学術情報

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Bone Augmentation Using 3D Cultured Chondrocytes in different Sites

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ABSTRACT

Objective: We have previously reported that mesenchymal stem cell derived chondrocytes (MSC-DCs) are effective for bone augmentation on cranial bone. In this study, we aimed to evaluate the effect of bone augmentation of maxilla bone using MSC-DCs.

Methods: Porous PLGA scaffold (porosity: 80%, average pore size 500 μm) was fabricated according to an established protocol. The animal experiments were performed at Hamri Co., Ltd., which has been approved by the AAALAC International. MSCs were isolated from femoral bone marrow aspirates obtained from 4 weeks old male F344 rats. For fabrication of 3D cultured MSC-DCs, MSCs were seeded to the PLGA scaffold and maintained with chondrogenic differentiation medium for 21 days. 11 weeks old male F344 rats were randomly allocated between 2 experimental groups. Maxilla bone group: The maxilla bone of first molar was exposed and MSD-DCs implant (3×3×1 mm) was implanted on the bone with no decortications. Cranial bone group: The cranial bone was exposed and MSC-DCs implant (ϕ9×2 mm) was implanted on the bone with no decortications. The bone tissue of each group was harvest after 2, 4, and 8 weeks and evaluated radiographically and histologically.

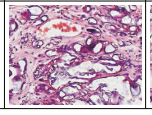
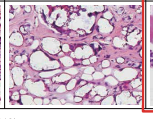
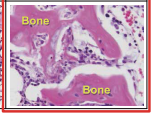
Results: Micro-CT image demonstrated that large amount of mineralized tissues were observed on both maxilla and cranial bone at 2 weeks. Increase in mineralized tissue volume was observed until 12 weeks. No significant differences were observed between the implant sites. Histological analysis revealed that bone formation by MSC-DCs were through endochondral ossification. The amount of newly form bone was depended on the size of MSC-DCs implant.

Conclusion: The 3D cultured MSC-DCs possess the ability to form new bone tissue on maxilla bone and cranial bone, which shows the potential of using this method clinically.

1 INTRODUCTION

In our previous reasearch, we have transplanted human mesenchymal stem cell derived chondrocytes (MSC-DCs) subcutaneously into back of Immunodeficient mouse, and showed high osteogenic ability of MSC-DCs transplant (Table 1). We also have transplanted MSC-DCs to rat segmental bone defect and reported that 15 mm gap, which is more than half of the femur, could be regenerated quickly（Fig. 1）.
From our previous research, effectivity of MSC-DCs transplant in the filed of orthopedics have been shown. However, effectivity of MSC-DCs transplant in dental field was not confirmed.

Table 1. Ecotopic bone formation assay in mouse subcutaneous tissue.

	Undifferentiated human MSCs	Bone differentiated human MSCs	Chondrogenic differentiated human MSCs (MSC-DCs)
Bone formation	×	×	○
H&E stain			

F. Jakob et al., *Maturitas*, **75(2)**, 118-124(2013).

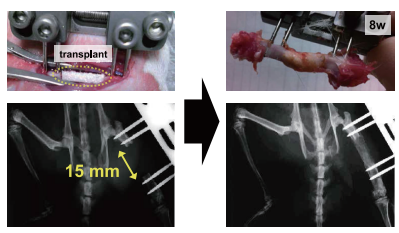


Fig. 1. Regeneration of rat segmental bone defect using MSC-DCs.
N. Harada et al., *Biomaterials*, **35(27)**, 7800-7810(2014).

Aim

To compare the effect of bone augmentation using 3D cultured MSC-DCs transplant in different sites (maxilla bone & cranial bone).

2 MATERIALS & METHODS

Scaffold

Features

- Poly（*dl*-lactic-co-glycolic acid）（PLGA）（LA/GA=75/25 mol%, Mw=120,000）
- Absorb by 3~4 months
- Radiolucent

Details

- Porosity ： 80%
- Pore size ： 180 ~ 790 μm （Average 500 μm）

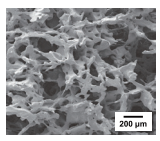


Fig. 2. SEM image of PLGA scaffold.

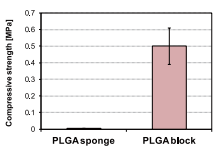


Fig. 3. Compressive strength of PLGA scaffold.

MSCs culture

Isolation and cultures

MSCs were isolated from femoral bone marrow obtained from 4 weeks old male Fisher 344 rats.
MSCs were cultured in αMEM containing 3 ng/mL FGF-2 and 10% FBS.

