1152 Is Cellular Distribution Crucial Factor for Bone Augmentation ?

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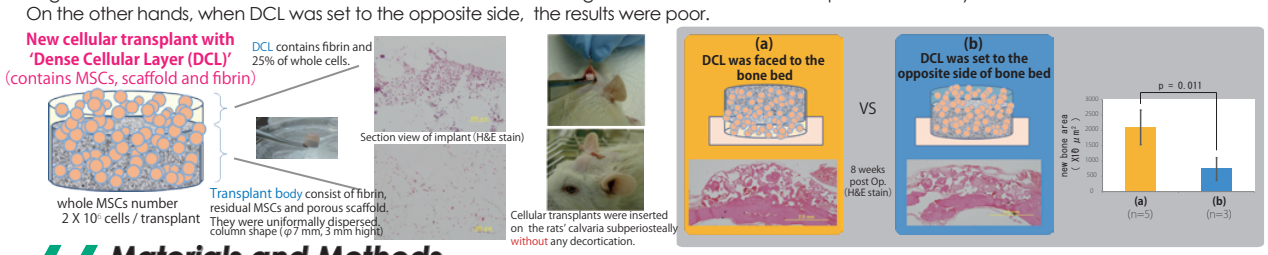
Introduction

To avoid invasive autologous bone collection, we tried to establish a horizontal augmentation method using mesenchymal stem cells (MSCs) and chemically fabricated scaffold. Previously in small size rat calvaria augmentation model, we were to first to use new cellular transplant which has Dense Cellular Layer (DCL) face to the bone surface to form bone vertically and reproducibly. It seemed that sufficient cellular distribution in the recipient-transplant interface is key to engraftment of transplant. In this study, clinical-sized (1.5ml) transplants were applied on rat calvaria, then we evaluated the effect of DCL position in the transplants for bone formation and osseointegration of titanium implant.

Our previous work

Our new cellular transplant was furnished with DCL, which to be faced to bone bed. DCLs consisted of MSCs and fibrin without solid scaffold. During the process of fibrin formation, DCL shrinks and the cell density is increased. In our previous work, in small rat calvaria augmentation model, we found that they could reproducibly augment the bone vertically. Transplant and calvaria were continuously jointed, and newly formed bone area in augmented bone was increased. DCL seemed to enhance the engraftment of the cellular transplants when they were faced to the bone bed. On the other hands, when DCL was set to the opposite side, the results were poor.



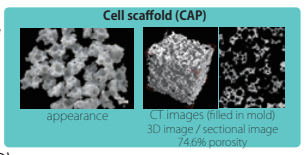
Materials and Methods

Animal study was carried out at laboratory of Hamri Co., Ltd. (Ibaraki, Japan), which has been approved by the Association for the Assesment and Accreditation of Animal Care (AAALAC) International. The experimental protocol was approved by the Institutional Animal Care and Use Commitee (IACUC) of Hamri Co.,Ltd.

Materials and Animals

Animals : 9 weeks old male Fisher 344/Jcl rats
MSCs :MSCs were isolated from femoral bone marrow obtained from 4 weeks old male IFisher 344/Jcl rats. MSCs were cultured in aMEM containing 3ng/ml FGF-2 and 10% FBS. Cells are subcultured for 2 times.
Rat Plasma : Rat blood collected from abdominal aorta of 4 weeks old male Fisher 344/Jcl rats, and anticoagulated by 3.13% citric acid aqueous solution. Then it centirfuged by 1000G for 10 minutes and supernatant plasma was collected.

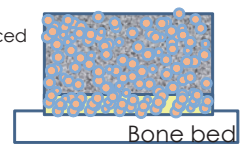
$CaCl_2$ aqueous solution: $CaCl_2$ was dissolved in 3.3% (w/w) in distilled water (Ohtsuka)
Cell scaffold :Multi porous carbonate apatite (CAP)granule (chemically fabricated) 0.5-1.5 mm sieved grade
Titanium dental implants for animal : φ3.0mm, 4.0mm hight, test material (GC Corporation)
μCT apparatus : TOSCANER-30K (TOSHIBA ITC)



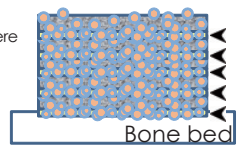
Methods

All transplants consist of Fisher 344/Jcl rat MSCs (25X10⁵ cells/transplant), CAP granule, and rat plasma. They were assembled in column-shape in the appropriate mold, and 10%(v/v) $CaCl_2$ aqueous solution added to make fibrin (coagulation). In transplant body (portion other than DCL, 16.2mm dia., 7.5mm height,), the three components (MSCs, CAP granule and fibrin) were uniformaly distributed. Three groups of transplants were prepared as below.

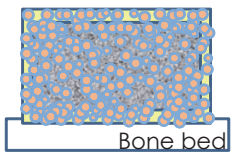
【Group A】
DCL (contains 6.25X10⁶ cells)was placed bottom of transplants.(N=3)



【Group B】
Five DCL (each contains 1.25X10⁶ cells) were placed evenly on the bottom and inside (arrowhead) of transplants.(N=4)



【Group C】
Whole transplant was covered with DCL (contains 12.5X10⁶ cells). (N=3)

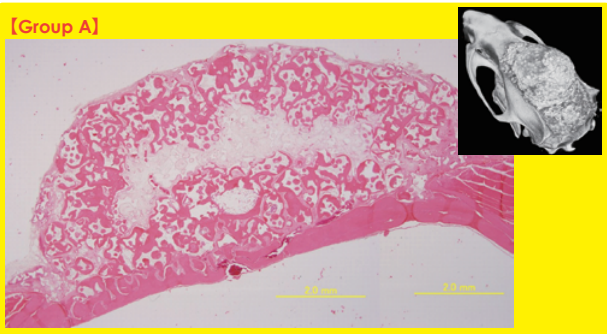


【Operation】
The parietal hair was shaved, and incision was made to the periosteum. Periosteum and skin were elevated to make the space of transplant. Then, cellular transplant was subperiosteally inserted without any decortication of calvarial bone. At this point, DCL was faced to the recipient bone. Ablated periosteum and skin were sutured.

