Tracking implanted mesenchymal stem cells in vivo with nano-crystal reagents

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Introduction
Previously, we've reported development of a scaffold free cell delivery system. With this system, we are able to surgically implant cells only construct into target tissue without use of biomaterials as carrier or scaffold. And we also obtained simultaneous regeneration of hyaline cartilage and subchondral bone by implantation of mesenchymal stem cell (MSC) based scaffold free construct to rabbit osteo-chondral defect. Still, it is little known for fate of the implanted stem cells. Several methods for trace implanted cells are widely used, yet, there are only a few systems for trace autologous implanted cells which are simple and safe.

In this study, we choose nanocrystal semiconductor fluorescent materials which are technically easy to load into cell and also relatively simple to detect in the cell. The purpose of this study is to detect the implanted mesenchymal stem cell in vivo by using the cell tracking reagent.

Material and Methods
MSCs were obtained from iliac crest of Japanese white rabbit. The Cells were expanded in monolayer until necessary number. The MSCs were labeled by Qt racker™ (Quantum Dot Corp. CA, USA) according with manufacture instruction. The labeled MSCs were mold into a cylinder shape with 4.5 mm in diameter and 4 mm in height. The autologous labeled-MSC plug was implanted into a patello-femoral groove of the rabbit. The knees were immobilized for one week. The rabbits were sacrificed at 6 weeks after operation. The harvested knee samples were photographed and scanned by micro CT (MCT). The samples were fixed in 4% Paraformaldehyde (PFA), decalcified by 0.5M EDTA. After dehydration in alcohol, the samples were embedded in paraffin and sectioned into 5 to 10 micrometer in thickness. For detecting labeled cell, sections were de-paraffined and observed by fluorescent microscope and confocal laser scanning microscope (FV-1000, Olympus, Tokyo, Japan). For histological evaluation, sections were stained with Safranin-O/Fast green for GAGs, and immunohistological stained anti-Type 1, and type II collagen.

Results
In gross appearance of the cartilage at 6 weeks, most of surface area of the defect was covered with cartilage-like tissue. Histologically, columnar distribution of the chondrocyte was observed. In the subchondral bone level, endochondral ossification occurred from the peripheral area of transplants. And small central area remained in chondrocyte. MCT showed further ossification was occurred in subchondral bone level. These results showed that this cell-tracking reagent did not have negative effects on regeneration of cartilage and bone. Confocal microscopy detected the labeled cells both in cartilage layer and subchondral bone layer. In the implanted muscle, we also detected labeled cells along with muscle fibers, suggesting that implanted MSCs were differentiated into myocyte or muscle-satellite cell.

Conclusion
This study showed that implanted mesenchymal stem cells were survived and differentiated in accordance with site specific information, and also demonstrated usefulness of this scaffold free cell delivery system. Further investigations are required yet, the nanocrystal semiconductor fluorescent materials are potentially useful tool for cell tracking in regenerative medicine.

The role of type V collagen as a pioneer ECM during renal Tissue Restructuring.

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Development of renal tissue requires various phases to induce micromovement and differentiation of mesenchymal cells. A mediator ECM is needed to maintain synergic extracellular environments for such kinds of phase changes. In this study, we investigated the function of type V collagen fiber on adult glomerular endothelial cells and developmental metanephros, and proved that type V collagen fiber is an intermediary ECM which may provide an environment for ECM and cell/tissue with plasticity and turnover and cause continuous phase changes during tissue development and morphogenesis. Culture of adult glomerular endothelial cells on type V collagen fiber showed that this ECM could not only induce the migration of cells dynamically, but also enhance the cell-cell interaction on the super structure of ECM. Moreover, type V collagen was found to turn over the stable phase of ECM structure, and provide a transitional environment for cells.

On the other hand, in vivo experiment for embryonic metanephros showed that type V collagen fiber shifted from the whole area of ureteric buds to the leading area of gomeruli during development. Interestingly, collagen V fiber was found existed around the growing gomeruli, whereas collagen IV located within the mature glomeruli, suggesting ECM phase has been changed during glomerular development. Culture of metanephros of E11.5 on collagen V fiber resulted in the vascular morphogenesis from mesenchymal cells, and collagen V fiber was found incorporated into the branching tip of ureteric buds. These facts suggest that type V collagen fiber plays a guiding role between interfaces of ureteric buds and glomeruli which leads tissue plasticity to induce microvascular formation and fusion between ureteric buds and glomeruli.