Biological effects of PLA-TCP nanocomposites on osteoblastic cells

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ABSTRACT

Synthetic degradable biomaterials, including polylactic acid (PLA) and tricalcium phosphate (TCP), are widely used for matrix scaffold. In present study, we produced nanocomposites blending PLA and TCP with various ratio. PLA was dissolved in an organic solvent, chloroform and then adding TCP with ethanol. Composite structures were successfully generated at all compositions. The osteoblastic cells attached and spread well on PLA-TCP blending nanocomposites. In particular, the cellular growth rate and alkaline phosphatase activity was higher on the PLA-TCP composites than on the pure PLA. Moreover, real-time gene expression of osteocalcin was prominent in PLA-TCP composite compared to the PLA. These results showed us that PLA-TCP blending polymeric nanocomposites are considered to be useful as bone cell supporting matrix for hard tissue regeneration.

Keywords: nanocomposites, osteoblast, polylactic acid, tricalcium phosphate

1 BACKGROUNDS

Regeneration of hard tissues with biomedical materials has been a ultimate goal in orthopedics and dentistry. Bone defects are usually treated by using either bioceramics or degradable polymers [1]. Recently, degradable polymers such as poly(lactic acid) (PLA) has been introduced as bone regenerative mateiral [2].

Albeit the polymer could bear an initial mechanical load and cell compatibility, in vivo biological performance has been regarded as the subject of debate, particularly with respect to degradable products, inflammation, and abrupt mechanical failure [3].

In order to improve the properties of the polymeric materials, bioactive ceramic components, such as tricalcium phosphates (TCP), were proposed as adjunctive biomaterials [4]. Considering the biological effects of composite materials on osteoblastic cells, we hereby utillized PLA as a basic degradable matrix and intentionally added TCP nanopowders to improve the biological performance toward hard tissue cells.

2 MATERIALS & METHODS

2.1 Cell cultures

Murine osteoblastic cell (MC3T3-E1) pellets were suspended and plated in flasks in α-MEM containing 10% FBS and 1% penicillin/streptomycin. The cells were concentrated by centrifugation at 12,000 rpm and 4℃.

2.2 Sample preparation

PLA was dissolved in an organic solvent, chloroform and then TCP nanopowder was added.

2.3 Scanning electron microscopy (SEM)

The samples were prepared in 48-well culture plates with 1 x 10^4 cells seeded on each sample. The morphology of cells attached to membranes was observed by a SEM after 7 days..

2.4 Proliferation assay

MTS solution was added into each well containing 500μl of medium and incubated at 37℃. The absorbance of formazan produced was measured at 490 nm with a microplate reader at the designated time point.

2.5 Differentiation assay

Thawed cell suspensions were and centrifuged at 12,000 rpm at 4℃. The protein content was determined by the Bradford method using BSA as a standard. ALP activity was measured by absorbance at 405 nm at the designated time point.

2.6 Polymerase chain reaction assay

Polymerase chain reactions were performed to determine genetic marker expression.

3 RESULTS

SEM revealed that osteoblastic cells were successfully attached and spread well on PLA-TCP blending nanocomposites (Fig 1).

MTS showed that the cellular growth rate was higher on the PLA-TCP composites than on the pure PLA (Fig 2).

Cell differentiation activity, which was determined by ALP assay, also presented the fact that the PLA-TCP nanocomposites were superior to the pure PLA (Fig 3).

Besides, real-time gene expression of osteocalcin (OC) was prominent in PLA-TCP composite compared to the pure PLA (Fig 4).