【Evaluation】
16weeks after operation, 3 rats of each group sacrificed and tissues were taken out. Titanium implant was inserted in augmented bone of residual animal of GroupB, and sacrificed at 52 weeks and tissue was retrieved (GroupB2). All tissues were scanned by micro-CT apparatus and sectioned for histology (coronal section, H&E and Villanueva Goldner stain).

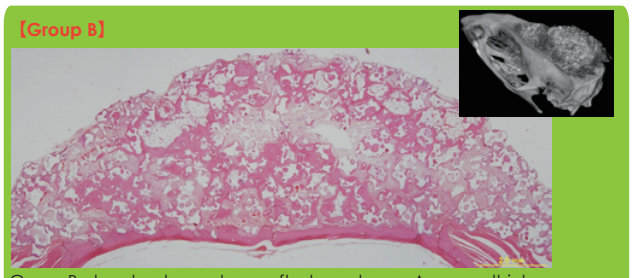
Results and Discussion

【Group A】



Dome shaped augmented bone engrafted to the bone bed. Average thickness of center of augmented bone was 4.69±0.76mm.(N=3) Bone formation occurred from outer side of the transplants. Bone marrow formation and blood vessel run into the graft observed.Ossification of center region was poor, but blood vessels were observed.

【Group B】



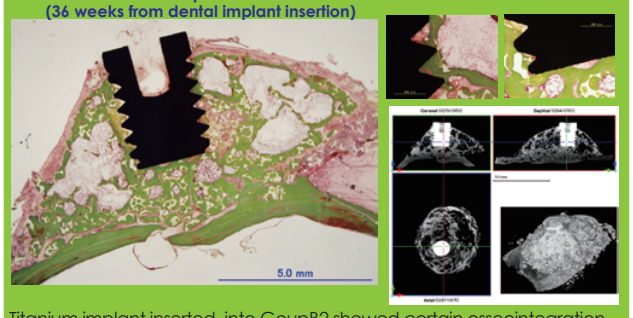
Group B also also showed engrafted new bone. Average thickness was 4.99±0.76mm.(N=3) Despite presence of DCL inner region, ossification did not occurred from center. But blood vessel and osteoblast like cells were observed in the area. So further ossification of the transplant to be expected.

【Group B2】

•Titanium dental implant insertion (16weeks after transplantation)

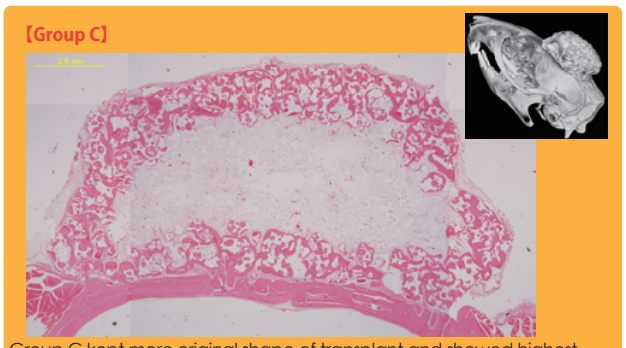
Titanium dental implant inserted at 16 weeks after transplantation. Calvaria and transplant did not move apart during implant insertion.

•52 weeks after transplantation
(36 weeks from dental implant insertion)

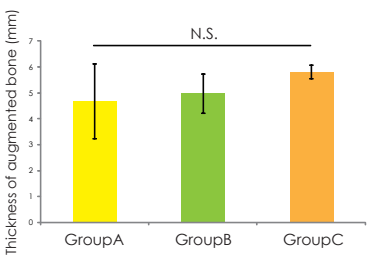


Titanium implant inserted into GoupB2 showed certain osseointegration and preservation of bone thickness. Bone marrow and trabecular bone formation was also proceeded comparison to the section of 16 weeks.

【Group C】



Group C kept more original shape of transplant and showed highest bone formation than others. Average thickness of center was 6.03±0.26mm.(N=3) Althogh there were more uncalcified region in the middle, blood supply also observed.



All experimented groups could achieve large dome-shaped bone augmentation 16 weeks or after. As with our previous work, Group A showed that bigger transplant with DCL facing the bone bed could augment clinical-sized bone. Group B showed that DCL placed in middle of transplant could not be a start point of bone formation. In all transplants, ossification occurred from the outer region. Although there were no significant difference observed, Group C showed highest augmented bone height and kept more of original shape of transplant. In addition to DCL-bone connection, connection of DCL to the surrounded connective tissue might also be a key factor to augment bone.
It is said that NO mobility and angiogenesis is important for bone augmentation. It is estimated that DCL of the transplant-recipient interface may induce the two-way migration of cells, it may lead to fixation and biological bridging. Then it is believed that transmission of stimuli for blood vessel formation or ossification occurs.

Conclusion

Our novel transplant which is whole surrounded by dense cellular layer showed highest bone augmentation. DCLfacing to the bone bed and surrounding connective tissue might be a key factor for stable and powerful bone augmentation. Also, the augmented bone could acquire certain osseointegration and performed for the animals lifespan, so traditional bone collection might be substituted by this new method in the future. (Some of the results of this study is patent pending.)