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CAP GRANULES-AGGREGATES FOR BONE VOID FILLER APPLICATIONS

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INTRODUCTION

Calcium Phosphates (CaP) materials are highly used as bone void fillers due to their properties of biocompatibility, bioactivity and osteconduction.

[1] Hydroxyapatite (HA) is a very popular material in dental and orthopaedic field, however other compositions, more quickly resorbable, have been developed in recent years (i.e. β-tricalciumphosphate (βTCP) or biphasic HA/βTCP). In this work the chemical-physical characterization of these bone grafts are presented in comparison with a new biphasic composition HA/tetracalciumphosphate (TTCP).Moreover preliminary in vivo test results are given.

MATERIALS & METHODS

CaP materials, obtained by droplet extrusion, were supplied in four composition (HA, βTCP, HA/βTCP, HA/TTCP) in porous granules and aggregates form (Eurocoating SpA, Italy). The morphology, surface roughness, internal porosity and average pore size were evaluated by SEM(JSM-5500,Jeol). For the evaluation of the specific surface area (SSA) BET (Nitrogen ASAP 2010 Micromeritics) and Hg-porosimetry analyses (CE 2000) were performed. The density was calculated with He-picnometry (1035 Micromeritics). XRD patterns were recorded on Rigaku Dmax III diffractometer by using Cu ka radiation. For FT-IR analysis, products were reduced to powder and analyzed with Thermo Nicolet Avatar 330. The solubility of each type of granule was evaluated through dissolution rate and dissolution behavior tests by measuring calcium and phosphate release in TRIS solution at pH 7.3. The cytotoxicity test was monitored toxic effects of granules on a cell line of mice fibroblasts Balb/c 3T3 in the cell culture under EN ISO 10933-5. Preliminary in vivo sinus graft study was performed for HA/TTCP granules following actual rules for animal care, and involved adult monkeys (Macaca Fascicularis).

RESULTS & DISCUSSION

Morphology and Microporosity

Spherically-shaped granules were manufactured with a uniform size, their diameter can be chosen in the range 300-1200 µm. Surface of granules is rough, with intra-granule micro-porosity. Aggregates were also prepared with diameters in the range 1.2-5 mm and macroporosity inter-granules (Fig.1)

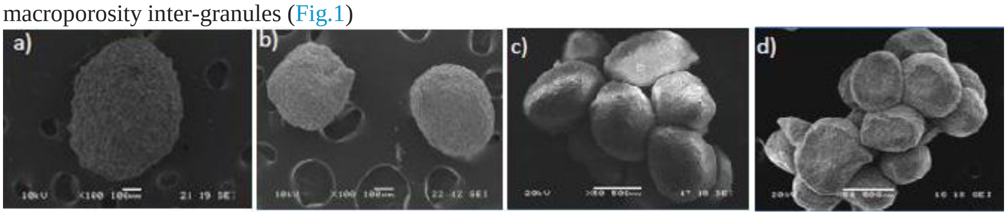
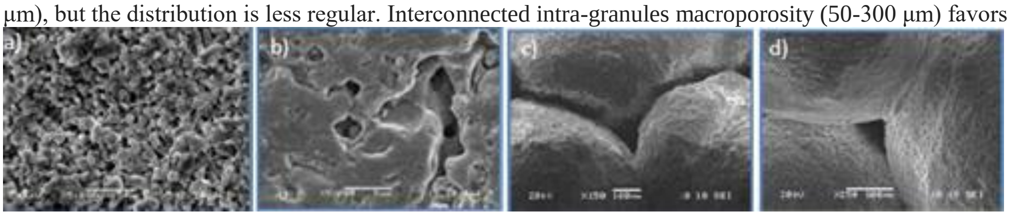


Figure 2 shows a comparison of CaP granules section that allows to analyze the internal microporosity. For HA and HA/βTCP granules micropores are homogenously distributed and very small (0,1-1 µm). βTCP and HA/TTCP spheres show bigger pore (1-10 µm), but the distribution is less regular. Interconnected intra-granules macroporosity (50-300 µm) favors the cell penetration.



Mineralogical composition

XRD analysis (Fig. 4 and Tab. 1) revealed the final composition of CaP granules; the sum of extraneous phases (CaO for HA, TTCP for HA/βTCP, αTCP and C2P2O7 for βTCP granules) was less than 5 wt%, thus fulfilling the requirements of ISO 13779-3 norm.

