The efficiency of genetically-modified mesenchymal stromal cells combined with a functionally-graded scaffold for bone regeneration in corticosteroid-induced osteonecrosis of the femoral head in rabbits

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INTRODUCTION: Core decompression (CD) with mesenchymal stromal cells (MSCs) is a promising treatment for early-stage osteonecrosis of the femoral head (ONFH). Recently, preconditioning of MSCs, using inflammatory mediators, is widely used in immunology and various cell therapies. We developed a 3-dimensional printed functionally-graded scaffold (FGS), made of β-TCP and PCL, for cell delivery at a specific location. The present study investigates the efficacy of cell-based CD treatments using genetically-modified MSCs that over-express PDGF-BB (PDGF-BB-MSCs) or genetically-modified MSCs that co-over-express IL-4 and PDGF-BB and are preconditioned by exposure to tumor necrosis factor-alpha and lipopolysaccharide for 3 days (IL-4-PDGF-BB-pMSCs) using the FGS for treating steroid-induced ONFH in rabbits.

METHODS: Twenty-four male mature New Zealand rabbits received a single intramuscular dose of 20 mg/kg of methylprednisolone acetate 4 weeks before surgery. Rabbits were divided into 4 groups: 1) the CD group, 2) the IL-4-PDGF-BB-pMSC group, 3) the FGS + PDGF-BB-MSC group, and 4) the FGS + IL-4-PDGF-BB-pMSC group. To create those groups, 1 million cells in 200 µl of HG with or without the FGS were inserted in the tunnel: 1 million IL-4-PDGF-BB-pMSCs alone, FGS with PDGF-BB-MSCs or with IL-4-PDGF-BB-pMSCs. Rabbits were euthanized 8 weeks after the surgery. Micro CT and histological analyses [hematoxylin and eosin (H&E) staining, tartrate-resistant acid phosphatase (TRAP) staining, and a cluster of differentiation31 (CD31) staining] were performed (Figure 1).

RESULTS:

For the area inside the CD, the bone volume in the CD group was higher than in the other 3 groups, and the bone volume in the IL-4-PDGF-BB-pMSC group was higher than in both FGS groups. The FGS + IL-4-PDGF-BB-pMSC group showed a tendency to accelerate bone ingrowth compared to the IL-4-PDGF-BB-pMSC alone group. For the area outside the CD, the BMD and BVF were not significantly different among the 4 groups. However, in the IL-4-PDGF-BB-pMSC groups, the BMD tended to be higher when combined with the FGS (Figure 2).

In the subchondral bone, there was no significant difference in the percentage of empty lacunae among the 4 groups. In the area surrounding the tunnel, the percentage of empty lacunae was significantly lower in the IL-4-PDGF-BB-pMSCs and FGS + PDGF-BB-MSCs groups compared to the CD group (Figure 3).

Although there were no significant differences in the osteoclast density in the subchondral bone among the 4 groups, we found that the osteoclast density was significantly increased in the area surrounding the tunnel in the IL-4-PDGF-BB-pMSCs and FGS + PDGF-BB-MSCs groups compared to the CD group (Figure 4).

When evaluating the subchondral bone, there was no significant difference in the CD31 positive microvessel formation among the 4 groups. However, the assessment of the area surrounding the bone tunnel showed that the FGS + PDGF-BB-MSC groups had significantly greater CD31 positive microvessel formation than the CD group (Figure 5).

DISCUSSION AND CONCLUSION:

All histological analyses showed that the effects of PDGF-BB-MSCs and IL-4-PDGF-BB-pMSCs were more pronounced in the area surrounding the bone tunnel than in the subchondral bone: decreased percentage of empty lacunae and increased osteoclast density. This local effect suggests that IL-4 or PDGF-BB signaling may preferentially occur in a paracrine manner. In addition, in both the PDGF-BB-MSC and IL-4-PDGF-BB-pMSC groups, the percentage of empty lacunae was increased, and the osteoclast density was decreased when the FGS was also used, compared to the group with no FGS. Cytokines such as IL-4 and PDGF-BB released from IL-4-PDGF-BB-pMSCs may bind to the FGS which continuously releases these factors into the area outside the CD. This slow-release function could have diminished the effect of these cytokines on the area outside the CD when the FGS was added. This observation may have important implications for the treatment of early ONFH based on the combination of MSCs and FGS. This emphasizes that treatments need to be applied in a temporally and specially optimal manner.

IL-4 and PDGF-BB released at appropriate doses and time points, in combination with the FGS, may constitute an important adjunctive therapy to CD for early stage ONFH.