Treatment of Focal Cartilage Defects With a Juvenile Allogeneic 3-Dimensional Articular Cartilage Graft

Frank McCormick, MD,* Brian J. Cole, MD, MBA,† Benedict Nwachukwu, BA,‡ Joshua D. Harris, MD,* H. Davis Adkisson IV, PhD,‖ and Jack Farr, MD§

DeNovo engineered tissue graft (recently renamed “RevaFlex”) is a novel cellular therapy currently under Phase III investigation for cartilage regeneration. It is an in vitro–grown 3-dimensional hyaline-like cartilage tissue containing viable cultured juvenile allograft chondrocytes. Once expanded in vitro, juvenile chondrocytes are grown on a temporary polycarbonate membrane that is separated from the final tissue-engineered product at the time of packaging. The living cartilage allograft provides a chondrogenic, chondro-conductive, and chondro-inductive milieu. These immature chondrocytes are metabolically highly active and capable of spontaneous matrix formation, but do not stimulate an immune response. This is theorized to allow greater production of hyaline-like cartilage as opposed to fibrocartilage. In addition to the potential regenerative benefits, DeNovo engineered tissue avoids donor site morbidity, has the potential for greater cost efficiency, and is a potential single-step procedure. The authors describe a surgical technique, with supporting biochemical composition data, and review preliminary Food and Drug Administration Phase I/II data on the safety and efficacy of this new cartilage repair modality.

Oper Tech Sports Med 21:95-99 © 2013 Elsevier Inc. All rights reserved.

KEYWORDS cartilage repair, cartilage restoration, DeNovo ET, juvenile allograft cartilage

Articular cartilage injury and repair has long been an area of therapeutic challenge.\(^1\)\(^-\)\(^3\) Owing to a limited ability for articular cartilage repair or self-regeneration, injury to articular cartilage elicits a blunted or absent healing response. If there is a response, the tissue generated is fibrocartilaginous scar.\(^2\)\(^-\)\(^4\)\(^,\)\(^5\) The inferior biologic and mechanical properties of this resultant fibrocartilage construct may lead to suboptimal function and gradual degeneration with secondary onset of pain and disability.

A spectrum of therapeutic approaches exists to promote the regeneration and healing of injured articular cartilage. Strategies range from conservative, to reparative approaches stimulating fibrocartilage formation, to the recent application of tissue-engineering and regenerative-based techniques.\(^6\)\(^,\)\(^7\)

One approach is partially forming immature hyaline-like cartilage in the laboratory followed by maturation of the construct in the patient’s knee. Current subsets of this technique include autologous chondrocytes grown on a membrane (MACI by Sanofi, Bridgewater, NJ; NeoCart by Histogenics, Waltham, MA; HYAFF Hyalograft C by Fidia Farmaceutici, Abano Terme, Italy) and allograft chondrocytes derived from juvenile donors that generate a neocartilage containing cells and sufficient self-generated matrix to allow handling and implantation. This latter construct is the subject of this review: DeNovo engineered tissue (ET) (ISTO Technologies, St Louis MO). The authors describe a surgical technique and review preliminary Food and Drug Administration Phase I/II data on the safety and efficacy of this new cartilage repair modality.

DeNovo ET

Overview

DeNovo ET graft is a novel cellular therapy currently under Phase III investigation for cartilage regeneration with Food and Drug Administration approval and oversight. As such, it
is not available for clinical use outside auspices of the clinical trial. DeNovo ET (initially referred to as NeoCartilage Implant but changed to avoid confusion with the Histogenics product, NeoCart) and more recently renamed RevaFlex, is an in vitro-grown 3-dimensional hyaline-like cartilage tissue containing viable cultured juvenile allograft chondrocytes. Qualitatively, the characteristics of the cultured implant matrix approach that of human articular cartilage from which the chondrocytes are isolated. This matrix may provide a chondrogenic, chondro-conductive and chondro-inductive milieu. The matrix components include sulfated glycosaminoglycan; type II, IX, and XI collagens; aggrecan; decorin; and water.

Juvenile allograft cartilage is utilized as the immature chondrocytes are metabolically highly active (compared with adult cartilage), capable of spontaneous matrix formation and do not stimulate an immune response. Compared with older autograft chondrocytes that appear fibrocytic when in culture, the juvenile cells retain their native phenotype post-expansion to resume production of type II collagen and aggrecan. This may allow greater production of hyaline-like cartilage in vivo as opposed to fibrocartilage. In addition to the potential regenerative benefits, DeNovo ET avoids donor site morbidity, has the potential for greater cost efficiency, and is a potential single-step surgical procedure.

