Ectopic bone formation by microstructured synthetic materials through *in vivo* protein adsorption

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Introduction

Microstructured porous calcium phosphates (CaP) not only support bone formation in orthotopic sites but can also give rise to bone formation in ectopic or non-osseous sites [1,2]. One explanation for this osteoinductive behaviour is that the enlarged surface area allows the binding of high concentrations of proteins such as BMPs. If *in vivo* concentration of proteins is a true mechanism of ectopic bone formation, materials other than CaP may also have osteoinductive properties provided that they have an enlarged surface area (micropores) and can bind similar proteins.

To test this hypothesis, we compared the ability of hydroxyapatite (HA), TiO_2 ceramic and Al_2O_3 ceramic to absorb various proteins and evaluated the osteoinductive capacities of these materials after intramuscular implantation in canines.

Materials and methods

HA ceramic with low surface area (HA-L) was prepared as described previously using PMMA [2]. Microstructured HA (HA-H), microstructured TiO_2 and microstructured Al_2O_3 ceramic were prepared using a diluted H_2O_2 solution and high temperature sintering. Ceramic cylinders (∅5x6mm) and ceramic particles (1.0-1.4mm) were prepared, cleaned and sterilized. The microstructures of the ceramics were observed with scanning electronic microscopy (SEM).

Using a micro-BCA kit, the abilities of the ceramics to absorb BSA, lysozyme and serum proteins from aqueous solutions were evaluated (3x1.0cc ceramic particles per material). The ability of the ceramics to concentrate rhBMP-2 from a DMEM with 1% FBS and 250ng/ml rhBMP-2 were examined with Elisa (3x1.0cc ceramic particles per material).

The osteoinductive capacity of the ceramics was evaluated by intramuscular (i.m.) implantation in 8 dogs (male, 10-15kg) for 3 months. Routine histology was performed on the harvested samples and histomorphometry was performed regarding the area percentage of the formed bone in the available space. Back scattered scanning electron microscopic observation (BSE) was performed regarding the mineralized bone.

Student's *t*-test was performed and statistical significance was defined as $p<0.05$.

Results

SEM indicated the presence of micropores in HA-H, TiO_2 and Al_2O_3, while no micropores were observed in HA-L (Fig. 1).

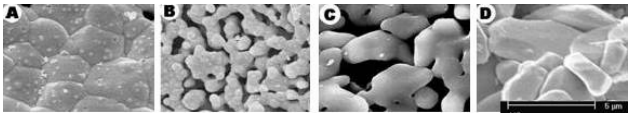

Fig. 1. SEM of HA-L (A), HA-H (B), TiO_2 (C) and Al_2O_3 (D)

The ability of the ceramics to absorb single proteins varied widely. HA-H has a higher affinity to BSA than TiO_2 and Al_2O_3 (Fig. 2A) ($p<0.05$), while Al_2O_3 absorbed more lysozyme than TiO_2 than HA (both HA-L and HA-H) (Fig. 2B) ($p<0.05$). The amount of the serum proteins absorbed to ceramics was different at a short time (after 1 day) ($HA\text{-}H>TiO_2>Al_2O_3$, $p<0.05$), while with an increased soaking time, the amount of serum proteins absorbed was not chemistry-dependent but surface area dependent (7 days after soaking) (Fig. 2C). All ceramics concentrated rhBMP-2 from the DMEM/FBS/rhBMP-2 solution. HA-H, TiO_2 and Al_2O_3 concentrated more rhBMP-2 than HA-L after 7-day soaking (Fig. 2D) ($p<0.05$).

Bone formation was found in all implants of HA-H, TiO_2 and Al_2O_3, but not in HA-L (Table 1). The percentage of the formed bone in the implants was not significantly different between HA-H, TiO_2 and Al_2O_3.

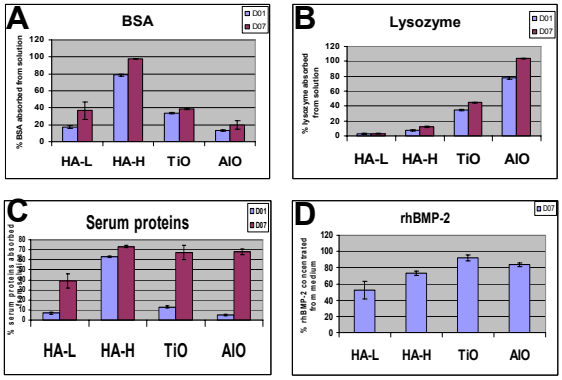

Fig. 2. Proteins adsorbed into 1.0cc ceramic particle from 4ml aqueous solution containing (A) 200ug BSA/ml, (B) 100ug lysozyme/ml, (C) 1% FBS, and rhBMP-2 concentrated into 1.0cc ceramic particle from 45 ml DMEM with 1% FBS and 250ng rhBMP-2/ml (D).

Table 1. Bone formation in ceramics after 3mth i.m. implantation

	HA-L	HA	TiO	AlO
Chemistry	HA	HA	TiO_2	Al_2O_3
Micropores	No	yes	yes	yes
macroporosity	50±5	29±3	32±6	47±3
Implants	8	8	8	8
Bone incidence	0/8	8/8	8/8	8/8
Bone ratio (%)	0	10±6	16±5	17±9

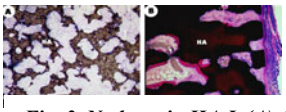

Fig. 3. No bone in HA-L (A) & bone formation in HA-H (B)

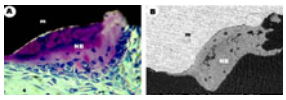

Fig. 4. Bone formation in TiO_2. A: Bonding osteogenesis; B: direct mineralization on TiO

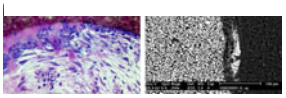

Fig. 5. Bone formation in Al_2O_3. A: Bonding osteogenesis; B: bone mineralization far from the surface.

Bone formation started on the surface of HA-H (Fig. 3B), TiO_2 (Fig. 4A) and Al_2O_3 (Fig. 5A). Bone mineralization started on surface of HA (Fig. 3B) and TiO_2 (Fig. 4A& 4B), but away from the surface of Al_2O_3 (Fig. 5B).

Discussion and conclusion

Although the affinity of single proteins to the ceramics depended on the chemistry, the amount of complex proteins (i.e. serum proteins) absorbed onto ceramics at a longer time was not dependent on the chemistry but likely the surface area. Microstructured ceramics absorbed more rhBMP-2 from a culture medium with 1% FBS, while all microstructured ceramics resulted in ectopic bone formation, regardless of their chemistries. It is possible that by *in vivo* concentrating more proteins such as BMPs on the enlarged surface, microstructured materials can induce ectopic bone formation.

References

1. Yuan H, *et al*, Biomaterials 1999; 20:1799-1806;
2. Habibovic P, *et al*, Biomaterials 2005; 26:3565-3575.