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Cartilage Transplants Hold Promise for Challenging Bone Defects

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Abstract

The challenges of healing have led investigators to question existing paradigms in the hopes of uncovering overlooked solutions. Such is the case in a recent study showing that introduction of a cartilage construct into a mouse tibial defect induces remarkable healing owing to the transformation of donor chondrocytes into new bone.

Despite major advances in our ability to treat bone fractures, no definitive procedure exists to heal critical-size (>3cm) defects, and long-term outcomes of current procedures suffer high rates of failure and complications.[1] These problems are further evidenced by the dissatisfaction reported following limb-salvage surgery, as the results of such procedures, in terms of the patient's quality of life, are no better than amputation.[2] Although the gold standard of care for bone defects is autograft of live tissue (i.e. transport of the patient's fibula to a segmental defect in their radius), this approach is not possible for most critical-size defect situations that would require too much host bone to transport. Thus, devitalized bone allografts are commonly used in combination with pharmacological, biological and cellular adjuvants to improve healing. Conventional thinking is that these cellular adjuvants should be osteoblastic, to promote primary bone healing via intramembranous bone formation with rigid fixation. Contrary to this established paradigm, Bahney *et al.* hypothesized that the introduction of a cartilage construct into a segmental defect efficiently heals the bone via endochondral ossification (Figure 1), and they have gone on to demonstrate the feasibility of this technique in a mouse model of tibial fracture.[3]

The similarities between endochondral ossification in embryonic bone development and during fracture healing are well known. Investigators have also shown that endochondral ossification follows ectopic transplantation of cartilage-like tissue derived from mesenchymal stem cells (MSCs).[4] Moreover, "bone organs", with mature vasculature and functional haematopoietic compartments, can be generated from ectopic transplantation of engineered hypertrophic cartilage.[5] The study by Bahney *et al.* expands on this previous work by use of a translational model of bone regeneration.[3] They harvested endochondral cartilage from callus tissue generated at the site of an unstable tibia fracture in mice. This

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cartilage construct was then transplanted as a graft into a critical-size tibia defect. The authors showed that the bone regenerate healed the defect with similar radiographic, biomechanical, and histologic properties to those observed with the live isograft control. More surprisingly, when they repeated these experiments using cartilage graft from genetically labelled mouse strains (*LacZ* and *GFP*) in order to assess cell fate in their model, they found that the majority of the regenerated bone was composed of donor cells, which had apparently transformed (dedifferentiated and then differentiated) into osteoblasts and osteocytes.

Another interesting finding in this study was the persistence of donor-derived hypertrophic chondrocytes at the fracture site.[3] Apoptosis of terminal hypertrophic chondrocytes is widely believed to be required for bone formation during embryonic development, and impairment of this process gives rise to rickets.[6] Thus, the observation that hypertrophic chondrocytes showed no sign of apoptosis suggests some remarkable quality of this fracture-callus-derived cartilage construct. As the authors point out, this observation warrants further study to formally elucidate the mechanism by which the donor cells undergo a morphological change from chondrocytes to osteocytes.

Bahney *et al.* also performed *in vitro* studies to better understand the role of angiogenesis and vascular endothelial cell effects on the morphological changes of cartilage explants, which is another revolving concept of fracture healing. Although it is well known that vascularization of the fracture callus is critical for its mineralization and remodeling into lamellar bone, recent studies have shown that the formation of fibrous tissue during allograft healing is associated with large-vessel (>100μm) arteriogenesis, which promotes fracture non-union.[7] Additionally, treatment with teriparatide, which increases cartilage formation at the host–graft interface, substantially inhibits arteriogenesis. Thus, another critical area for future study is the importance of the hypoxic environment, generated by cartilage in the early phase of bone healing, to inhibition of the chronic inflammation and fibrosis that usually causes the bone non-union that follows massive allografting.

Some limitations to the study also warrant discussion. The first is the challenge of translating results from mice, which have extraordinary bone-healing potential, to humans. One of the most serious complications following reconstructive surgery for a massive bone defect is re-fracture.[1] Thus, beyond the obvious issues of scale and long-term outcome, the novel approach developed by Bahney *et al.* seems to rely on the persistence of cartilage at the fracture site, which could be highly susceptible to fracture, and potentially to hypertrophic non-union in humans.

Another question is whether something intrinsic to cartilage produced in fracture callus exists that engenders it with unique bone-healing properties that cannot be attained by differentiated MSCs or other chondrocytes. As Bahney *et al.* described, human MSCs embedded in a hydrogel scaffold produced cartilage-like matrix with robust expression of *type II* and *type X collagen* and *MMP-13* only in vitro, and the bone-healing potential of this construct is unknown. Even though human articular cartilage and chondrocyte allograft transplantation products are currently used for cartilage repair, the results of this study do not support their use for bone repair. Thus, even if human fracture-callus-derived cartilage

proves to be highly osteogenic for this purpose, how it could be obtained to treat patients remains unknown.