Surgical Technique
DeNovo ET has the potential to allow for a single-step surgical procedure if the lesion size and grade can be accurately predicted by magnetic resonance imaging (MRI) or prior recent arthroscopic surgery. The patient is placed on the operating table in the supine position with a tourniquet on the proximal thigh (not inflated) and patient is then prepped and draped in the usual sterile manner. A diagnostic arthroscopy is performed to identify defect size, location, and any concomitant pathology (Fig. 1). Care is taken to ensure the defect is of suitable size for this type of cartilage regeneration and has potential for defect shouldering. The Phase I pilot study required a functional meniscus, intact ligamentous status, and the absence of clinically relevant malalignment. The intended use of the DeNovo ET implant is for the treatment of up to 2 unilateral, symptomatic femoral (condyles or trochlea) articular cartilage lesions (International Cartilage Repair Society [ICRS] Grades 3 and 4) in the knee ranging from 1-5 cm² after peripheral debridement, no more than 6 cm² for 2 lesions combined, and no less than 1 cm² for at least 1 lesion as confirmed by arthroscopic evaluation at the time of treatment surgery. In the case of 2 lesions, they must be in separate compartments of the same knee.

Defect Preparation
A mini-arthrotomy is performed on the index knee, based on the location and size of the defect. The defect is then located and may be highlighted with a sterile surgical marker for documentation (Fig. 1). The defect area is then prepped with a 15-blade scalpel, followed by a sharp curette to create well-defined vertical walls at the defect perimeter (ie, shoulder). The zone of cartilage injury following debridement typically

Figure 1 Arthroscopic evaluation demonstrates an isolated 16 × 13 mm isolated trochlear defect in a 35-year-old male athlete. (Color version of figure is available online.)

Figure 2 A medial arthrotomy is performed to access and debride the trochlear defect. A scalpel and curette are used to create vertical walls and exposure of the subchondral bone without bleeding. (Color version of figure is available online.)

enlarges beyond the index size estimates with proper debridement. Care must be taken to avoid disrupting the subchondral bone (Fig. 2). If bleeding of the subchondral bone occurs, the defect is treated with a layer of fibrin glue (FG). Hemostasis must be achieved (with tourniquet deflated if used initially) prior to implantation of the DeNovo ET graft. Frequent saline irrigation is helpful to prevent cartilage desiccation and debris removal, and is avoided after graft placement.

Defect Sizing
Each DeNovo ET packet consists of a 22-24-mm diameter circular graft. A foil defect-sizing template is useful to outline the perimeter of the defect (Fig. 3). Defect depth is measured directly as the implant must inset relative to the surrounding defect margins. Alternatively, one may use a sterile glove or suture packaging. Next, the DeNovo ET graft is customized to...
the dimensions of the defect template using a Metzenbaum or tenotomy scissors (Fig. 4).

**Graft Fixation**

A thin, fresh layer of FG is placed within the defect. We then place the graft in the fresh fibrin-covered defect using pick-ups. The implant should be slightly recessed relative to surrounding native cartilage (0.5 mm) (Fig. 5). Once the implant is in place, we wait 5 minutes for the FG to fully cure. One can test implant stability by ranging the knee through full motion.

**Wound Closure**

The incision is closed in standard layers. Drains are not used so as to avoid disrupting the graft.

**Postoperative Management**

For tibiofemoral lesions, patients should typically undergo a 4-6-week period of protected or nonweight-bearing with continuous passive motion and rehabilitative exercises performed during this period. Flexion is limited for patellar-trochlear defects during this time frame, but ambulation in extension is allowed as tolerated.

**Preliminary Results**

DeNovo ET is a novel tissue-engineered articular therapy currently under study but demonstrating early positive results. A Phase I/II, prospective, open label, nonrandomized single-arm clinical study for DeNovo ET was initiated in November 2006. As part of the study, 12 subjects with ICRS Grades 3-4 articular cartilage knee lesions of the femoral condyle or trochlear groove were treated with the DeNovo ET implant. Clinical efficacy was assessed via patient-reported outcome measures, MRI and elective second-look arthroscopy with biopsy. Subject outcome measures showed, on average, improvement in all scores from baseline to 12 months.

A total of 9 knees were evaluated at 12 months with a second-look arthroscopy and biopsy to visually grade the DeNovo ET repair. In 6 of 9 (66.7%), cartilage repairs were graded as grossly normal or nearly normal using ICRS post-cartilage repair grading (Fig. 6). In 7 of 9 (77.8%), the degree of lesion repair was graded as “level with surrounding cartilage” and the remaining 2 of 9 (22.2%) were graded as 75% repair of lesion depth. MRI studies correlated well with observed outcomes at second-look arthroscopy, demonstrating maturation of the implant with respect to both volume of fill and quality of repair tissue.

Biopsy specimens were also obtained from the center of the lesion during the second-look arthroscopies. Specimen sections were stained to evaluate the morphologic...
Preliminary histopathologic evidence suggests that the implant has significant intermediate-term survivability with the potential to grow into and integrate with surrounding host tissue without evoking an inflammatory response. There was no known observed immunologic response through 6 months to the implanted juvenile allograft as determined by clinical follow-up or laboratory assessment of T-cell subsets and anticollagen antibody responses.

