Introduction: The repair of osteochondral defects poses a great challenge because it requires grafts that can withstand physiological loading, and restore the 3-dimensional architecture of the articular cartilage and underlying bone, with the development of a stable interface [1]. We hypothesized that composite grafts consisting of a layer of in vitro-grown engineered cartilage [2] supported by an osteoconductive biomaterial [3] would have mechanical properties allowing for fixation into large, deep osteochondral defects and would provide a biological template that, in the presence of host-derived biochemical and physical signals, could be remodeled into an osteochondral tissue that was well integrated with adjacent host tissues.

Methods: Engineered cartilage was prepared by isolating articular chondrocytes from young (3 month) New Zealand White (NZW) rabbits, amplifying them (8 days without passage) in flasks, and cultivating them on fibrous polyglycolic acid (PGA) meshes for 4-5 weeks. Composites were made by suturing either engineered cartilage, cut to 7 x 5 x 1 mm, or cell-free PGA mesh to Collagraft, a biomaterial consisting of type I collagen, hydroxyapatite and tricalcium phosphate [3]. Standardized defects 7 x 5 x 5 mm were created bilaterally in the femoropatellar groove of adult (8 month) NZW rabbits and randomly allocated to 3 groups: (I) natural healing; (II) implantation of composites of cell-free PGA and Collagraft; and (III) implantation of composites of engineered cartilage and Collagraft. Rabbits were sacrificed at 6 weeks and 6 months and a portion of the distal femur was EDTA-decalcified, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, alcian blue, or van Gieson. The architecture of the repair tissue was assessed from 5 parameters determined by computerized image analysis: 1. defect width at the surface (W), 2. average thickness of the cartilaginous region (T=total cartilaginous area normalized by W), 3. average thickness at the center of the cartilaginous tissue (Te=the central 10% of the cartilaginous area normalized by 0.10 W) 4. the ratio of central and average thicknesses (Te/T, that would equal 1 if the repair cartilage was uniformly thick), and 5. the bone fraction of the region extending from the surface to a depth of 1 mm (Bs). The present work reports the structural properties of the resulting repair tissue, as assessed from all available data (histological sections prepared from 10 of 17 six week and 10 of 18 six month samples); analysis of the other histological, biochemical and biomechanical data is still in progress.

Results: Six week repair tissue in group III consisted of a 1.02 ± 0.21 mm thick surface zone that resembled hyaline articular cartilage and was 4.4-fold thicker than adjacent host cartilage (0.23 ± 0.06 mm), and a region at the base of the engineered cartilage marked by an inflammatory response that was associated with decreased alcian blue staining. Six month repair tissue in group III resembled native osteochondral tissue (Fig. 1) and consisted of chondrocytes aligned in vertical columns overlying a clearly defined tidemark and subchondral bone (Fig. 1, inset). Cartilage represented a 0.12 ± 0.02 mm, uniformly thick zone (Tc/T = 1.06 ± 0.15) at the articular surface, and bone represented the major fraction of the region extending to a depth of 1 mm (Bs = 81 ± 6 %). The repair tissue, although relatively poorly integrated with host cartilage, was extremely well integrated with host bone laterally and from below such that in some specimens it was very difficult to distinguish the original defect borders (Fig. 1). Other specimens still appeared to be in the process of remodeling and contained islands of engineered cartilage that were identifiable by the occasional presence of polymeric fibers. Although 6 week repairs were comparable in all three groups, 6 month repairs in groups I and II were inferior to those in group III. In particular, defects not repaired with the engineered cartilage component were filled mainly with fibrocartilage that was shaped like an inverted cone and appeared to interfere with reconstruction of the subchondral plate and underlying bone. Repair tissues in groups I and II consisted of randomly oriented cells (round and elongated), and exhibited degenerative changes (fibrillation, fissures). The cartilaginous zones were thicker centrally than laterally (Tc/T = 3.30 ± 0.20 and 2.54 ± 0.35 in groups I and II, respectively). The bone fractions in the region extending to a depth of 1 mm were relatively low (Bs= 68 ± 13 % and 50 ± 13 % in groups I and II, respectively).

Discussion: Design requirements of grafts for the repair of large, full-thickness osteochondral defects include: (a) mechanical properties that allow for customized shaping and fixation at the defect site, and (b) biochemical properties that permit or even induce host remodeling. In previous rabbit studies, thin (~0.20 mm) cartilaginous grafts either dislodged or resorbed over 3 months [4] whereas thick (4 mm) cartilaginous plugs failed to vascularize or remodel over 6 months [5]. In the present study, engineered composites composed of a layer of engineered cartilage significantly thicker than that found in vivo, overlying an osteoconductive biomaterial provided a biological template for the repair of large, deep osteochondral defects. The cartilaginous component of the composite, which was cultivated in vitro for 4-5 weeks prior to implantation, could be readily shaped using a scalpel, while the mechanical properties of the Collagraft component permitted composite fixation at the defect site. In vivo, under physiological loading conditions, the composite was remodeled into osteochondral tissue with characteristic architectural features. In particular, 6 month repair tissues consisted of a cartilaginous surface, a reconstituted subchondral plate at the appropriate depth, and underlying trabecular bone. The remodeling process appeared to involve a vascular invasion of the engineered cartilage from below such that only its cartilaginous articular surface was preserved. Possible factors stimulating the remodeling process include the allogenicity of the chondrocytes and the biochemical properties of the engineered cartilage; these hypotheses, and the long-term fate of the grafts, are the subjects of future investigations.

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References:

Fig. 1: 6 month repair in group III, H&E stain; bar: 1 mm or 370 μm for inset.