academic Publishers*

1. Introduction

Owing to their biocompatibility and osteoconductivity, various calcium phosphate biomaterials have been developed as bone substitutes. Among these are hydroxyapatite ceramic (HA), tricalcium phosphate/hydroxyapatite ceramic (BCP), tricalcium phosphate ceramic (TCP) and calcium phosphate cement (CPC) [1–7]. An ideal bone substitute should be osteoinductive, while it is generally thought that calcium phosphate biomaterials do not exhibit bone-inducing capacities [1–7]. Over the past few years, however, several publications have emerged that reported calcium phosphate-induced osteogenesis in soft tissues [8–18]. Many of our experiments have shown osteoinduction by calcium phosphates, which appeared to be at least partially related to the animal species [12–18]. Herein, we provide an overview of several studies in which calcium phosphate-induced osteogenesis was examined in dog muscle, with special emphasis on material properties.

2. Materials and methods

2.1. Materials

Nine kinds of materials were tested in the studies as indicated in Table I. Material 1 was prepared by Mitsubishi Ceramic Int. Co. (Japan), X-ray diffraction (XRD) analysis showed that it was HA, and no micropores could be found on the pore surface under SEM (Fig. 1a). The other materials were prepared by Sichuan Union University (SCUU), China. Calcium phosphate ceramics were made by the following procedures: apatite powders with different Ca/P ratio (1.67 for HA, 1.60 for TCP/HA and 1.50 for TCP) were wet-synthesized and foamed to a green body by H_2O_2 solution. The green body was subsequently sintered at high temperature (1100–1200 °C) to obtain the ceramics. The chemical constitution was checked by XRD [12, 14]. TiO_2 ceramic was made from TiO_2 powder with the same foaming and sintering procedure as that used to prepare calcium phosphate ceramics. Porous ceramics with macropores and micropores on the macropore surface (Fig. 1b) could be made by this method. Calcium phosphate cement was made by mixing powders containing α-TCP, DCPD and a small amount of HA with phosphate solution containing Na^+ and K^+; when implanted as a paste, the cement was prepared just before implantation; when implanted as prehardened form, the cement was prepared 2 d before implantation. Apart from cement paste, other implants with suitable size (diameter 3.5 mm × 8.5 mm to 5 mm × 6 mm) were prepared and cleaned with distilled water and sterilized by autoclaving at 121 °C for 30 min before implantation.

2.2. Surgical procedure and histological preparation

Surgery was performed under general anaesthesia and sterile conditions on adult dogs. The number of materials, the implantation sites and the harvest time are shown in Table I. The harvested samples were fixed in 10% buffered formalin, dehydrated in a series of alcohol solutions, and embedded in methyl methacrylate (MMA). Un-decalcified sections (10–20 μm)

*Author to whom all correspondence should be addressed.
Present address: IsoTis BV, Professor Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands, also Biomaterials Research Group, Leiden University.

TABLE I Summary of bone formation in different materials

No.	Material	Producer[a]	Sinter. temp. (°C)	Micropore[b]	Impl. site[c]	Time[d]						
						30 d	37 d	45 d	60 d	90 d	150 d	180 d
1.	HA	J	1200	−	DM					0/4		0/4
2.	HA	C	1200	+	DM	0/4		0/4	0/4	2/4		4/4
3.	HA	C	1100	+	DM	1/4		2/4		4/4	4/4	4/4
4.	TCP/HA	C	1200	+	DM	0/4		2/4	4/4			
5.	α-TCP	C	1100	+	DM	0/4		0/4			0/4	
6.	β-TCP	C	1100	+	DM	0/4		2/4			4/4	
7.	H-Cement	C	(Pre-hardened)	+ [6,19]	TM		1/2		2/2			1/2
8.	P-Cement	C	(Paste)	+ [6,19]	DM					0/4		2/4
9.	TiO_2	C	1200	+	DM		0/2		0/2			0/2

[a]J, Mitsubishi, Japan; C, Sichuan Union University, China.
[b](−) without micropore; (+) with micropores.
[c]DM, dorsal muscle; TM, thigh muscle.
[d]2/4, bone formation was found in 2 of 4 implants.

