

## Enzymatic Synthesis of Amorphous Calcium Phosphate-Chitosan Nanocomposites and its Processing into Hierarchical Structures

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The requirements for the production of suitable supports for the growth of cells and tissues involve the use of biocompatible and/or biodegradable materials and the processing of the components into a porous matrix of adequate morphology. The use of organic and inorganic materials has been widely explored, but lately, the use of organic-inorganic composite materials is attracting even increased attention. Controlling the length scale at the organic-inorganic interface within the nanometer range produces a wealth of both novel structural features and enhanced properties arising from the synergistic interaction of the individual constituents; i.e., consequence of the strong mechanical interface between mineral substrate and polymer matrix [1]. Biomineralization offers the opportunity to produce highly organized nanocomposite structures, controlling specific architectures over extended length scales for a wide range of compositions [2]. Here in, we have applied an enzymatically assisted route (e.g., the urease assisted hydrolysis of urea) for the preparation of nanocomposites. The base generated by urea hydrolysis promoted both CHI gelation and calcium phosphate precipitation at biological temperatures (~37 °C) [3]. Macroporous hierarchical structures were thereafter obtained by a cryogenic process (named ISISA, ice segregation induced self-assembly) that simply consist on the unidirectional freezing (at -196 °C) of the hydrogel nanocomposite [4, 5]. Upon freezing, the ice formation (hexagonal form) causes every solute originally dissolved/dispersed in the hydrogel to be segregated from the ice phase. After freeze-drying, the resulting hierarchical structures consists on well aligned micrometer-sized pores in the freezing direction corresponding to the empty areas where ice crystals originally resided, being the macrostructure supported by the matter (e.g., calcium phosphate nanoparticles dispersed within CHI matrix) accumulated between adjacent ice crystals [6].

### References:

- [1] (a) Mann, S. *Angew. Chem. Int. Ed.*, 39, (2000) 3392-406. (b) Sanchez, C.; Arribart, H.; Giraud-Guille, M. M. *Nat. Mater.*, 4, (2005)277.
- [2] (a) Sanchez, C.; Julián, B.; Belleville, P.; Popall, M. *J. Mater. Chem.* 15 (2003), , 3559-3592. (b) Tai, K.; Ulm, F.-J.; Ortiz, C. *Nano Lett.* (6),2006, 2520-2525.
- [3] (a) Sondi, I.; Matijević, E. *J. Coll. Inter. Sci.* 238, (2001), 208-214. (c) Chenite, A.; Gori, S.; Shive, M.; Desrosiers, E.; Buschmann, M. D. *Carbohyd. Polym.* 64, ( 2006), 419-424.
- [4] For a recent review see: Gutierrez, M. C.; Ferrer, M. L.; del Monte, F. *Chem. Mater.* 20,(4), (2008), 634-648.
- [5] See also: (a) Mukai, S. R.; Nishihara, H.; Tamon, H. *Chem. Commun.* 874, (2004). (b) Gutierrez, M. C.; Hortigüela, M. J.; Amarilla, J. M.; Jimenez, R.; Ferrer, M. L.; del Monte, F. *J. Phys. Chem. C*, 111, (2007) 5557, and references there in. (c) Gutierrez, M. C.; Garcia-Carvajal, Z.; Hortigüela, M. J.; Yuste, L.; Rojo, F.; Ferrer, M. L.; del Monte, F. *J. Mater.*

*Chem.* 17(29), (2007), 2992-2995. (d) Gutierrez, M. C.; Garcia-Carvajal, Z.; Yuste, L.; Rojo, F.; Ferrer, M. L.; del Monte, F. *Adv. Funct. Mater.* 2007 (DOI: 10.1002/adfm200700093) (e) Abarrategui, A.; Gutierrez, M. C.; Moreno-Vicente, C.; Hortigüela, M. J.; Ramos, V.; López-Lacomba, J. L.; Ferrer, M. L.; del Monte, F. *Biomaterials* 29, (2008), 94-102.

[6] Gutierrez, M. C.; Jobbagy, M.; Ferrer, M. L.; del Monte, F. *Chem. Mater.*, 20 (2008), 11-13.

**Figures:**

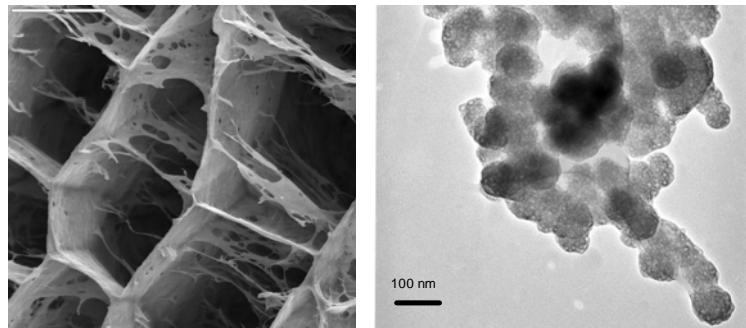


Figure 1: SEM micrograph of hierarchically structured amorphous calcium phosphate-chitosan nanocomposites (left, bar is 50  $\mu\text{m}$ ). TEM micrograph of amorphous calcium phosphate aggregates entrapped within the chitosan network gel (right, bar is 100 nm).