The Repair Of Articular Cartilage Defects Using A Transglutaminase 4 And Hydrogel Embedded With Synovium-derived Stem Cells In Rabbit Model

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Introduction: Recently, injectable hydro gels that are in situ formed after injection at the defect site have received much attention. Hyaluronic acid (HA) and type I collagen are major components of articular cartilage and fibrin glue is widely used in the tissue engineering of cartilage. We previously revealed that synovium-derived stem cells (SDSCs) had a great proliferation potential and a multi-lineage differentiation potential in vitro, and a good adhesive effect of Transglutaminase 4 (TG4) by cross-linker enzyme. The in situ crosslinking between gel and tissue extracellular matrix are preventing cells from dissipation. TG4 also maintain the carried cells to be viable and proliferative. We had developed newly injectable hydrogel-typed scaffold and attempted to develop a novel compound of TG4-hydrogel with SDSCs. In this study, we investigated the effect of human SDSCs embedded with injectable hydrogel and TG4 on the repair of articular cartilage defects.

Methods: Isolation and culture of cells: Synovium was isolated from the knee joint of patient undergoing total knee arthroplasty. After digestion with collagenase, released cells were cultured. Preparation of recombinant human TG4: Bacmid DNA was prepared with the recombinant of pVL 1392 (Baculovirus Transfer Vector containing a 6xHis tag) and cDNA of TG4. Sf21 cells were transfected with Bacmid DNA. Purification of the 6His-tagged TG4 proteins was performed chromatography on the Ni-NTA resin and the harvested protein was measured its TG activity using [1, 4 \(^{14}\)C]-putrescineincorporation into N, N'-dimethylcaseinassay. Preparation of TG4-hydrogels with SDSCs: Injectable hydrogel was a solution containing fibrinogen (Green Cross, Korea), 10 mg/ml HA (LG CI, Korea) and 3 % type I collagen (RMS innovations, UK). SDSCs were suspended at 2 \(\times\)10^7 cells/ml in a solution and homogeneously mixed. Thrombin/CaCl\(_2\) solution was then added to form a hydrogel. The groups were 1) hydro gel only (hydrogel solution + thrombin/CaCl\(_2\)) 2) hydro gel + SDSCs, 3) hydro gel + human fibronectin (FN) 4) hydro gel + FN + SDSCs + TG4 100 μg/ml (TG4-100), 5) hydro gel + FN + SDSCs + TG4 400 μg/ml (TG4-400). in vitro Cell proliferation: Viable cells in the hydro gels were assessed using the Live/Dead Viability kit and the cell number was measured using the MTS method. GAG and DNA amount assay: The synthesized GAG was determined by binding to DMB dye and total amount of GAG was normalized to the amount of DNA by indole assay. Chondrogenic differentiation: To determine the differentiation capacity of SDSCs in hydro gel, constructs were cultured in chemically defined chondrogenic medium. Histology: Samples from each time point were stained with safranin O for proteoglycan detection. The expression of type I, II collagen was detected by immunohistochemical staining. RT-PCR: The cartilage specific ECM genes expression was analyzed by RT-PCR. In vivo cartilage formation: Osteochondral defect was made in the trochlear groove and medial condyle (Φ: 4mm, depth: 3 mm) of the knee joint of rabbit with a custom-made surgical tool.
The hydrogel and thrombin solution was injected using a dual syringe. Statistical analysis: All experiments were hydro gel/SDSCs performed in triplicate and the results were analyzed using paired t-test. P values less than 0.05 were considered significant.

**Results:** Most of the encapsulated SDSCs in hydro gel remained viable, changed morphology and proliferated during the 7 days. GAG synthesis from encapsulated cells in hydro gel was shown to have increased up to 21 days. Chondrogenic differentiation exhibited chondrocyte-like cells embedded in a proteoglycan-rich ECM and positive staining for type I and II collagen was shown. RT-PCR also revealed the expression of cartilage specific ECM genes. In the rabbit defect model, cartilage defect injected TG4-hydro gel with SDSCs was found to have filled with regenerated cartilaginous tissue by 12 weeks after injection. The injected TG4-hydro gel with SDSCs and hydro gel with SDSCs were observed as red fluorescence in the defect Margin of the defect completely integrated with regenerated tissue.

**Discussion:** The present study was to fabricate and characterize a novel biocompatible hydrogel composed of HA, type I collagen and fibrin glue that could be used as an injectable cell carrier for cartilage repair. Our TG4-hydro gel can be useful as an injectable cell carrier for cartilage repair. It shows the chondrogenic differentiation potential of cells treated enzyme-hydrogel. This TG4-hydro gel can be a good candidate for the cartilage regeneration carrier. We confirmed repair of articular cartilage defects of the SDSCs in TG4-hydro gel in vivo, as well as in vitro. TG4-hydro gel demonstrated better potential for in vivo chondrogenesis in a rabbit model. This finding indicates the feasibility of TG4-hydro gel for cartilage repair.

**Significance:**
2) Lee et al: Tissue engineering, 2012
4) Kuwahara et al: Tissue engineering, 2010

![Figure 1. Proliferation of SDSCs embedded with hydro gels.](image)
Figure 2. Images of the condyle of the rabbit knee surgery after 1 week (a) and 12 weeks (b). Red fluorescence: the injected hydro gel with SDSCs, TG4-hydro gel with SDSCs.