**Discussion**

At the present time, the efficacy of DeNovo ET is being assessed as part of a multicenter adaptive Phase III randomized controlled trial, using microfracture as the comparator. The subject population selected for study participation will consist of 225 men or women between the ages of 18-60 years diagnosed with ICRS Grade 3 or 4 cartilage lesions of the distal femur. The primary goal of the Phase III investigation is to confirm the safety of allogeneic juvenile chondrocytes (Neocartilage Implant) for cartilage repair and to demonstrate superiority to the standard of care comparator treatment (microfracture) at 36 months.

Juvenile-derived chondrocytes are reported to display superior chondro-regenerative potential compared with chondrocytes derived from adult donors. In one laboratory study, juvenile-derived chondrocytes expanded through 5-15 population doublings produced statistically significant greater levels of sulfated glycosaminoglycan (S-GAG) in agarose culture relative to adult chondrocytes also taken through >5 population doublings. Another study, investigating cartilage defect repair in adult rabbits by transfer of fetal allografts reported complete integration at the graft-host junction for defects of the distal femur and less success with patellar defects, citing mechanical instability as the reason for failed repair of patellar grafts. Other investigators have reported that human juvenile chondrocytes provide inductive signals capable of promoting chondrogenic differentiation of human bone marrow–derived mesenchymal cells during in vitro coculturing, without expression and synthesis of type I collagen. Given the potent biological activity of juvenile chondrocytes, commercial attention toward harnessing their regenerative potential for in vitro production appears attractive. Currently, an improved understanding of chondrocyte biology has facilitated the development of in vitro techniques for the reproducible manufacture of living cartilage allografts (DeNovo ET) using juvenile chondrocytes. Further, comparative studies have shown that juvenile donor cells are 100-fold more active in vitro when compared with their adult counterparts. Given the evidence, the DeNovo ET construct is expected to have a higher level of plasticity with a greater potential to stimulate a healing reaction in the host and to facilitate integration with adjacent articular cartilage and underlying subchondral bone. In our limited observation, the DeNovo ET construct interaction results in significant remodeling and in the long-term may ultimately result in complete or partial replacement of the implant by host articular cartilage. This hypothesis will have to be proven through long-term prospective follow-up. In addition to the stated biologic advantages, in vivo immunogenicity studies have shown that DeNovo ET–derived juvenile chondrocytes do not promote graft rejection immune responses in unrelated recipients. This is in agreement with other published work, suggesting that the allograft cartilage does not evoke an host immunologic response and makes the application of juvenile chondrocytes particularly inviting.

The biochemical composition of the DeNovo ET implant produced from juvenile chondrocytes has been shown to be most consistent with hyaline articular cartilage. In one study, investigators reported on a model for producing hyaline-like neocartilage in vitro. Investigators showed through biochemical and microscopic characterization that tissue-engineered cartilage disks produced from juvenile chondrocytes cultured in a serum-free medium revealed collagenous matrices characteristic of hyaline articular cartilage; mature chondrocytes did not show similar hyaline generative potential.

DeNovo natural tissue (NT) graft is a comparable treatment option for focal articular cartilage defects in the knee. In contrast to DeNovo ET, DeNovo NT is obtained directly from a juvenile allograft donor joint and the cartilage is then aseptically minced and packaged by the tissue processor. The particulated allograft is mixed intraoperatively with FG before being implanted in the recipient’s prepared articular lesion. DeNovo NT is subject to laws regulating human cells, tissue and cellular tissue-based products as defined per Food and Drug Administration Code of Federal Regulations Title 21 Part 1271, and as such does not require premarket approval. There are currently 4 ongoing postmarket studies focused on reporting the safety and clinical efficacy of DeNovo ET.
NT for the repair of cartilage lesions of the knee and talus. Published evidence to date is limited but has shown the potential for robust hyaline-like cartilage regrowth as visualized through second-look arthroscopy, histopathologic biopsies, and clinical outcome.\textsuperscript{16–20} DeNovo ET may provide a technical advancement in the treatment of focal cartilage defects as it provides a contiguous implant capable of resurfacing lesions of up to 5 cm\textsuperscript{2}. Juvenile cells within the graft have been shown to produce chondro-inductive factors that may facilitate integration with surrounding host tissue.\textsuperscript{9} However, the clinical efficacy of this tissue engineered product has not been elucidated.\textsuperscript{15}

\section*{Conclusion}

We present a new technique for the repair of cartilage defects in the knee using the DeNovo ET implant. This application has the benefit of a single-step surgical solution using biologically active, immunologically inert, allograft chondrocytes within a hyaline-like tissue. Preliminary evidence suggests that DeNovo ET has the potential for reducing pain, improving function and recreating hyaline-like articular cartilage.

\section*{References}