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IN VIVO EVALUATION OF SYNTHETIC POROUS CALCIUM PHOSPHATES

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BACKGROUND

Clinical success has been obtained by grafting the maxillary sinus with different synthetic materials. New bone formation after sinus graft procedure was demonstrated through histological assessment in animals and in humans. Calcium phosphates (CaP) as hydroxyapatite (HA), betatricalcium phosphate (βTCP) and their combinations (HA/βTCP), and an innovative composite material HA/TTCP with higher mechanical strength have been proposed as synthetic bone grafts. Granules with spherical morphology allows easy injectability into bone voids; intra-granules macroporosity is fundamental for tissue and vascular ingrowth thus determining fast implants osteointegration.

MATERIALS & METHODS

CaP materials were supplied in four composition (HA, βTCP, HA/βTCP, HA/TTCP) in porous granules and aggregates form (Osprolife, Eurocoating SpA). The morphology and internal porosity and average pore size were evaluated by SEM (JSM-5500, Jeol). XRD patterns were recorded on Rigaku Dmax III diffractometer by using Cu ka radiation.
For the evaluation of the specific surface area (SSA) BET (Nitrogen ASAP 2010 Micromeritics) analyses was performed.
In vitro studies: solubility was evaluated on 300-600 µm granules in TRIS (pH 7.3) solutions. To confirm granules purity and absence of any toxic effects, cytotoxicity tests were performed on a cell line of mice fibroblasts Balb/c 3T3 according to EN ISO 10933-5.
In vivo studies: bone implantation study was planned and designed in agreement with ISO 10993-6. Granules were implanted in femurs of New Zealand white rabbit and explanted 6, 12 and 52 weeks after surgeries. Histological study was performed on retrieved biopsies and µCT images were acquired. Moreover a preliminary study was carried out on 2 adult primates (Macaca Fascicularis): sinus augmentation was performed using HA/TTCP granules (300-600 µm).

RESULTS & DISCUSSION

Spherically-shaped granules were manufactured with a uniform size, their diameter can be chosen in the range 300-1200 µm. Granules surface is rough, with intra-granule micro-porosity (0.1-10 µm). Aggregates were prepared with diameters in the range 1.2-5 mm, having same microporosity, but also inter-granules macroporosity (50-300 µm)(Fig.1).

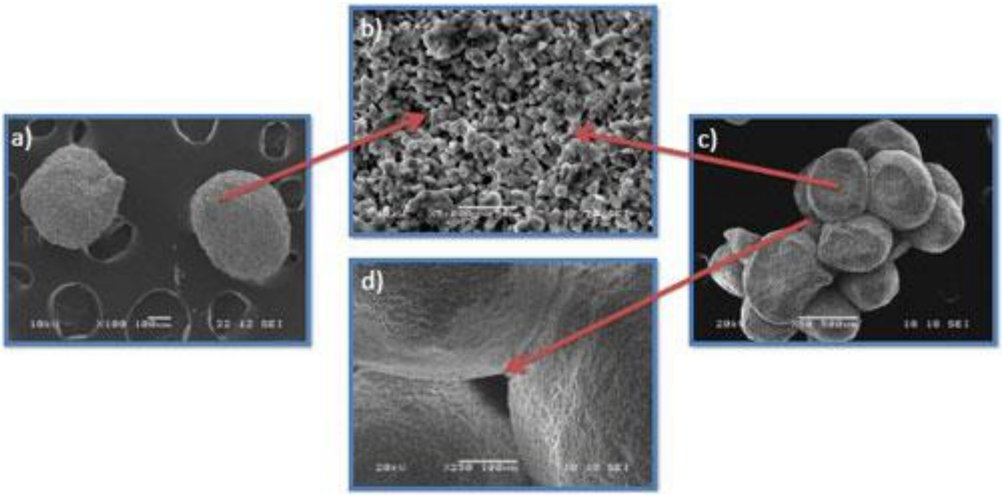

Figure 1. SEM micrographs of granules (a), internal microporosity (b), aggregates (c) andintergranules macroporosity of aggregates (d).

XRD analysis (Fig. 2) revealed the final composition of CaP granules; the sum of extraneous phases (CaO for HA, TTCP for HA/βTCP, αTCP and $C_2P_2O_7$ for βTCP granules) was less than 5 wt%, thus fulfilling the requirements of ISO 13779-3 norm.