S. Tsutsumi et al., *B.B.R.C.*, **288**, 413(2001).

Seeding to PLGA scaffold

After subculturing for 3 times, MSCs were seeded at 5×10^5 cells per scaffold by filter method.

K. Yamanaka et al., Dent. Mater. J., **34(1)**, 78-85(2015).

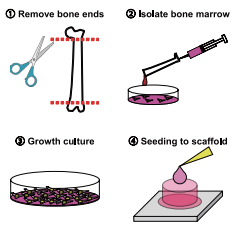


Fig. 4. Isolation and cultures of MSCs.

MSC-DCs preparetion

Chondrogenic differentiation of MSCs

① Cell seeded scaffolds were collected in 15 mL centrifuge tubes.
② Induction with chondrogenic inducing medium（9 mL）.
③ Maintenance in cell culture incubator at 37 °C for 21 days.
（Rotary cell culture at 1 rpm）

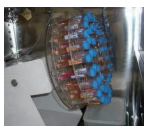


Fig. 5. Rotary cell culture system.

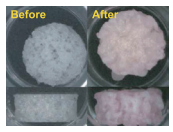
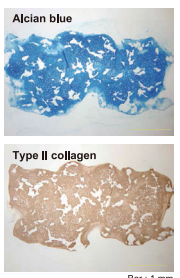


Fig. 6. MSC-DCs transplant.

Table 2. Composition of chondrogenic inducing medium.

Composition	Concentration
Base medium（αMEM）	—
D-glucose	4.5 mg/mL
Dexamethasone	10^{-7} M
Ascorbic acid 2-phosphate	50 μg/mL
Insulin	6.25 μg/mL
Transferrin	6.25 μg/mL
Selenic acid	6.25 μg/mL
Linoleic acid	5.33 μg/mL
Bovine serum albumin	1.25 mg/mL
Pyruvic acid	100 μg/mL
TGF-β3	10 ng/mL
BMP-2	500 ng/mL

M. Horie et al., *Stem cells*, **27(4)**, 878-887(2009).



Bar : 1 mm

Fig. 7. Closs-sectional view of MSC-DCs.

Transplant

Transplant site

Maxillary first molar buccal alveolar bone and clanial bone of 11 weeks old male Fisher 344 rats（no decortications）.

Transplant size

- Maxilla bone group ： 3 × 3 × 1 mm
- Cranial bone group ： ϕ9 × 2 mm

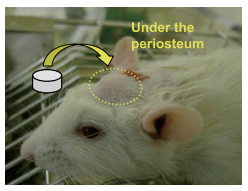


Fig. 8. Transplant procedure.
(Cranial bone)

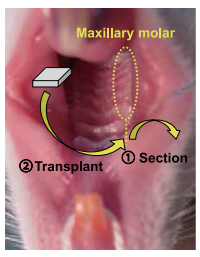


Fig. 9. Transplant procedure.
(Maxilla bone)

Evaluation

After harvesting, each groups were fixed in 10% formaldehyde, and micro CT images were obtained using a micro focus X-ray CT system (TOSCANER 30000 μhd, TOSHIBA). Each groups were demineralised with 10% EDTA solution for 2 weeks, embedded in paraffin, and sectioned. The sections were subjected to H&E, Alcian blue Type I collagen, Type II collagen and TRAP staining.

3 RESULTS

Micro CT evaluation

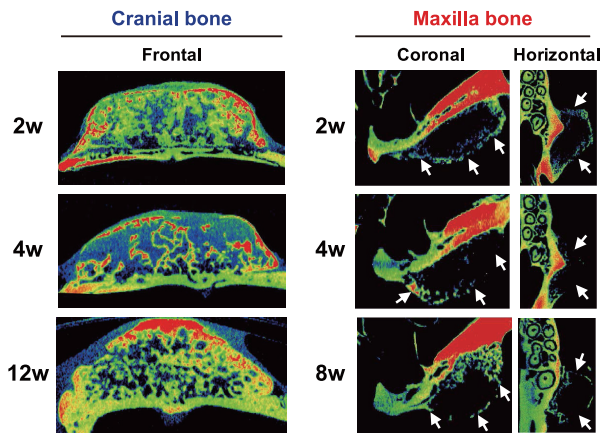


Fig 10. Micro CT evaluation.