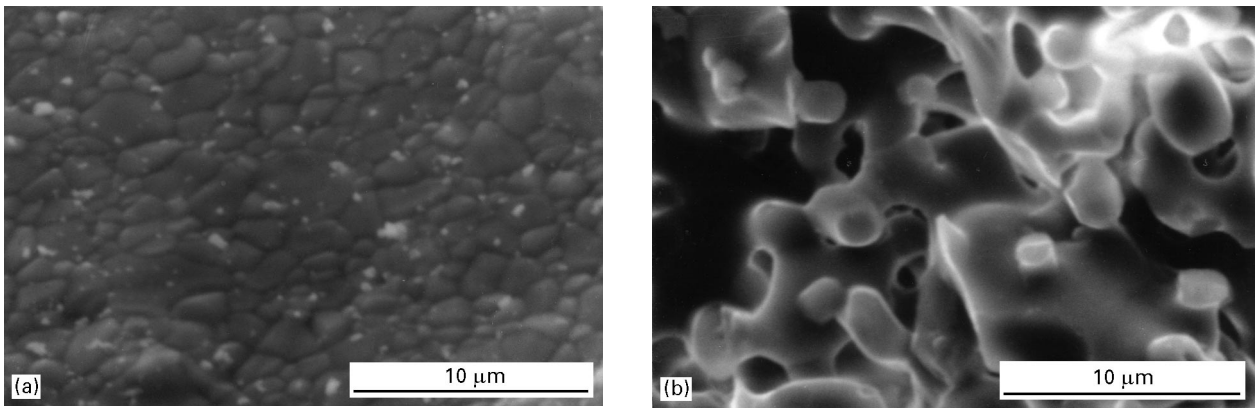


Figure 1 Microstructure on the pore surface of calcium phosphate biomaterials. (a) Smooth pore surface of material 1 without micropores; (b) typical rough pore surface of ceramic made by SCUU (β-TCP ceramic).

were made and stained with methylene blue and basic fuchsin.

Some decalcified sections were analyzed. In that case, fixed samples were decalcified in an acid compound (1000 ml solution containing 8.5 g sodium chloride, 100 ml formalin, 70 ml 37% hydrochloric acid, 80 ml formic acid, 40 g aluminum chloride and 25 ml acetic acid glacial), dehydrated in alcohol and embedded in paraffin; decalcified sections were stained with haematoxylin and eosin (HE). BSE (back-scattered electron microscopy) observation was made on un-decalcified sections coated with carbon.

3. Results

The results with regard to osteoinduction are summarized in Table I. It is clear that the osteoinductive potential varies significantly between the type of materials examined. No bone formation was found in any implants of HA without micropores (material 1), TiO_2 ceramic (material 9) and α-TCP (material 5). Only bone-like tissue could be detected in HA sintered at 1200 °C (material 2) at 90 and 180 d (Fig. 2), while bone formation could be found in HA sintered at 1100 °C (material 3) as early as 30 d (Fig. 3). As reported before [14, 15], bone formation could be found at 45 d post-operatively in TCP/HA (material 4). Bone formation could also be found in pores and deep rugged surface of cement, both implanted as prehardened form and paste (Fig. 4). Bone formation in β-TCP started before 45 d, while, at longer time implantation, the induced bone in β-TCP ceramic demineralized at the interface between bone tissue and ceramic (Fig. 5).

A bonding osteogensis process, in which the osteogenic precursor cells became attached to the pore surface, aggregated, proliferated, differentiated, produced bone matrix and ossified, could be observed in calcium phosphate-induced osteogenesis. Bone formation started from the pore surface and proceeded to the pore center; the induced bone bonded to calcium phosphate materials and no chondrocyte could be detected during the osteoinduction.

4. Discussion

Osteoinduction of different calcium phosphate biomaterials has been found in rabbits, goats, pigs, dogs, monkeys and baboons [8–18]. Different times for bone formation and different amounts of bone induced by the same material in different animal models indicate that osteoinduction of calcium phosphate biomaterials is animal-dependent [12, 13, 16, 17]. On the other hand, different tissue responses of different

calcium phosphate biomaterials in the same animal suggests that osteoinduction of calcium phosphate biomaterials is also material-related [11, 12]. However, the characteristics of calcium phosphate biomaterials affecting the osteoinductive potential is not yet clear. The present results suggest that the following factors are important in calcium phosphate biomaterial-induced osteogenesis.