Another challenge for this novel cartilage-construct approach to bone healing is the need to demonstrate its superiority over more practical cell sources; MSCs, for example, have been widely used experimentally and have great potential for cell-based therapies for a diverse range of diseases.[8] Likewise, induced pluripotent stem cells (iPS) also hold promise for cell-based therapies.[9] In a 2011 study, genetic stem cell technology was used to directly induce cartilaginous tissue from dermal fibroblasts by use of two cell-reprogramming factors (c-Myc and Klf4) and a chondrogenic factor (SOX9), without generating iPS.[10] On the basis of results obtained with their cartilage graft model, Bahney *et al.*[3] concluded that the chondrocytes in the graft construct, which do not express Oct4, re-expressed this pluripotent transcription factor during their transformation into bone cells. This is an interesting observation because other studies that utilized iPS cells as osteoprogenitors showed that Oct4 was not expressed during cellular transformation,[9, 10] suggesting that the cartilage grafts differentiate through a different pathway.

In conclusion, the paper by Bahney *et al.*[3] highlights many unknowns in the field of bone healing for large segmental defects, and underscores the need to challenge existing paradigms and clinical practices. The demonstration, for the first time, that cartilage grafts efficiently heal critical-size bone defects in a mouse model by mimicking embryonic endochondral ossification could be an important milestone. Nonetheless, future studies are needed to translate these finding and elucidate the mechanism by which the donor cartilage differentiates into bone, which could be the next breakthrough in this field.

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Biographies

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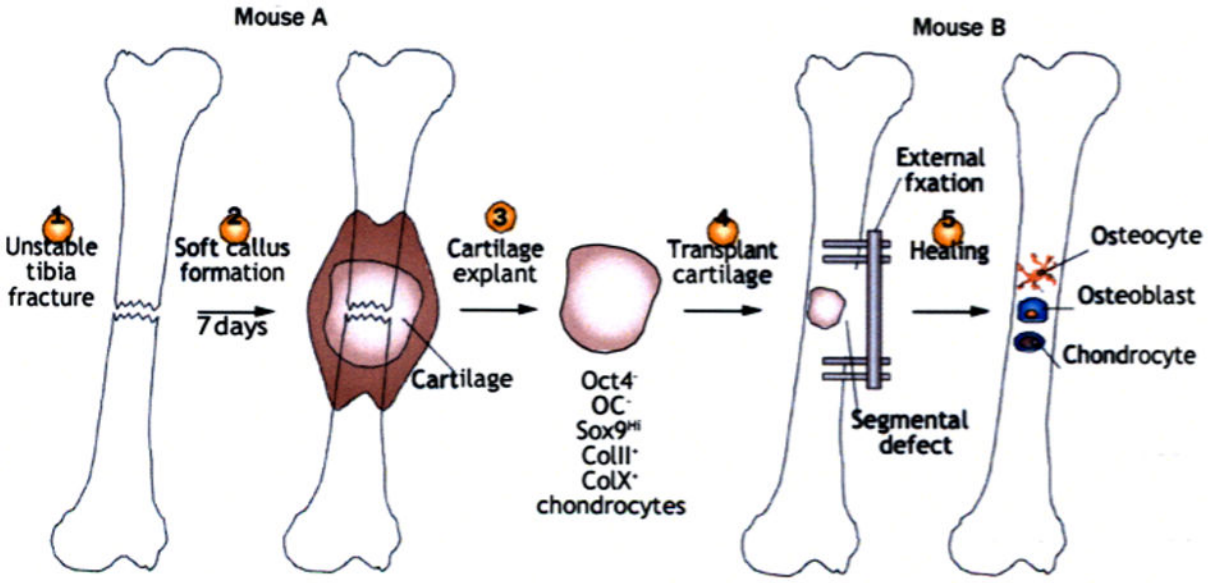

Figure 1. Cartilage grafts for segmental defect healing

The generation of an unstable fracture (1) in mouse A produces a lot of cartilage in the soft callus tissue by day 7 (2). The explanted fracture callus/cartilage is made into a graft (3), and transplanted (4) into a tibial segmental defect in mouse B that heals with external fixation. During this healing process (5), the grafted chondrocytes de-differentiate into Oct4+ progenitor cells, and then differentiate into hypertrophic chondrocytes, osteoblasts and osteocytes in the regenerate tissue. Abbreviations: col II, type II collagen; col X, type 10 collagen; OC, osteocalcin; Oct4, octamer-binding protein 4.