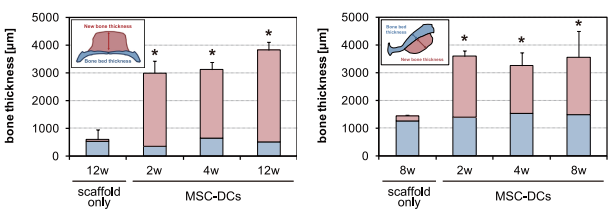


Fig 11. Bone thickness（$*p < 0.05$）.

Histological evaluation

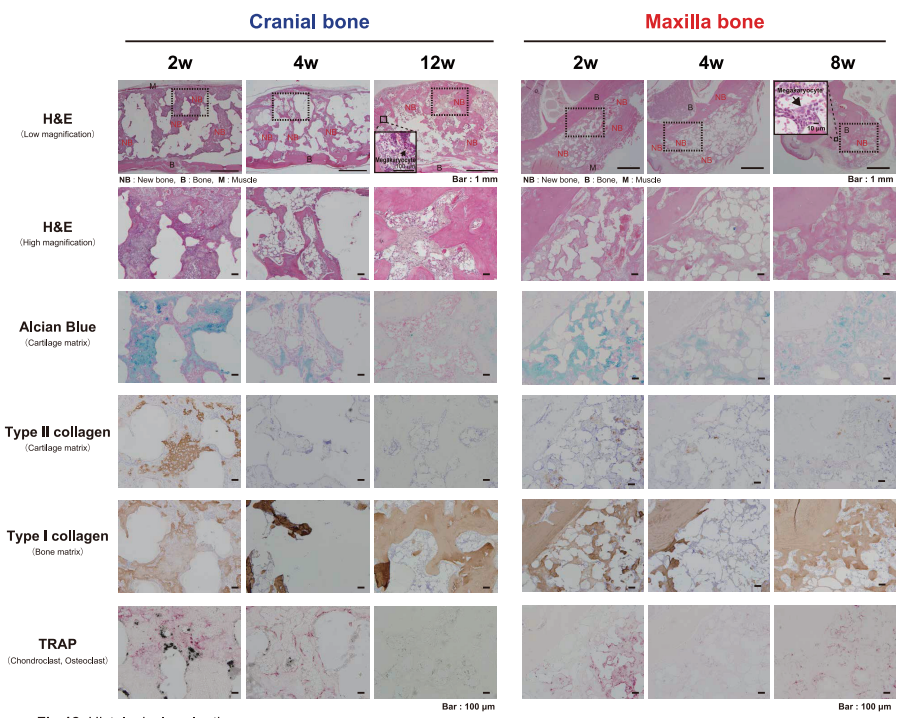


Fig 12. Histological evaluation.

4 DISCUSSION

It is shown that bone formation mechanism of bone augmentation using MSC-DCs transplant is similar to that of endochondral ossification. Since type II collagen of cartilage matrix has a different peptide chain, it is unlikely to change to type I collagen. Moreover, it is also unlikely that transdifferentiation between chondrocyte and osteoblast happens. We think that cartilage matrix surrounding MSC-DCs transplant once degrade and new bone matrix is formed afterwards, instead of cartilage changing to bone. Therefore, it is inferred that new bone is formed by host derived osteoblast, after MSC-DCs became hypertrophied and calcified by the effect of blood component, and degraded by chondroclast.
MSC-DCs transplant has ischemic tolerance specific to cartilage, so it does not necrose at bad blood circulation. This feature of MSC-DCs transplant may have a great advantage in generating large amount of bone.

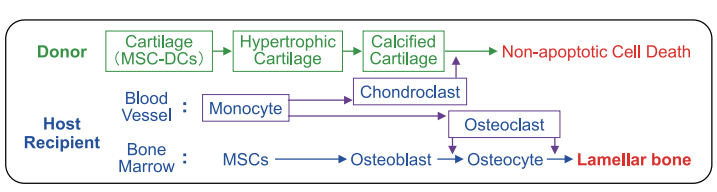


Fig. 13. Bone formation mechanism using MSC-DCs transplant.

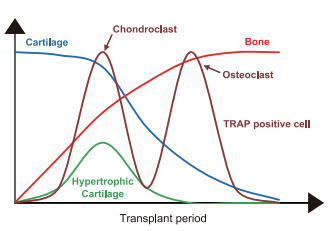


Fig. 14. Schematic graph illustrating bone formation mechanism.

5 CONCLUSION

The 3-D cultured MSC-DCs possess the ability to form new bone tissue on the rat maxilla bone and cranial bone, which shows the potential of using this method clinically.

Paradigm Shift ?