In-Vitro biological response of calcium phosphate ceramics synthesized by using recycled eggshell

N-S Oh[1], S-W Song[1], J-H Choi[1], S Oh[2], S-J Lee[3], M-H Lee[4]
[1]Inha University, Incheon, 402-751, South Korea.
[2]University of Tennessee Health Science Center, Memphis TN 38163, USA.
[3]Mokpo National University, Mokpo, 534-729, South Korea.
[4]Korea Institute of Ceramic Eng. & Tech., Seoul, 153-081, South Korea.

Introduction

In orthopedic, maxillofacial surgery and dental areas, freshly harvested autologous cancellous bone is regarded as a golden standard for a bone grafting due to the good and fast osseous integration. However, the autografting of the bone is limited due to the donor site morbidity and limited availability. It is generally acknowledged that calcium phosphate ceramics like hydroxyapatite (HA) and tricalcium phosphate (TCP) are a good candidate for bone substitutes due to their chemical similarity to bone mineral. Additionally, bone substitutes should have degradation characteristics that allow the organism to replace the foreign material by fully functional bone in a balanced time schedule [1, 2].

In this work, the HA and β-TCP powders were synthesized by a new wet-chemical method using eggshell and phosphoric acid. The biocompatibility of synthesized natural HA, HA/β-TCP (50:50) and β-TCP derived from eggshell was compared with those of as commercial chemical powder with hBMSCs.

Materials and Methods

Uncrushed and washed raw eggshell was calcined in an air atmosphere at various temperatures up to 1000 °C, for 1 h at each temperature. To synthesize calcium phosphate powders, phosphoric acid was mixed to the eggshell calcined at 900 °C. The mixing ratios (wt %) of the eggshell and phosphoric acid were changed from 1:1.0 to 1:1.7. The mixtures were ball milled with zirconia ball media under isopropyl alcohol solvent for 12 h, for homogeneous mixing and to prevent agglomeration of the calcined eggshell. After drying at 90~100 °C for 24 h, the dried mixtures were heated at various temperatures at 4 °C/min heating rate, in an air atmosphere. The ball-milled powders were uni-axially pressed and sintered at 1200 °C for 2 h. Development of crystalline phases of the mixtures and morphological characteristics were studied X-ray diffractometer scanning electron microscopy.

The *in-vitro* cytotoxicity and cell attachment of sintered disks were examined using hBMSCs. Cell response was characterized by MTT assay, Alkaline phosphatase stain and RT-PCR analysis.

Results and Discussion

Pure HA was synthesized in the mixing ratio of 1:1.1 wt% at 900°C for 1 h. The crystallization of HA was started at 800°C in the 1:1.1 mixing ratio, and the HA phase was continued up to the high temperatures. In the ratio of 1:1.3 and 1:1.5 wt%, β-TCP was effectively synthesized at 900°C. In the 1:1.5 ratio, β-TCP phase was detected at 700°C, and complete crystallized β-TCP was observed above 900°C. At the higher temperature than 1000°C, the β-TCP was gradually decreased and α-TCP was observed. The HA and β-TCP disk does not exert cytotoxic effect on the hBMSCs undergoing osteoblastic differentiation. In addition, the hBMSCs are adhered on the surface of synthesized natural HA and β-TCP disk as successfully as on the culture plate or as commercial chemical HA and β-TCP disk. The hBMSCs adhered on either synthesized natural HA, β-TCP or as commercial chemical HA, β-TCP disk displays undistinguishable actin arrangement and cellular phenotypes, indicating that synthesized natural HA, β-TCP does not disrupt normal cellular responses. Analysis of differentiation of the hBMSCs cultured on culture plate, synthesized natural HA, β-TCP and as commercial chemical HA, β-TCP disk shows that three matrices are able to support osteoblastic differentiation of the hBMSCs as accessed by alkaline phosphatase (ALP) staining.

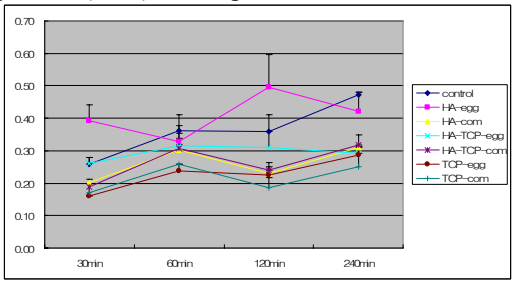

Fig 1. Cell attachment ratio of each groups

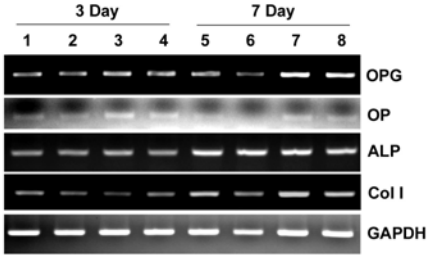

Fig 2. RT-PCR analysis of bone maker genes 1,5 Culture plate; 2,6 HA; 3,7 HA-TCP; 4,8 TCP

References

[1] Yaszemski MJ, Payne RG, Hayes WC, Langer R, and Mikos AG., Biomaterials 1996; 17; 175.
[2] Younger EM, Chapman MW, J. Orthop. Trauma. 1989; 3; 192.

Acknowledgements

This work was supported by grant No. R01-2005-000-10989-0 from the Basic Research Program of the Korea Science & Engineering Foundation.