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Delivering MC3T3-E1 cells into injectable calcium phosphate cement through alginate-chitosan microcapsules for bone tissue engineering

Key words: Injectable scaffold, Calcium phosphate cement, Osteoblast, Microencapsulation, Cell release, Chitosan

INTRODUCTION

- Tissue engineering approaches provide promising alternatives for bone repair.

- Among these bone substitute biomaterials, preformed scaffolds have excellent biological and mechanical properties but their use usually requires machining the graft or reaming of the surgical site to achieve a precise fit in the bone cavity. Injectable polymeric carriers are advantageous when used for seeding cells or injected in minimally-invasive surgeries but they are too weak for load-bearing applications .

- Solidified calcium phosphate cement (CPC) has two significant advantages. First, it can be converted to hydroxyapatite, the major inorganic component of human bone. Second, it possesses a certain degree of strength, which is important to define the shape of the regenerated tissue. However, the degradable time of CPC seems to be too slow to match the formation of new bone.

- To improve the effectiveness of CPCs, osteoblasts and stem cells embedded within alginate beads have been seeded into CPC paste and alginate hydrogel has been shown to protect the implanted cells from the mixing and injection forces, as well as from the CPC setting reaction.

- In the present study , a new type of cell- encapsulating microcapsule, alginate-chitosan (AC) microcapsule, that could release the cells throughout the CPC scaffold and concomitantly create macropores of several hundred micrometers in diameter in situ, was developed and completely mixed with the CPC paste.

METHOD

MC3T3-E1 cells were embedded in alginate-chitosan microcapsules using an electrostatic droplet generator

Test of MC3T3-E1 cell viability after microencapsulation

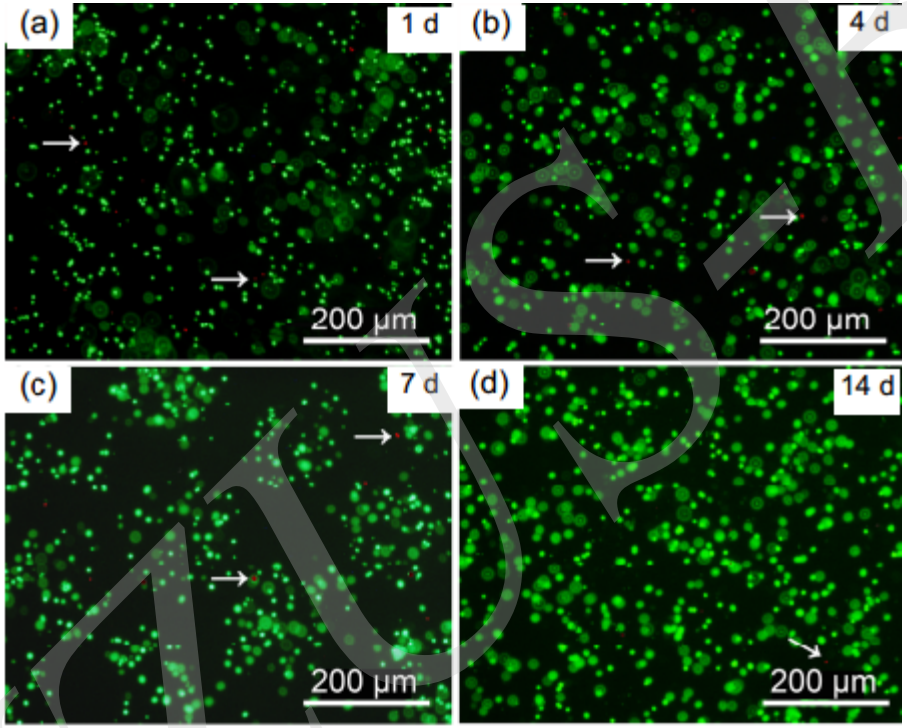
Cell proliferation:
A CCK-8 (Dojindo, Beijing, China) was used for this assay at Days 1, 4, 7, 14, and 21 after encapsulation.

Live and dead cells:
0.002 mmol/L calcein-AM and 0.004 mmol/L PI were added to each specimen . After 15 min incubation at 37 °C, the cells were observed by epifluorescent microscopy .

