INTRODUCTION:
Autologous chondrocyte transplantation (ACT) is a routine technique to regenerate cartilage tissue in chondral lesions [1]. Instead of using periosteal patch, the method can be enhanced by seeding the culture-expanded chondrocytes into a biodegradable 3-dimensional scaffold (matrix-associated ACT). These new generation ACT techniques lead to less surgical time, morbidity, and abolish periosteal patch hypertrophy. Even though extensive research in the field of cartilage tissue regeneration, the goals of successful cartilage repair has not yet been completely achieved and none of the techniques today can be called as "the golden standard".

This study aims to improve the current standard of care by introducing new techniques for improvement of regenerated articular cartilage by producing scaffold constructs that enable the maintenance of chondrogenic capacity of the ex vivo expanded autologous chondrocytes. Collagen type II is the major component of cartilage and, according to our previous studies, could be an eligible matrix material for matrix-associated autologous chondrocyte implantation [2, 3]. But due to its weak mechanical properties, type II collagen by itself is not optimal for tissue-engineered reconstruction of injured cartilage. In this preliminary in vitro study, we have used novel biomaterials based on biodegradable polylactide polymers (PLDLA; poly-L-D-lactic acid) in combination with the recombinant human type II collagen gel. With these hybrid constructs we aim to provide suitable environment for chondrocytes to proliferate and phenotypically express chondrocytic functions within a 3D-environment that also provides an adequate load-sharing structural matrix.

METHODS:
Chondrocytes were harvested from patellofemoral groove cartilage of bovine knees and digested with collagenase in sterile conditions. To fabricate hydrogels with chondrocytes entrapped within, recombinant human type II collagen (FibroGen Europe, Helsinki, Finland; 3mg/ml in 10mM HCl) was combined with growth medium (DMEM/F12 supplemented with: 10% Fetal bovine serum, 1% Penicillin-Streptomycin, 1% L-glutamine 200mM, 1% Fungizone AmphotericinB, and 50µg/ml L-Ascorbate acid) containing the desired number of passage 0 chondrocytes. After gelation in +37°C the gel-cell constructs were moved onto poly-L-D-lactic acid scaffolds (PLDLA 96/4, Tampere University of Technology, Tampere, Finland), and the hybrids cultured for 9 to 21 days. Different protocols were used to maximize infiltration of the gel into the PLDLA-scaffold. Cell viability and infiltration depth of gel-cell construct into the PLDLA-scaffold were analyzed. Chondrocytes were also cultured on the PLDLA-scaffolds without the rh type II collagen gel, and analyzed for cell infiltration onto the scaffold, viability and collagen type II production.

RESULTS:
The PLDLA-scaffold provided a biotolerant substrate upon which the bovine chondrocytes adhered. A vast majority of the chondrocytes cultured on these PLDLA-scaffolds was alive and maintained their rounded phenotype while growing parallel to the PLDLA-fibers (Fig. 1 A). Part of the cell population was elongated in morphology while wrapped around the PLDLA-fibers. The cultured chondrocytes secreted collagen type II, suggesting preservation of chondrocyte phenotype (Fig. 1 C).

When the bovine chondrocytes were entrapped in and cultured inside the rh type II collagen gel, the chondrocytes retained their viability and rounded phenotype well (Fig. 1 B). The rh type II collagen gel supplemented with the chondrocytes was evenly distributed along the PLDLA-scaffold (Fig. 1 D). Penetration depth of the chondrocytes inside the PLDLA-scaffold was 100-500µm with or without rh type II collagen gel. This indicates that the porosity of the PLDLA-scaffolds allow free infiltration of the gel into the scaffold.

DISCUSSION:
These preliminary in vitro results indicates the maintenance of chondrogenic phenotype on the tested PLDLA-scaffolds and PLDLA-rh type II collagen hybrids. The results encourages for further studies, and suggest that biodegradable PLDLA-scaffold inoculated with human recombinant type II collagen gel might provide mechanically rigid chondrogenic structures for repair of large articular lesions. The hydrogel portion of the implant serves as chondrogenic environment while the PLDLA-scaffold provides mechanical support. These hybrids are now being tested in vivo for their cartilage producing capacity.

REFERENCES: