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SYNTHETIC POROUS CALCIUM PHOSPHATE GRANULES FOR BONE SUBSTITUTES

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Calcium phosphates (CaP) are suitable as bone substitutes due to their biocompatible, bioactive and osteoconductive characteristics. Hydroxyapatite (HA), β-tricalcium phosphate (βTCP) and their combination (HA/βTCP) are the most used compositions for bone substitutes. In addition, tetracalcium phosphate (TTCP) is a bioceramic that shows in vivo solubility similar to βTCP, but with increased mechanical strength [1]. In the present study are characterized different porous granules suitable as bone lacunae fillers for non load-bearing biomedical applications. Spherical morphology is better than the irregular morphology as the former has greater flexibility in filling irregular lacunae and the latter can be related to inflammatory processes. [2] The combination, HA/TTCP, was attempted and was compared with HA, βTCP and HA/βTCP manufactured with the same technique. Size control (300-1200 μm), composition (pure and biphasic calcium phosphates) and internal micro-porosity modulation (0.1-10 μm) are possible with the presented process. These parameters affect specific surface area and final biomaterial solubility.

MATERIALS & METHODS

CaP materials were supplied in four composition (HA, βTCP, HA/βTCP, HA/TTCP) in porous granules form (Eurocoating SpA).

Characterization

The morphology, surface roughness, internal porosity and average granules 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, operating at 40 kV and 30 mA. For FT-IR analysis, granules were reduced to powder and analyzed as KBr pellets 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.

RESULTS & DISCUSSION

Morphology and Microporosity

The round morphology and an example of sub-millimeter dimension for each type of CaP granules are shown in Figure 1.

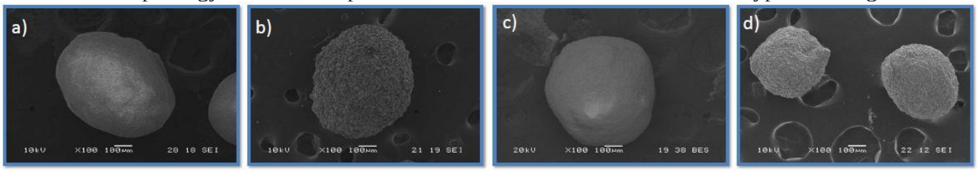

Fig. 1 - SEM micrographs of spherical a) HA, b) βTCP, c) HA/βTCP ad d) HA/TTCP granules

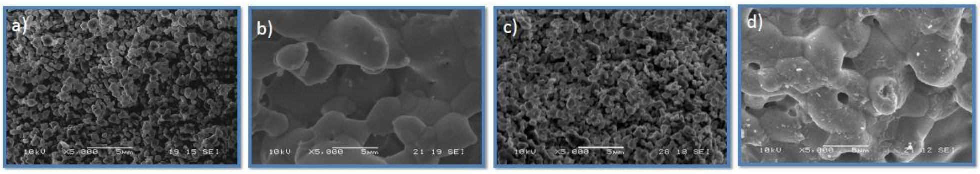

Fig. 2 - SEM micrographs of a) HA, b) βTCP, c) HA/βTCP and d) HA/TTCP granules surface

SEM pictures shown in Figure 2 allow to point out the surface roughness. HA and HA/βTCP granules have similar homogenous superficial morphology, while βTCP and HA/TTCP spheres are less smooth. It is useful to recall that surface roughness is important for improving idrophillicity and initial adsorption of physiological fluids and blood. Figure 3 shows a comparison of CaP granules section that allows to analyze the internal microporosity.

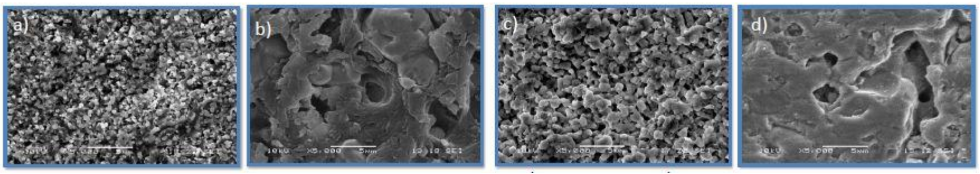

Fig. 03 - SEM micrographs of a) HA, b) βTCP, c) HA/βTCP and d) HA/TTCP granules section.

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.

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.

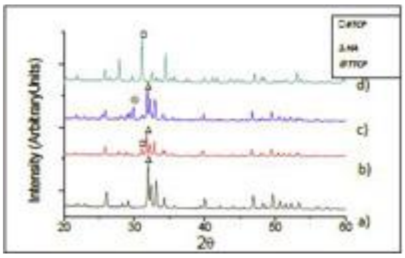

Figure 4. XRD pattern of SEM micrographs ofa) HA, b) HA/βTCP, c) HA/TTCP and d) βTCP granules

%wt	HA	βTCP	TTCP	CaO	$Ca_2P_2O_7$	αTCP
βTCP	0,0	[illegible]	0,0	0,0	1,0	1,0
HA/TTCP	[illegible]	0,0	45,4	0,0	0,0	0,0
HA/βTCP	60	38,8	1,2	0,0	0,0	0,0
HA	[illegible]	0,5	0,0	0,2	0,0	0,0

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

FT-IR spectra of crushed CaP granules confirmed the presence of typical bands of calcium phosphate (PO43-) and the absence of CO32-, this indicating that all organic phases are burnt out during sintering (Fig. 5 and Fig. 6).

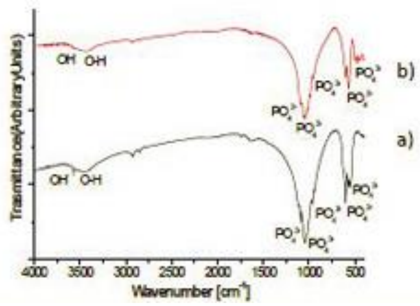

Figure 5. FT-IR spectra of a) HA and b) HA/TTCP granules.

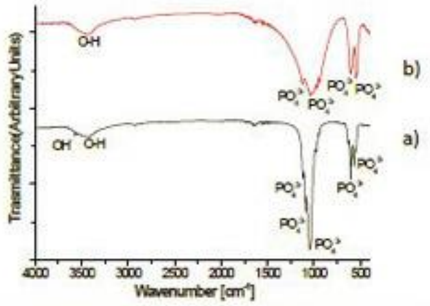

Figure 6. FT-IR spectra of a) HA/βTCP and b) βTCP granules.

BET , Hg-porosimetry and He-picnometry analyses

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)

Solubility test

Ca^{2+} (Fig. 7) and PO43- 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.

Sample	SSA_BET [m²/g]	SSA_Hg Porosimetry [m²/g]	Average pore size [µm]	Porosity [%]	Apparent density [g/cm³]
HA	3,92	3,70	0,19	47,59	0,44
βTCP	0,67	0,93	3,40	55,76	0,51
HA/βTCP	2,51	2,36	0,29	49,62	0,47
HA/TTCP	0,48	0,30	0,25	11,11	0,88

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

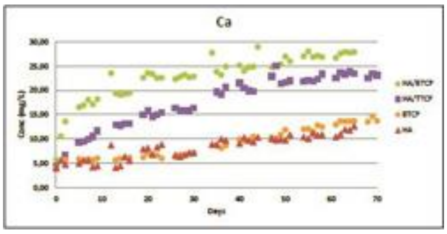

Figure 6. FT-IR spectra of a) HA/βTCP and b) βTCP granule

Cytotoxicity test

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

CONCLUSIONS

In this work CaP granules with controlled size, microporosity and different chemical composition were obtained by droplet extrusion method. All granules are spherical with porous surface and internal microstructure can be modulated to favor or limit the dissolution of bone substitute. In vivo test are in progress.

ACKNOWLEDGEMENTS

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