

Research Article

# Osteogenic Differentiation of Mesenchymal Stem Cells/Polymer Composites with HA In Vitro

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Received 14 January 2011; Accepted 3 February 2011

**Abstract** Synthetic polymer (RADA16:PuraMatrix®) is an artificial peptide and self assembling. RADA16 is able to provide three dimensional constructs which hold mesenchymal stem cell (MSCs). We combined the MSCs into the polymer and fabricated MSC/RADA composites. The composites showed osteogenic differentiation in the culture condition. Supplementation of hydroxyapatite ceramics (HA) in the composites (MSC/RADA/HA composites) improved the mechanical property and demonstrated the extracellular bone like matrix formation in the culture condition. These results indicate the important property of HA ceramics for the proliferation and differentiation of MSCs in the polymer. The MSC/RADA/HA composites could be available in the field of bone tissue engineering.

**Keywords** hydroxyapatite; synthetic self-assembling peptide; mesenchymal stem cell (MSCs); three-dimensional culture; RADA16:PuraMatrix®(3D-Matrix JAPAN TM)

## 1 Introduction

RADA16 is a new class of biomaterials, artificial self-assembling peptide-based hydrogel. In solution, these peptides rapidly form nanometer-scale fibers and assemble into three-dimensional hydrogel scaffolds when the peptide solution is exposed to physiological medium or salt solution. Because these hydrogel scaffolds contain a nanofiber network and water content of more than 99% (w/v), the hydrogel scaffold can mimic the natural extracellular matrix (ECM) and might combine with various kinds of cells in a three-dimensional manner. When osteogenic cells such as mesenchymal stem cells (MSCs) are used, the composites could be available for bone tissue engineering. However, the problem of the composites is the weak mechanical property.

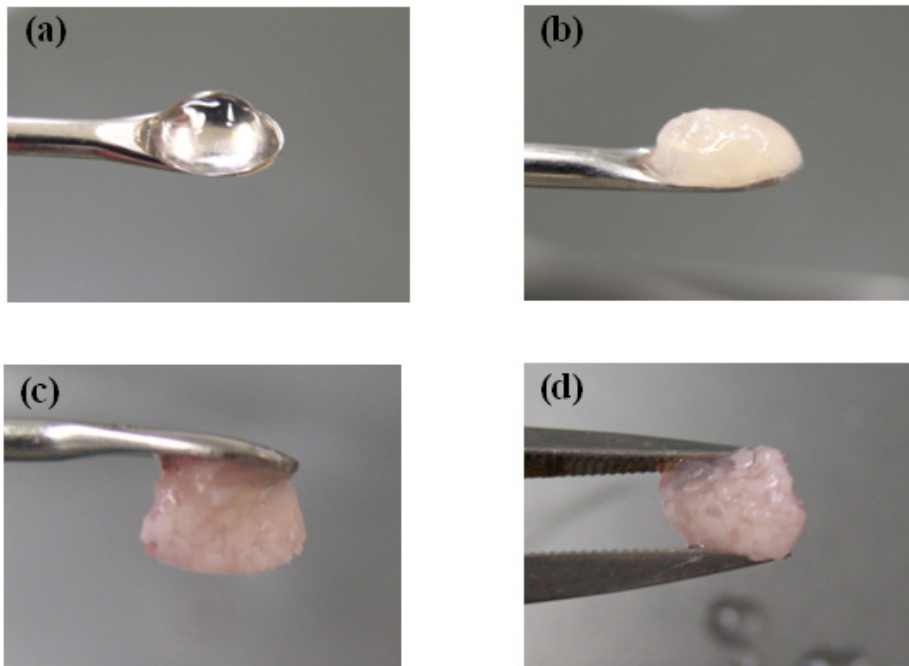
In this study, we tried to promote the mechanical property. Hydroxyapatite ceramics (HA) are biocompatible

and osteoconductive materials and MSCs/HA composites were widely used in bone tissue regeneration [5]. We also reported the MSCs/RADA16 composites could show in vitro spatial distribution of mineralized bone matrix [1]. Therefore, we attempted to fabricate the composites of MSCs/RADA16/HA to investigate their osteogenic properties.

