INTRODUCTION:
Cartilage repair continues to be a challenge, despite the progress made in engineering cartilage. One of the major obstacles hindering progression is the integration of the engineered constructs with the recipient cartilage. Cartilage integration is a unique, complex tissue repair process, involving cell proliferation, apoptosis and matrix production. It is necessary to develop an in vitro model which simulates the biological process of cartilage repair and offers access to therapeutic manipulation. The purpose of this study was to develop a novel in vitro approach, which can be conveniently applied to improve the integration of tissue engineered cartilage with articular cartilage. This model, a cartilage integration construct, consists of a cartilage explant and isolated chondrocytes.

METHODS:
Human articular cartilage was harvested from the distal femur, infrapatellar surface, and tibial plateau following total knee arthroplasty. Tissue that would be the explant (native cartilage portion) was first cut with a 6mm dermal punch down to the bone (Figure 1A); followed by another cut, within the 6mm circle, with a 3mm dermal punch. A cartilage ring, with a 6mm peripheral diameter and 3mm inner diameter, was removed as a cartilage explant (Figure 1B).

Fig. 1 Assembly of the cartilage constructs
Cartilage explants were stored in chondrocyte medium (supplemented DMEM (Dulbecco modified Eagle’s medium) with 10%FBS, Penicillin + Streptomycin, Ascorbic Acid, and Glutamine) in an incubator at 37°C and 5% CO² in air for later use. The rest of the cartilage that was to be used for chondrocyte isolation was excised using a surgical scalpel and minced into 1mm³ fragments, then treated with 0.25% pronase in DMEM for 1 hour, and finally digested with 0.4% type II collagenase in DMEM overnight. The resultant chondrocytes were counted with a hemocytometer and chondrocytes were concentrated to 125,000 cells per µL of the medium.

Cartilage constructs were formed in the bottom of a 12-well culture plate via the following procedures. Low melting-point agarose (1%) was dissolved in PBS buffer by autoclave. When cooled down to 37°C, agarose solution (400µL) was layered at the bottom of the well to form a bed (1mm in thickness) for the construct. Explants were then immediately placed in the center of the well and the plate was taken to a refrigerator (4°C) for 5 minutes for the gelation of agarose. Residual liquid of medium and agarose was aspirated from the inner ring of the explants using a 10µL pipette. About 10µL of the suspended chondrocytes were added into the center of the explant ring.

Chondrocyte medium was then cautiously introduced at the wall of the plate up to the level of the construct (Figure 1C). The top of the construct was not immediately covered with medium, in order to allow the chondrocytes to settle and prevent overflow outside of the explant. After incubation overnight, additional medium was added to cover the constructs. Medium was carefully changed every 2-3 days for the duration of the culture. For a control, explants were placed in the culture well without agarose, and identical quantities of chondrocytes were added in the same manner as previously described. After 4 weeks, the constructs were fixed with 4% paraformaldehyde and sectioned for immunohistochemistry for type II collagen.

RESULTS:
In the control, when explants were directly placed on the bottom of the culture plate, chondrocytes in the center of the explants migrated or flowed out of the construct, and were seen along the periphery of the explant after overnight culture (Fig 2A). The explants did not stay on the bottom of the culture plate, but were floating in the medium. In contrast, when explants were placed on top of the agarose gel, there were no chondrocytes outside the explant ring (Fig 2B). Chondrocytes were confined to the center ring of the explant at a high density (Fig 2C). Over time in culture, the center of the explant became less transparent as cells proliferated, aggregated, and accumulated extracellular matrix. Through more than 4 weeks of culture, the explants were stably anchored in agarose gel. By 4 weeks, cell death was noticed at the surface of the explant. Chondrocytes in the center of explants were alive but there was no significant extracellular matrix produced that was comparable with the explant (Fig 2D).

DISCUSSION:
The ultimate outcome of cartilage repair depends on a successful integration between the host cartilage and the newly formed reparative tissue. To study cartilage integration, an in vitro model has several advantages over an in vivo study. The integration process can be closely monitored and the conditions for integration can be precisely controlled. An in vitro model of cartilage integration also enables the use of human articular cartilage, which is of clinical significance.

The model described in this study simulates a clinical situation and provides a 3-D environment for the chondrocytes to integrate with articular cartilage. The novelty of this model is the application of low melting-point agarose to prevent “leaking” of chondrocytes to the outside of the explant. Chondrocytes were in layers and later congregated, simulating a pellet culture. The chondrocyte phenotype is stabilized by the high density of cells, ensuring that the chondrocytes are producing hyaline cartilage matrix.

This study presents a suitable model to study the integration of tissue-engineered constructs with native cartilage. It is particularly useful for studies addressing the cartilage zonal issues and chondrocyte subpopulations in cartilage repair.