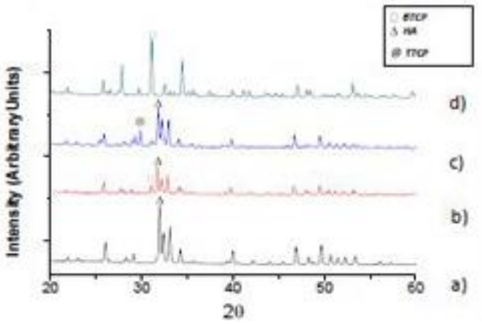

Figure 2. XRD pattern of SEM micrographs of a) HA, b) HA/βTCP, c) HA/TTCP and d) βTCP granules

Dissolution behavior

Ca^{2+} (Fig. 3) and $PO_4{}^{3-}$ concentration released from granules in TRIS solution referred to 60 days confirmed that HA granules are the less resorbable even with the highest SSA. βTCP granules were also found slowly resorbable due to low SSA. Granules with biphasic compositions are dissolved faster than monophasic ones. Moreover, between biphasics, HA/βTCP (higher SSA) showed to be more resorbable than HA/TTCP.

Finally biphasic HA/TTCP granules resulted more resorbable than monophasic granules in spite of less favorable physical characteristics.

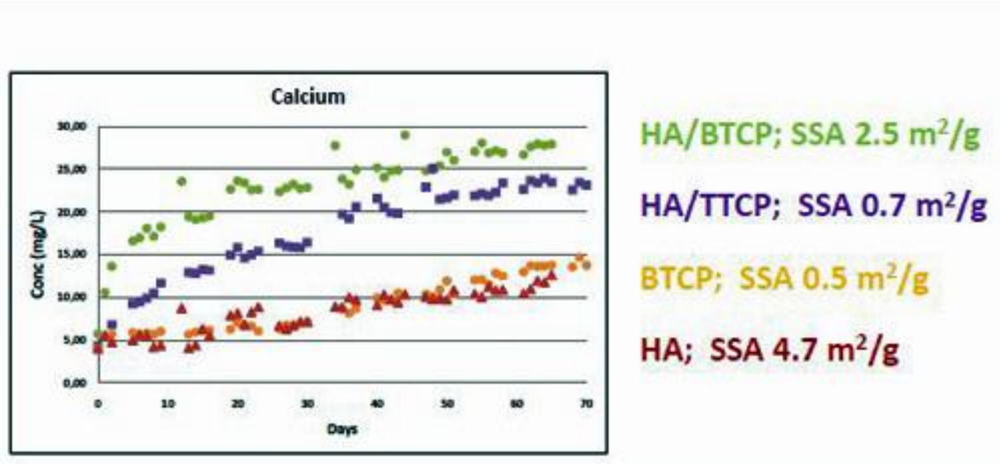

Figure 3. Ca^{2+} concentrations released from each type of granules in TRIS solution at pH 7.3

Cytotoxicity behavior

Cells showed a vitality trend confirming non cytotoxic all compositions of OsprolifeTM granules and aggregates (i.e. HA; βTCP ; HA/βTCP; HA/TTCP).

Bone response in rabbits

Histological findings at 12 weeks confirm the results obtained after 6 weeks of implantation: neither local nor systemic toxicity due to HA, βTCP, HA/βTCP and HA/TTCP granules were macroscopically observed.

Histomorphometric analysis on the retrieved samples demonstrated that these biomaterials are biocompatible, resorbable and highly osteoconductive. Abundant bone formation was observed in femurs treated with CaP granules (Fig.4 and Fig.5).

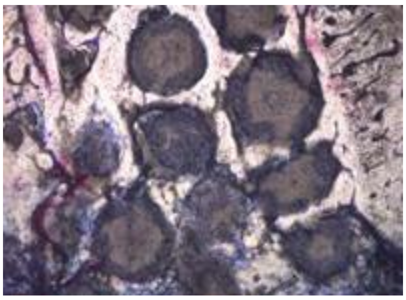

Figure 4. Non decalcified slice forhistomorphometric analysis: bone ingrowth within HA/βTCP granules is pronounced just 6 weeks after implantation. Large resorption fronts are extend all around granules, as the toluide blue staining show.