1. Microporosity. Evidence can be found in the differences among material 1, 2, 3, 4, 6, 7 and 8; the

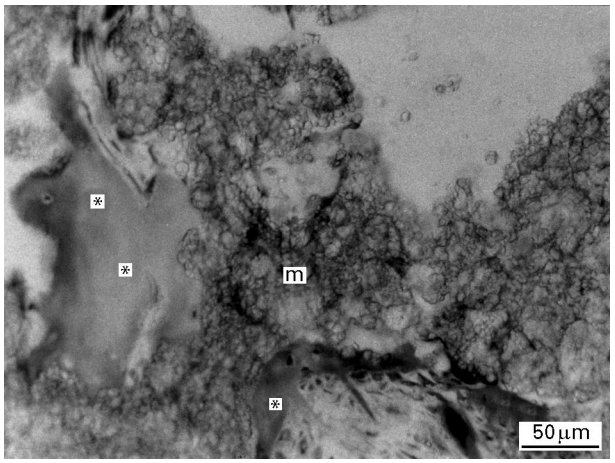


Figure 2 Bone-like tissue in HA (material 2, 1200 °C) at 180 d: un-decalcified section, methylene blue and basic fuchsin staining. m, ceramic; *, bone-like tissue.

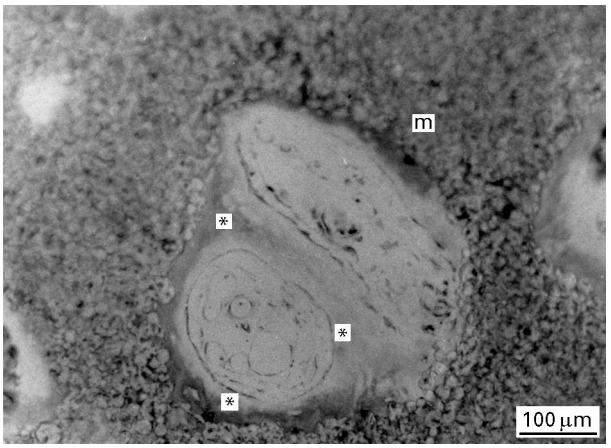
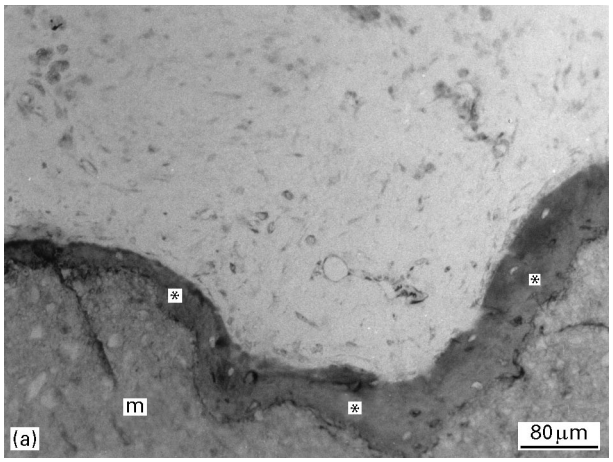


Figure 3 Bone formation in HA (material 3, 1100 °C) at 45 d; un-decalcified section, methylene blue and basic fuchsin staining, m, ceramic; *, bone.