CPC-cell construct preparation

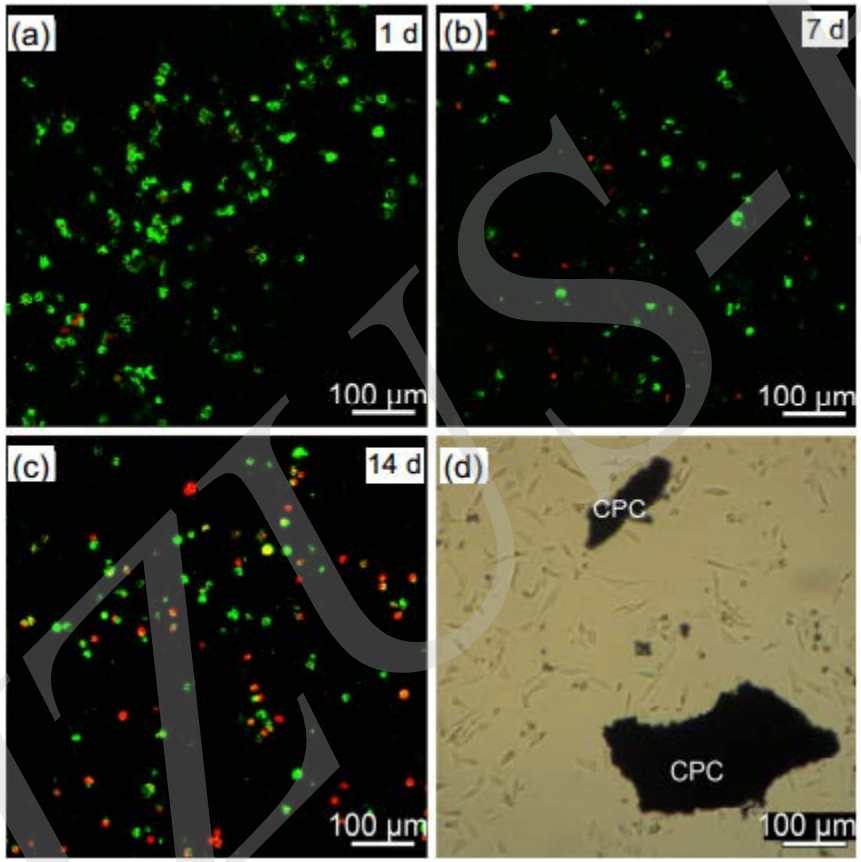
- Microencapsulated MC3T3-E1 cell release after mixing with CPC paste
- Viability and osteogenic differentiation of released MC3T3-E1 cells
- Cell attachment evaluated by environment scanning electron microscopy
- Staining of mineralized nodules formed by released MC3T3-E1 cells

RESULTS AND CONLUSIONS



At Days 12 and 14, microencapsulated MC3T3-E1 cell viability was observed to have increased. Very few dead cells were evident during the entire culture period.

