A 3D TYPE I COLLAGEN / HYDROXYAPATITE NANOPARTICLES COMPOSITE FOR BONE REGENERATION

A.Y. Pataquiva Mateus [1,2], M.P. Ferraz[1,3], E.J. Herrero[4], F.J. Monteiro[1,2]
[1] INEB— Instituto de Engenharia Biomédica, Laboratório de Biomateriais, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal.
[2] Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e Materiais, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal.
[3]Universidade Fernando Pessoa, Praça 9 de Abril 349, 4249-004 Porto, Portugal.
[4] Hospital Universitario Puerta de Hierro, Unidad de Biomateriales, Servicio de Cirugía Experimental, San Martín Porres 2, 28035 Madrid, Spain.

Introduction

Collagen (COL) is the major structural protein of connective tissue such as skin, bone, cartilage, tendons and ligaments.COL is present in 19 varieties in the human body. It constitutes about one-third of the total body protein in mammals. Because of its biological properties and easy availability, COL type I is widely used as a biomaterial, on multiple physical forms such as sponges, films and membranes, wire, fabrics. Among relevant properties of COL are low immune response, low toxicity, the ability to promote cellular growth and attachment, homeostasis and the ability of COL solution to reconstitute *in vitro* the microfibrillar structure found in natural tissues [1].

Synthetic hydroxyapatite (HA) has excellent biocompatibility and bioactivity due to its chemical and structural resemblance to mineral bone and tooth. It has been clinically used as sintered bulk ceramic, porous structures, granules and coatings. Nano-sized HA formulations, with properties closer to those of living bone, are presently being studied, as HA particles size is a relevant factor for i*n vitro* cell activity, and is a parameter of great importance for injectability and handling. Desired characteristics of synthesised HA are fine and uniform particle size, in the nanometre range, phase homogeneity and minimized degree of particle agglomeration. Although many chemical processing routes have been employed to prepare fine HA powders, in this work chemical precipitation has been chosen as it is the most commonly used alternative [2].

Materials and Methods

Solutions of calcium hydroxide (Ca(OH)2) and ortho-phosphoric acid (H3PO4, 85%), both of analytical grade, were used as reactants for the preparation of HA nanoparticles. Firstly, 1L of an aqueous suspension of H3PO4 (0.6M) was slowly added drop by drop to 1L of an aqueous suspension of Ca(OH)2 (1M) with addition of sodium dodecylsulphate (10g) while vigorously stirring for 2h at room temperature. Concentrated NaOH was added until a final pH of 10.5 was reached. The white and opaque solution obtained was washed using de-ionized water and was dried at 80°C for 24 h.

Type I Collagen was obtained from the tail tendons of young *Wistar* rats. After washing in 1% (w/v) NaCl solution, the tendons were dissolved in 0.5M acetic acid for 3-4 days at 4ºC. After adding pepsin (0.5mg/ml) in 0.5M acetic acid was incubated for 24h and with a subsequently centrifugation at 5000rpm for 1h at 4ºC. Solid NaCl was added to the pepsin-soluble portion to a final concentration of 1M. After centrifugation and re-suspension in acetic acid the solution was dialyze against 0.5M acetic acid at 4ºC to remove salt [3]. Fig 1 shows an interconnective macroporous sponge obtained from a collagen solution lyophilised for 48h.

The solution of COL/nanoHA was prepared adding 0.5 – 5% w/w nanoHA on a collagen solution and put in moulds after constant agitation avoiding the materials separation. Solution was frozen at -80ºC for 24h and follow was lyophilized for 48h.

Results

Samples were taken for SEM observation, after gold sputtering at an accelerating voltage of 10 kV. Preliminary observations presented sponges of COL/HA, with controlled pore size and fully interconnective macroporosity, well distributed through out the all sponge, with particles both integrating the pores walls, but also with many aggregates being present at the surface. This structure is now being fully physically characterized

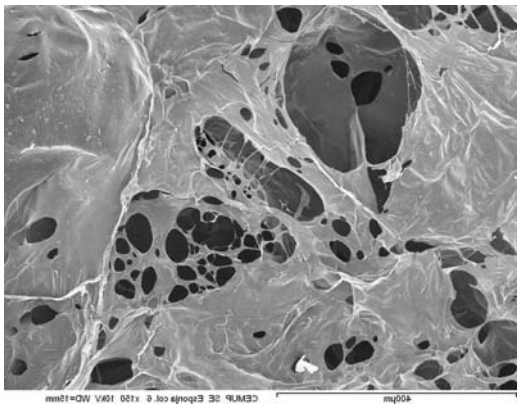

Fig. 1: Scanning electron micrograph of COL/HA sponge (x150)

Composite materials of COL and nano-sized HA

The use of collagen type I and nano-particulate HA for the generation of new bone substitutes is a promising approach for the generation of new bone substitutes to mimic structural and morphological features of natural bone, and to obtain materials with improved mechanical and biological properties.

In this context we prepared composite materials from collagen and nano-sized HA, described above. SEM and TEM results of the composites show morphological characteristics that suggest a potential use as bone tissue engineering soft scaffolds, due to the presence of collagen, as an attractive cell scaffold, and of nanoHA generating multiple binding sites for adhesion of several macromolecules of interest.

References

1) Sionkowska, A. Polymer Degradation and Stability. 68 (2000) 147-151.
2) Jarcho, M., Clinical Orthopeadics Related Research. 157 **(**1981) 259-278.
3) Rainey, J., Wen, C., Goh, C. Matrix Biology. 21 (2002) 647-660.

ACKNOWLEDGMENTS

Alis Pataquiva Mateus is grateful to FCT for awarding her a PhD scholarship SFRH/BD/16616/2004. This work was performed under contracts POCTI/SAU/BMA/56061/2004 and CRUP – Acções Integradas Luso – Espanholas 34/06, 2006.