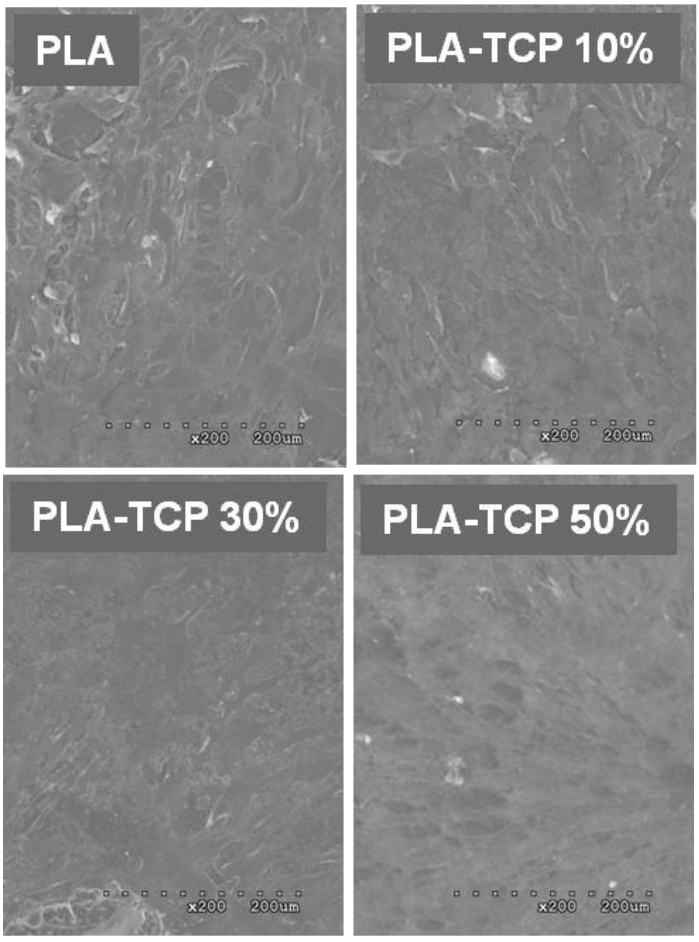

Fig 1. SEM of osteoblastic cells on PLA-TCP nanocomposites.

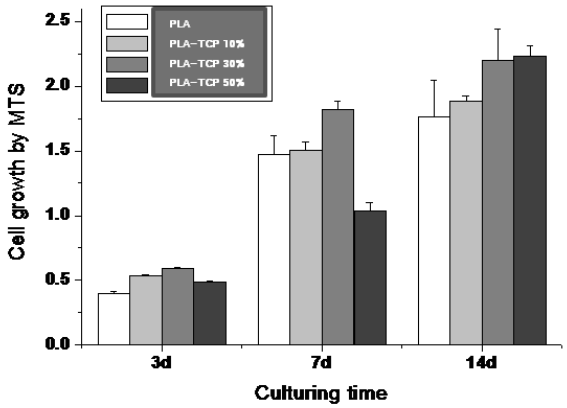

Fig 2. MTS assay showed that PLA-TCP nanocomposites increase the osteoblastic cell proliferation rate at the designated time.

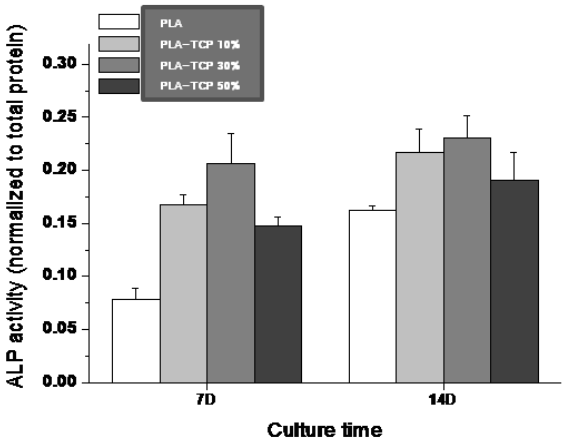

Fig 3. PLA-TCP nanocomposites showed a superior osteoblastic cell differentiation rate at the designated time.

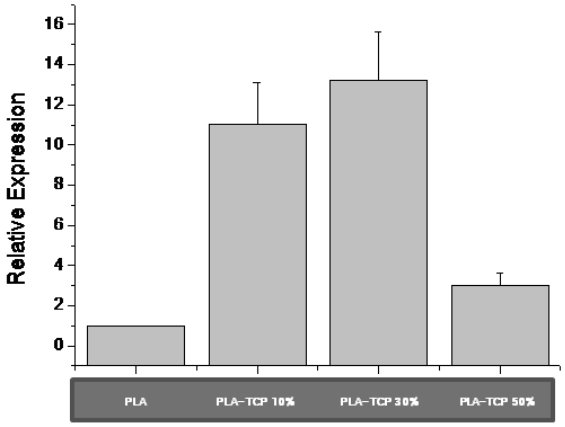

Fig 4. PLA-TCP nanocomposites showed prominent gene expression of osteocalcin.

4 SUMMARY

PLA-TCP nanocomposites had favorable effects on the proliferation and differentiation of osteoblastic cells and promoted osteocalcin gene expression.

ACKNOWLEDGEMENTS

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