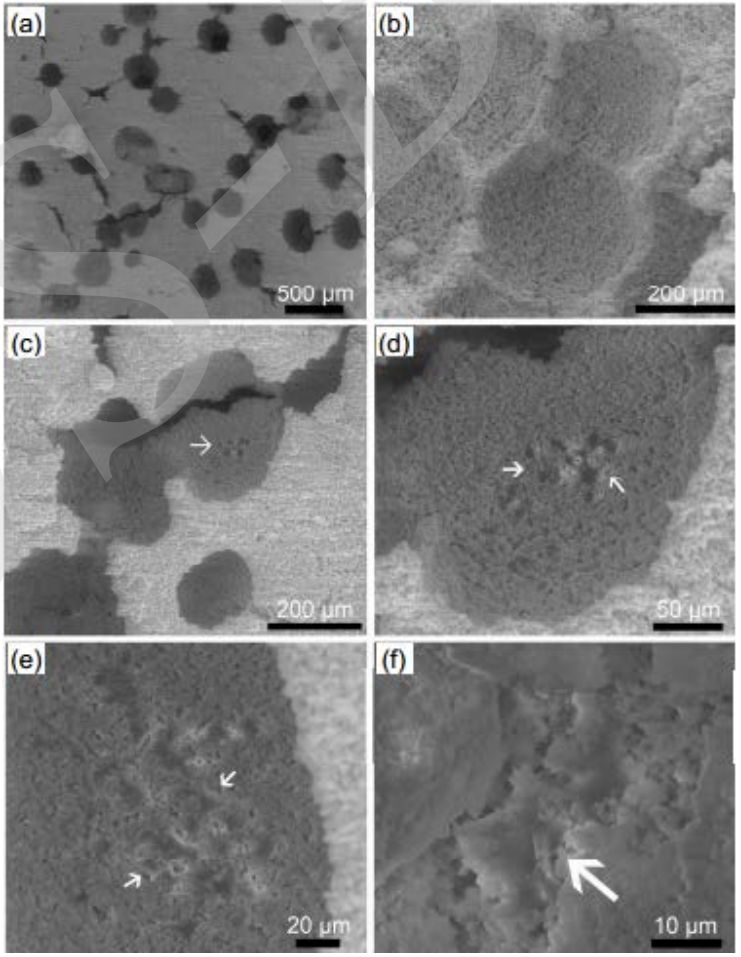
RESULTS AND CONLUSIONS



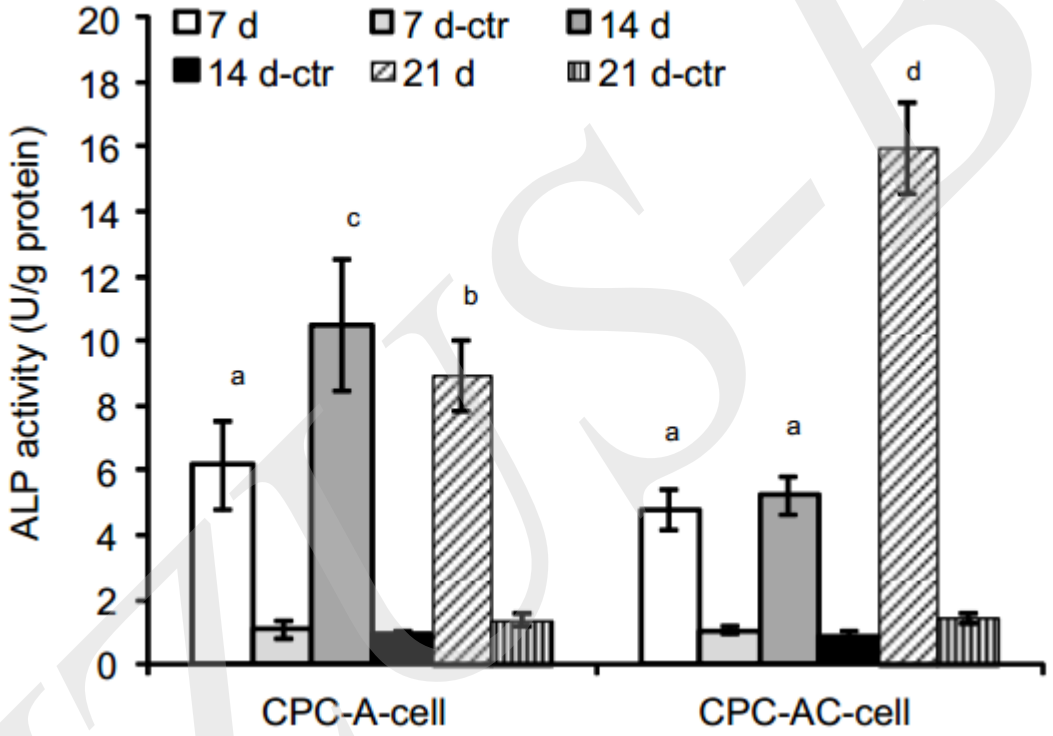
The released cells attached to the setting CPC scaffolds, survived, differentiated and formed mineralized nodules.

RESULTS AND CONLUSIONS

- Cells grew in the pores concomitantly created by the AC microcapsules in situ within the CPC.
- Pores formed by the AC microcapsules had a diameter of several hundred microns and were spherical compared with those formed by alginate microcapsules.



RESULTS AND CONLUSIONS



At Day 21, cellular ALP activity in the AC group was approximately four times that at Day 7 and exceeded that of the alginate microcapsule group ($P<0.05$).

In conclusion, AC microcapsule is a promising carrier to release seeding cells deep into an injectable CPC scaffold for bone engineering.