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In vitro biocompatibility of a nanohydroxyapatite and biphasic nanohydroxyapatite/ß-tricalcium phosphate

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(B)

Background and Aim

Biphasic calcium phosphate (BCP) bioceramics belong to a group of bone substitute biomaterials that consist of an intimate mixture of HA and b-TCP, of varying ratios. BCPs are recommended for use as alternatives or additives to autogenous bone for orthopaedic and dental applications (1, 2).

The biodegradation of HA in physiological environments is too low to achieve the optimal formation of bone tissue even if it has excellent biocompatibility and bioactivity (2,3). On the other hand, b-TCP shows fast release of Ca^{2+} and PO_4^{3-} ions when exposed to physiological fluids and could be considered as bioactive. However, the fast dissolution profile of b-TCP drastically reduces the surface area available for bone cell proliferation and its applications in medicine are limited. Optimum bioresorbability is found when appropriately mixing both phases to give a biphasic calcium phosphate (4, 5).

Objectives

The study aimed to evaluate the biocompatibility of biphasic nano-hydroxyapatite/b-tricalcium phosphate with two different ratios (HA60:TCP40, HA70:TCP30) in vitro.

Materials and Methods

Materials

The nanoHA and nanoHA/ß-TCP with two different ratios of nanoHA/ß-TCP (HA100, HA70, HA60) were prepared and tested.

The materials were used in a 5 x 5 x 3 mm^3 block and incubated in Osteoblast cell line MC3T3-E1 (ATCC) passage 2-3 in proliferating medium containing alpha-MEM medium (Gibco), with the addition of 10% fetal bovine serum, 2% penicillin/streptomycin, and 0.2% Fungizone.

Cell loading and proliferation were assessed using a WST-1 colorimetric assay. Cellular morphology and adhesion to the scaffolds and cell viability were assessed optically using SEM. The alkaline phosphatase assay and osteocalcin assay were used to detect the early and late stage of osteoblastic cell differentiation. Detail of experiments are presented in Table 1.

The results were presented as mean ± SD. (n=3). The difference between various test biomaterials were analyzed by an analysis of variances and multiple comparison test by Turkey HSD. The level of statistical significance is set at $p < 0.05$.

Table 1. Details of experiments

Biocompatibility tests	Day1	Day3	Day14	Day21
Cell loading efficacy the number of metabolically active cells	x			
Cellular adhesion and morphology SEM/CLSM	x			x
Cell proliferation assay (WST-1)	x	x		
Cell differentiation assay ALP	x	x		
Osteocalcin synthesis				x