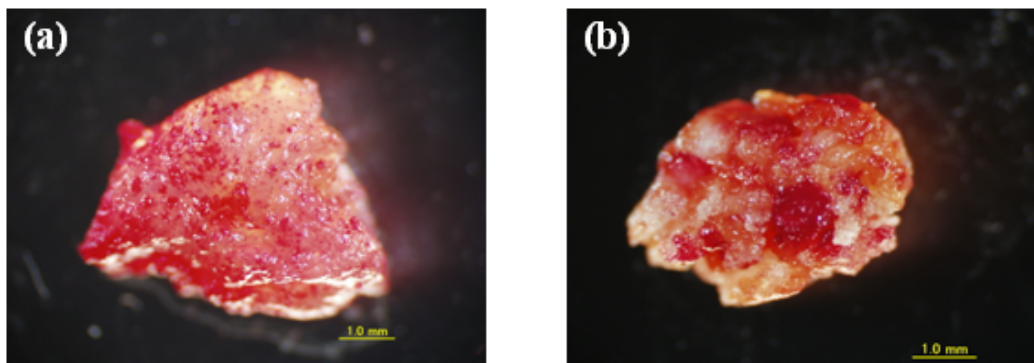
## 2 Materials and methods

Rat bone marrow plugs from femora of 7-week-old male Fischer 344 rats were flushed out and suspended in minimum essential medium. These bone marrow cells were cultured in T-75 flask [3,4]. The adherent cells (MSCs) were initially cultured up to 80% confluence, harvested, and resuspended in 10% sucrose solution. For fabrication of MSC/RADA composites, the cell suspension was mixed with 2% RADA16 and applied into cell culture insert (BD falcon) in 24-well cell culture plate and gelled. For fabrication of MSC/RADA/HA composites, 5 mg HA (CELLYARD™) granule was impregnate in the mixture of MSCs in the RADA16 solution and gelled as described above. These composites were subcultured in osteogenic medium with dexamethasone (Dex) [2]. ALP staining was done at 14 days, and the enzyme activity measurements were done at 7, 14, 21 days. The ALP activity was represented by the amount of p-nitrophenol released from substrate of p-nitrophenyl phosphate (pNPP) after 10 minutes of incubation.

For histological analysis, after 2 weeks subculture with Dex, the composites were fixed with 10% formaldehyde and decalcified with K-CX solution (Falma, Tokyo, Japan) and embedded in paraffin. They were cut into sections in 4 μm thicknesses and stained with hematoxylin and eosin (Muto Pure Chemicals, Tokyo, Japan).



**Figure 1:** Photos of 1% RADA16 (a), MSC/RADA composite (b) and MSC/RADA/HA composite (c, d). The composites were cultured for 2 weeks in the osteogenic medium with Dex (b).



**Figure 2:** ALP stain of MSC/RADA composite (a) and MSC/RADA/HA composite (b). The composites were cultured for 2 weeks in the osteogenic medium with Dex.

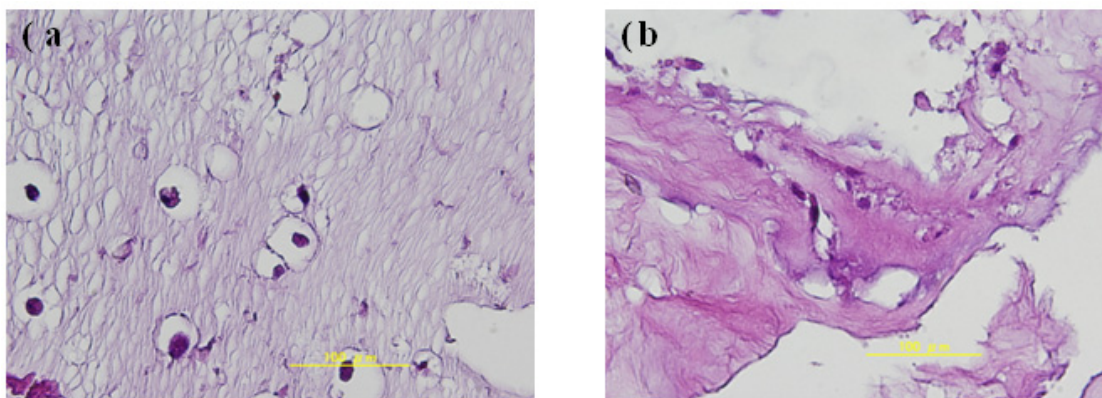
### 3 Results and discussion

After 2 weeks subculture with Dex, we could hold the MSC/RADA/HA composites by tweezers; in contrast, we could not hold the MSC/RADA composites (Figures 1(a), 1(b), 1(c)). Therefore, the MSC/RADA/HA composites were easy to handle due to their improved mechanical property, which might be advantageous for surgeons in clinical situations of hard tissue repair.