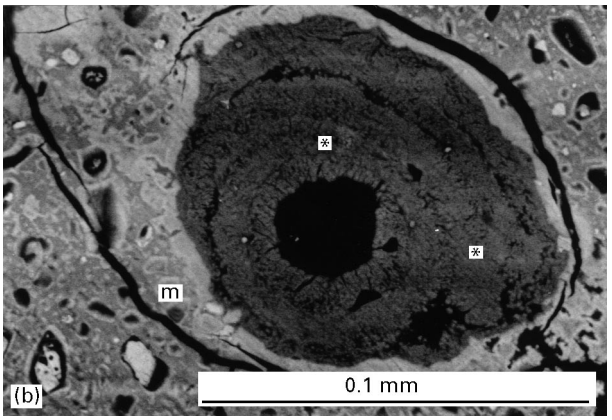
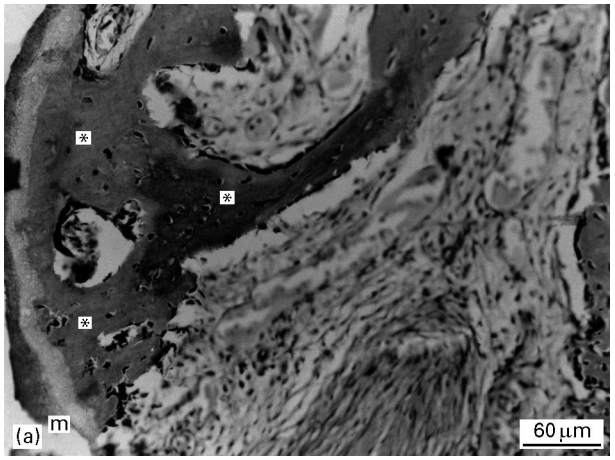


Figure 4 Bone formation induced by calcium phosphate cement. (a) Bone formation in deep rugged surface of prehardened cement at 60 d; un-decalcified section, methylene blue and basic fuchsin staining. (b) Bone tissue formed in pores of cement implanted as paste at 180 d (back-scattered electron image). m, cement; *, bone.



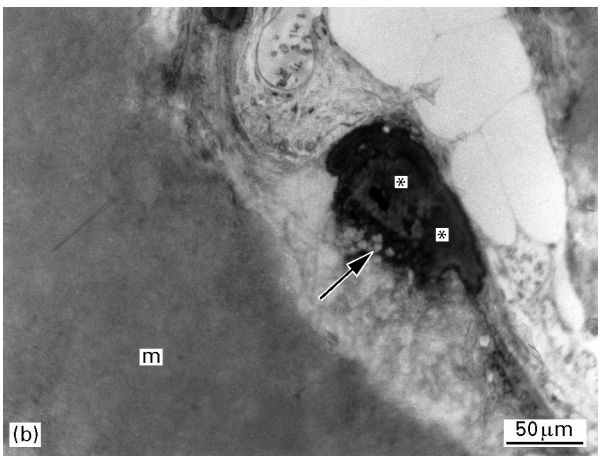


Figure 5 Bone formation in β-TCP. (a) 45 d, decalcified section, HE staining; (b) 150 d, un-decalcified section, methylene blue and basic fuchsin staining, note bone demineralization (arrow); m, ceramic; *, bone.

absence of micropores in material 1 resulted in the absence of bone induction even after 180 d.

2. Sintering temperature. Low sintering temperature is beneficial to calcium phosphate-induced osteogenesis, as indicated by the differences in results between materials 2 and 3, sintered at 1100 °C; normal bone formation was always found in material 3 [16, 17], while only bone-like tissues could be found at a longer time in material 2 which was sintered at 1200 °C.

3. Mild dissolution of the materials. Less osteoinductive potential of HA (material 2) than that of TCP/HA (material 4) was found in previous study [12, 14, 15] and this study; the reason might be that the mild dissolution of TCP/HA provides a Ca/P environment needed for bone formation. On the other hand, rapid dissolution of the materials is detrimental, as indicated by materials 5 and 6. No bone formation was seen in α-TCP; while initial bone induction could occur in β-TCP (Fig. 5). However, the normal bone at early time (Fig. 5a) in β-TCP reversed to bone degeneration at longer time (150 d) (Fig. 5b), because no bone marrow or bone remodeling could be detected, but clear bone demineralization could be observed.

4. Three-dimensional environment. In materials 7 and 8, bone formation only occurred in the deep rugged surface of the cement and in pores into which soft tissue could grow. Bone formation in the ceramics also occurred in the pores.

5. Material specificity. Osteoinduction could not be found in ceramics other than calcium phosphate biomaterials. Bone formation was not observed in TiO_2 ceramics (material 9), although they had micropores on the macropore surface.

5. Conclusion

We have shown that several kinds of calcium phosphate biomaterials can induce bone formation in soft tissues. The results presented herein indicate that osteoinduction can be a property of calcium phosphate biomaterials when they exhibit specific chemical and structural characteristics.

Acknowledgment

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