Results

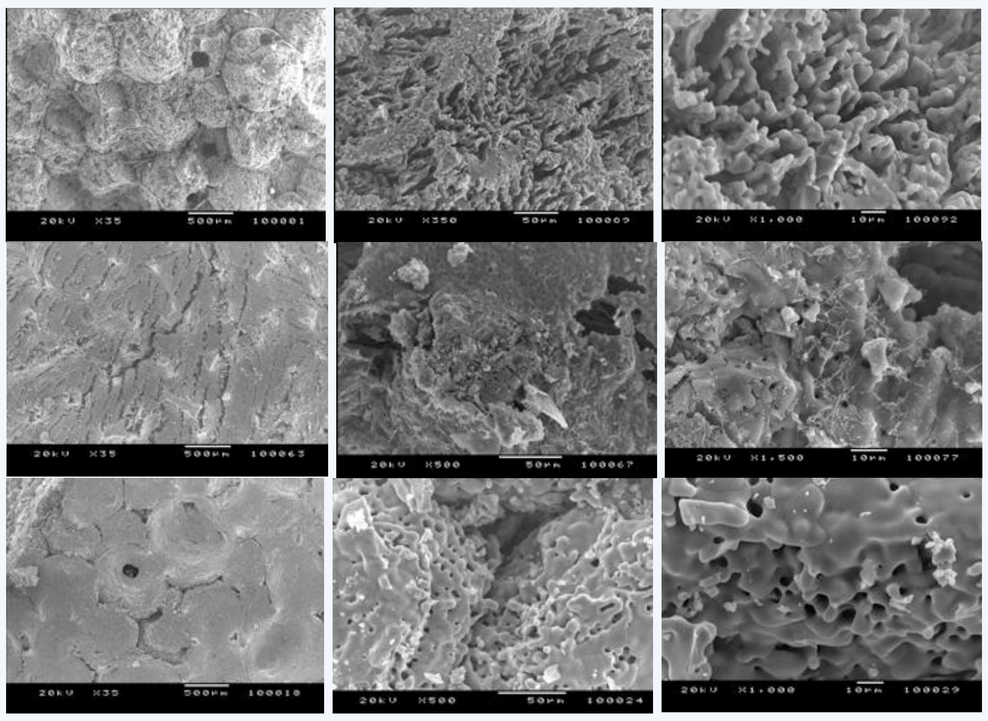

Figure 1 SEM images of (A) HA100, (B) HA70 and (C) HA60

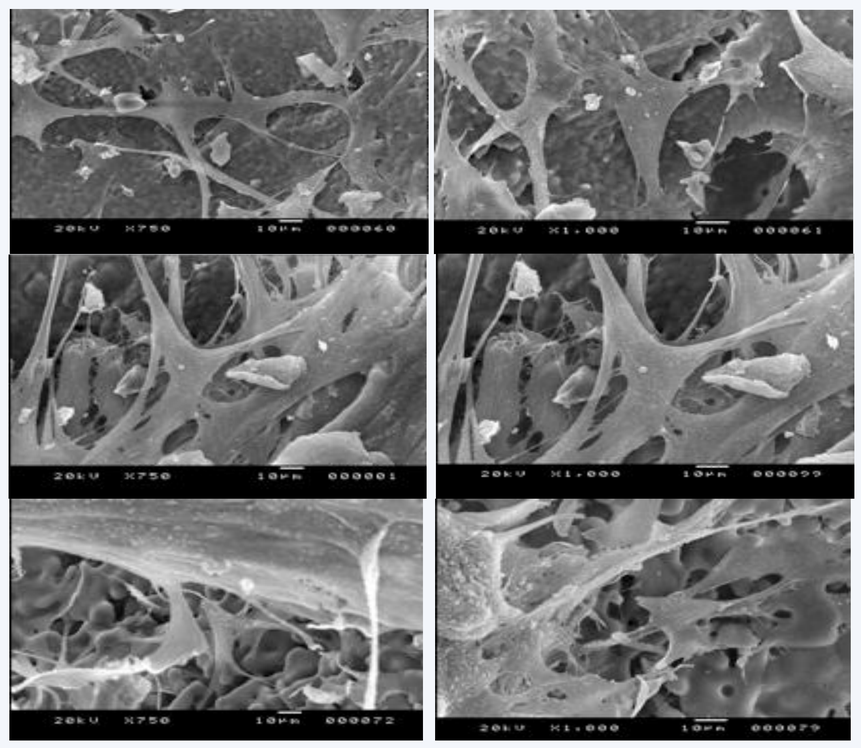

Figure 2 SEM images of osteoblastic cells morphology after 1 day on the surface of (A) HA100, (B) HA70 and (C) HA60

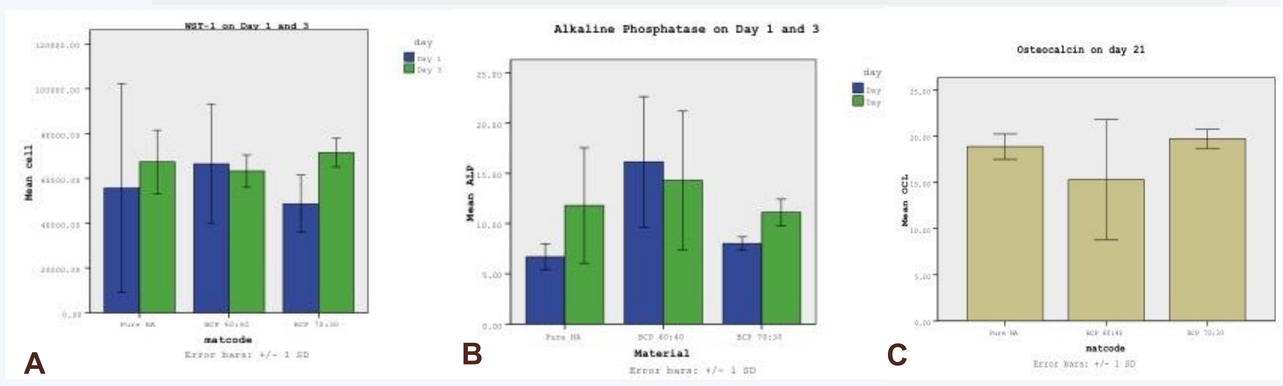

Figure 3, A) Mean cell number B) Alkaline phosphatase C) Osteocalcin

Conclusions

It can be concluded that higher TCP ratio enhanced better cell proliferation and the early phase of cell differentiation while higher ratio of HA yielded better osteocalcin production at the late state of osteoblast differentiation. BCP with HA:TCP 70:30 should be further investigated in the animal study.

References

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