Translational Study of Autologous Bone Marrow Stem Cell with / without TGF-beta 1 Over Express Fumarate Hydrogel For Chondral Defect Repairing

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Objectives

To analyse (1) in vitro pore sizes and mechanical stiffness of the freeze-dried and rehydrated freeze-dried OPF hydrogel respectively, (2) condensed BMSC morphologies in OPF scaffold seeded by a new cell seeding method - freeze-dried and rehydrated freeze-dried OPF hydrogel respectively, (3) TGFb3 over express plasmid construction and genetically modified (TGFb3 over express) BMSCs function in in vitro chondrogenic model, (4) in vivo gross morphological and histological outcome of defects implanted with BMSCs-OPF scaffold 4 months in a porcine model, and perform (4) qualitative analysis of highly-magnified histological sections and (5) quantitative statistical analysis of the neo tissue.

Materials and Method

The pore sizes and mechanical stiffness of the freeze-dried OPF hydrogel scaffold and compressive stiffness of its rehydrated form were measured. Full length human TGF-b3 gene was inserted into mammalian express plasmid pcDNA4/TO. The plasmid was transformed into BMSCs to generate genetically modified BMSCs. The function of the genetically modified BMSCs was test in in vitro chondrogenic model with TGF^- media. Morphology of the BMSCs in OPF scaffold was observed at 24 hours after cells were seeded with naked eye and phase microscope. Three dimensional structure of the fabrication was observed through confocal microscope and cross section by histological method. In vivo osteochondral defect repair was tested from 6 defects in 8-month-old Prestige World Genetics (PWG) micropigs. Scaffold alone was used as control groups. Gross morphology, histology and variation analyses were performed at 4 months postoperatively.

Results

The freeze-dried OPF hydrogel's pore sizes ranged from 20um to 136um in diameter. The rehydrated freeze-dried scaffold had a similar mechanical stiffness to fresh OPF hydrogel. The genetically modified BMSCs can complete chondrogenic differentiation without extra TGF support from cell culture media. The BMSCs were condensed into many pellet – like cell masses with diameter of dozens to hundreds micrometer in OPF scaffold. More than 95% percent of the cells were available. BMSCs had a good compatibility with OPF scaffold in vitro. The BMSCs-scaffold can repair up to more than 99% defect area with more than 80% hyaline-like cartilage at 4 months. It was statistical significantly ($P<0.0001$) more than the 54% neo-tissue filling ratio and the 39% hyaline-like cartilage in control group. Genetically modified BMSCs group had an elevated subchondral contour line.

Conclusion

OPF hydrogel scaffold alone can only partially enhance defect healing. BMSCs-OPF fabrication can fully repair chondral defect with hyaline-like cartilage in micropig osteochondral model with less effect for the subchondral bone regeneration. Genetically modified BMSCs, in this scope, has such a strong influence on the repair of degenerated subchondral bone that result in over elevation of its contour line.