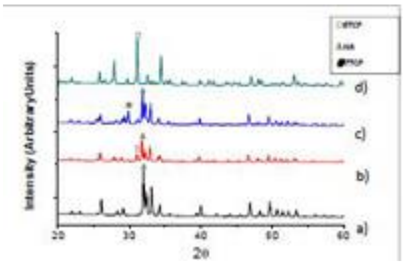


Figure 3. XRD pattern of SEM micrographs ofa) HA, b) HA/βTCP, c) HA/TTCP and d) βTCP granules

%wt	HA	βTCP	TTCP	CaO	$Ca_2P_2O_7$	αTCP
100 βTCP	0.0	98.0	0.0	0.0	1.0	1.0
60 HA/40TTCP	55.6	0.0	45.4	0.0	0.0	0.0
60HA/40βTCP	60	38.8	1.2	0.0	0.0	0.0
100 HA	99.3	0.5	0.0	0.2	0.0	0.0

Table 1. Quantitative analysis of CaP granules and detection of foreign phases

Specific surface area and porosity

The highest SSA was obtained for HA and HA/βTCP granules that have more small micropores than βTCP. HA/TTCP spheres are the more dense and have the lowest SSA (Table 2).

Dissolution behavior

Ca2+ (Fig. 4) and PO4 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 biphasic, 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.

Sample	SSA [m²/g]	Average pore size [nm]	Porosity [%]
HA	4.7	190	48
βTCP	0.7	3400	56
HA/βTCP	2.5	290	50
HA/TTCP	0.5	250	11

Table 2. SSA, average pore size, % porosity and density data for all families.

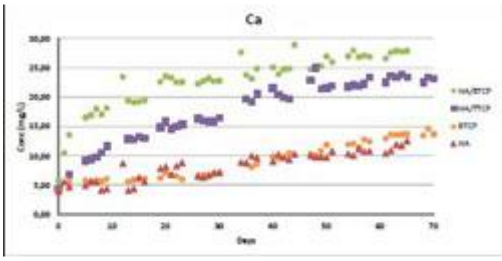


Figure 4. Ca2+ concentrations released from each type of granules in TRIS solution at pH 7.3.

Cytotoxicity

Cells showed a vitality trend confirming non cytotoxic all the granules composition (i.e. HA; βTCP ; HA/βTCP; HA/TTCP)

Preliminary "In vivo" test on new HA/TTCP granules

After healing several newly formed bone trabeculae were observed. At 14 weeks the new BV is 17% and it increases until 27% at 17 weeks. Bone graft contact reaches 64%. For HA/TTCP composition Graft Volume is 29% at 17 weeks, thus confirming it as a slowly resorbable synthetic biomaterial as shown Table 3 and illustrated in Fig. 5.

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 3. Histo-morphometric data after 14 and 17 weeks.

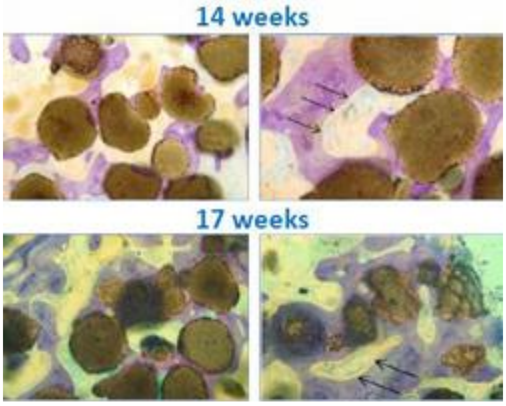


Figure 5 Histological appearance of trabecular bone in-growth within HA/TTCP.
Arrows indicate osteoid formation.

CONCLUSIONS

In this work are shown CaP granules with controlled spherical size, porous surface, internal microporosity and different chemical composition. All these features can be modulated to favor or limit the dissolution rate of bone substitute. Preliminary in vivo study on HA/TTCP granules in primates gave excellent result at two months. HA/TTCP is a promising biomaterial to improve new bone formation and reduce graft healing time.

ACKNOWLEDGEMENTS

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REFERENCES

[1] V. Dorozhkin,BIO ,2011, 1, 1-51