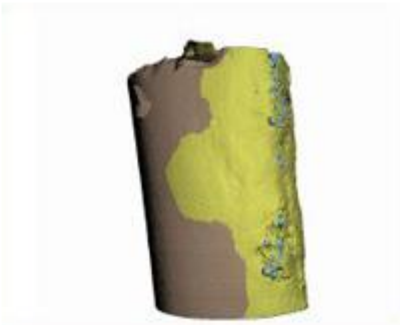

Figure 5. 3D images from microCTscans. βTCP granules (in blue) are well integrated in the newly formed bone (in green) 6 weeks after implantatio

Preliminary study on new HA/TTCP granules in Macaca Fascicularis

At 14 weeks new bone were 17% of total volume and it increased to 27% at 17 weeks. At this time Bone-graft contact reached 64%. Histologies showed newly formed bone trabeculae. Graft Volume was 29% at 17 weeks, thus confirming HA/TTCP as a slowly resorbable synthetic biomaterial. Results are shown in Table 2 and examples of histology are illustrated in Fig. 6.

Explantation time	BV Bone Volume [%]	VB Vital bone [%]	GV Graft Volume [%]	BGC Bone Graft Contact [%]
14 weeks	54.74	17.39	37.35	39
17 weeks	56.93	27.58	29.36	64

Table 2. Histo-morphometric data after 14 and 17 weeks.

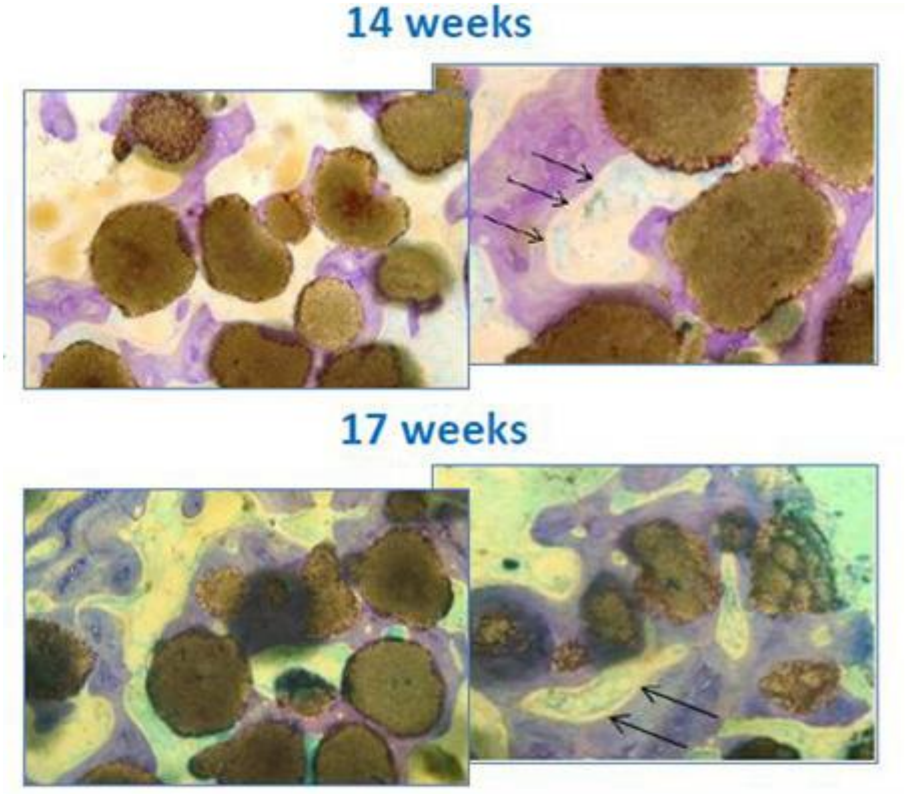

Figure 6 Histological appearance of trabecular bone in-growth within HA/TTCP granules. Arrows indicate osteoid formation

CONCLUSIONS

Results suggest that Osprolife granules and aggregates proposed in the present work can be used as fillers for bone regeneration in small, non load bearing and confinable lacunae. Their features facilitate bone tissue ingrowth and bone void filling as shown by preliminary in vivo experiments. Biomaterials appear well integrated in newly formed bone showing good osteoconductive properties.

ACKNOWLEDGEMENTS

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