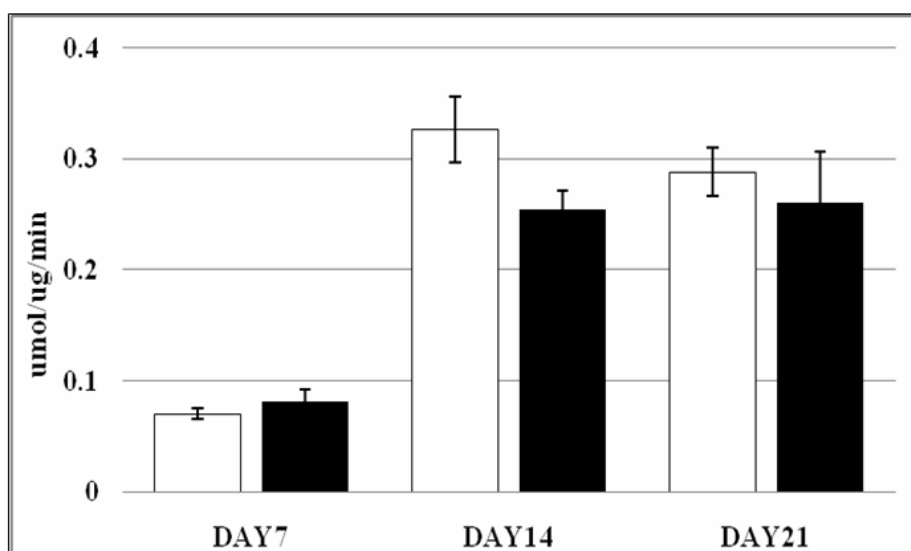
In the ALP staining at 14 days, both MSC/RADA and MSC/RADA/HA composites showed high activity, which was seen homogeneously around the entire surface of the composites (Figures 2(a), 2(b)). The strong stain indicates

the osteogenic differentiation of both composites during the subculture with Dex.

To analyze the cellular components as well as extracellular matrix, we fixed the 14 days subcultured composites and made histological section. As seen in Figure 3(a), we confirmed the uniform distribution of MSCs in the pore regions of both composites. Interestingly, we could detect extracellular bone like matrix together with osteocytic cells in the MSC/RADA/HA composites (Figure 3(b)). This suggests that the HA surface supported the attachment and proliferation of MSCs followed by their osteogenic differentiation.



**Figure 3:** Histological sections of of MSC/RADA composite (a) and MSC/RADA/HA composite (b). The composites were cultured for 2 weeks in the osteogenic medium.



**Figure 4:** ALP activity of MSC/RADA (white column) and MSC/RADA/HA (black column) composites cultured in the osteogenic medium at 7, 14, 21 days.

The osteogenic differentiation of both composites was further confirmed by the results of alkaline phosphatase activity (ALP). We measured the ALP activity per DNA contents of MSC/RADA and MSC/RADA/HA composites. At 14 and 21 days, ALP activity of both composites showed high ALP activities (Figure 4).

These results showed that the synthetic polymer of RADA16 has biocompatible nature, which allows the proliferation of MSCs followed by osteogenic differentiation. Thus, the composite of MSCs and RADA16 (MSC/RADA) might be available for hard tissue repair. However the problem is the weak mechanical property. As seen in Figure 1, we could not hold the composites with tweezers. In this regard, impregnation of HA into the polymer improved the mechanical property and also might enhance

the mineralized bone matrix formation on the surface of HA. Therefore, THE MSC/RADA/HA composites can be available for bone tissue engineering.

#### 4 Conclusions

This study showed that the mesenchymal stem cells (MSCs) can be cultured in the synthetic polypmer of RADA16 and hydroxyapatite ceramics granules improved the handling property of the composites. The culture of the composites in the presence of Dex showed osteogenic differentiation and the HA granule may have promoted the bone matrix formation in the composites. All the results indicate the clinical significance of the composites to be used for hard tissue regeneration.

**References**

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