**Abstract**

**Objective:** This clinical study was conducted to evaluate a cartilage repair procedure of transplanting particulated juvenile cartilage tissue allograft (*DeNovo® NT Natural Tissue Graft*).

**Design:** A multi-center, prospective, single arm, 25-subject clinical study was designed to evaluate clinical outcomes such as IKDC and KOOS scores, as well as extent and quality of repair with MRI and optional biopsies at various time points post-implantation (up to five years). To date, 19 patients with one or two chondral lesions on the femoral condyles and/or trochlea have been treated with this cartilage repair technique.

**Results:** The first group of nine patients has been evaluated with up to 18 months post-op follow-up. No revision has been performed to date. Significant improvements in clinical outcomes over the pre-op baseline data have been observed. MRI data at 12 months post-implantation indicated good defect filling.

**Conclusions:** The present report describes clinical data of a cartilage repair procedure of transplanting particulated juvenile cartilage tissue allograft to treat cartilage lesions of femoral condyles and trochlea. Clinical outcome data as measured at up to 18 months post-implantation and MRI data indicate early positive outcome of this technique.

**Introduction**

Articular cartilage lesions of the knee are common. While some estimate that over one million knee cartilage lesions are seen annually in the US, the number of symptomatic patients with cartilage lesions requiring surgical repair is about 250,000 annually. Currently in the US, the surgical treatment options for these symptomatic patients are debridement/lavage, marrow stimulation, osteochondral autograft transplantation, fresh osteochondral allograft and autologous chondrocyte implantation. More recently, a cartilage allograft transplantation option with particulated juvenile cartilage tissue allograft has become available. In the past, allograft transplants were limited to osteochondral grafts as graft incorporation to host tissue was possible only at the bony level. That is, the desired live articular cartilage transplant needed to remain attached to its underlying subchondral bone. It was generally accepted that cartilage graft without the subchondral bone attached would not be incorporated to either host cartilage (failure of lateral integration) or host subchondral bone (failure of basilar integration). In fact, both autologous and allogeneic osteochondral grafts do not heal at the lateral margins (i.e., no cartilage-to-cartilage integration is seen): only the basilar bone portion heals, which keeps the overlying cartilage cap in its proper position. The host “heals” to the transplant through creeping substitution of the patient’s bone into the transplanted bone. In some cases, however, it has been suggested that host immunological responses to the bony portion of the osteochondral allograft led to possible non-incorporation and necrosis of the graft bone. Bugbee reported that the greater the amount of transplant material, the more patients become “antibody positive” and that the failure rate is higher in this subset of patients. Historically, the majority of failures reported in early osteochondral transplant clinical series were related to failure of the dead bone (thicker segments of bone failed with collapse similar to avascular necrosis). Thus, the amount of bone transplanted has been progressively decreased to the point that the technique is now termed osteochondral shell transplantation with cartilage-bone composite thicknesses of only 6-8mm. The concept that cartilage could be transplanted without its underlying bony component and heal would be considered inappropriate even a few years ago by most cartilage surgeons. However, a thorough literature review reveals that a German publication reported that cartilage allograft transplantation can lead to cartilage defect healing by cutting articular cartilage into small pieces. That is, chondrocytes in cartilage pieces might be able to escape from the extracellular matrix, migrate, multiply and form new extracellular matrix (hyaline-like cartilage tissue) and integrate with surrounding host tissues.
There was also a study where autologous osteochondral graft was turned into paste and the resulting material was implanted to repair cartilage defect, which was concomitantly treated with microfracture. Animal studies showed that the combination of bone and cartilage in a paste formed bone and cartilage while cartilage pieces alone formed cartilage. Furthermore, animal studies in the SCID mouse, goat and horse models showed that particulated adult cartilage grafts can remodel to form a continuous volume of repair tissue. Independent studies showed that new extracellular matrix can be formed between two adjacent juvenile cartilage cubes in an explants culture study (Fig. 1) and particulated juvenile cartilage grafts healed chondral defects on trochlea areas of the horse knee joints (Fig. 2).

In parallel with this particulated cartilage research, there have been studies evaluating the effect of age on cartilage healing. These studies demonstrated that injured immature cartilage can heal spontaneously while injured adult cartilage cannot heal. Furthermore, marked differences in biological responses between juvenile and adult cartilage have been found, which suggest that transplantation of juvenile chondrocytes could potentially lead to improved cartilage defect healing:

- Immature articular cartilage tissue has a significantly higher cell density than mature articular cartilage.
- Immature chondrocytes are superior at producing extracellular matrix than mature chondrocytes as measured by the rate of production of sulfated glycosaminoglycan (S-GAG).
- Immature chondrocytes show significantly greater mRNA levels for Type II and Type IX collagen.

The present paper will detail the results to date of an IRB approved prospective study of particulated juvenile cartilage allograft (DeNovo NT Natural Tissue Graft) (Zimmer, Inc., Warsaw, IN/ISTO Technologies, Inc., St. Louis, MO) transplanted in human knees, with a subset of patients currently at 18 months after surgery. The objective of this clinical study was to evaluate clinical performance of this cartilage repair technique.

**Methods**

The prospective case series investigation was IRB approved. Symptomatic patients over the age of 18 with one or two contained focal chondral lesions involving the medial femoral condyle, the lateral femoral condyle or the trochlea were eligible to participate. In addition, the study participants needed to have near normal alignment (varus or valgus 5 degrees or less from neutral mechanical axis), functional menisci and a stable knee. Clinically qualified patients were consented for participation in the study and final satisfaction of the inclusion/exclusion criteria was confirmed at arthroscopy before the patient was implanted with DeNovo NT graft. The key inclusion and exclusion criteria are listed on the following page.
Inclusion Criteria:

• Voluntary signature of the IRB approved Informed Consent,
• Male or female subjects between the ages of 18 to 55 years,
• Pretreatment arthroscopic confirmation indicating one or two contained lesion(s) are equal to an ICRS Grade 3a, 3b, 3c, 3d of the femoral condyle or trochlear groove and OCD lesions (Grade 4a) with healed bone base, which is non-sclerotic and no loss of bone greater than 6mm measured from the surrounding subchondral plate,
• Has peripheral cartilage debridement to healthy cartilage that results in a lesion(s) with an area of greater than or equal to 1 cm² and less than or equal to 5 cm²,
• PCL, LCL and MCL in the affected knee are stable and the ACL is stable or can be stabilized as a concomitant procedure,
• Ipsilateral knee compartment has intact menisci (or requires partial meniscectomy resulting in stable menisci).

Exclusion Criteria:

• Clinical and/or radiographic disease diagnosis of the indexed affected joint that includes:
  • Osteoarthritis or avascular necrosis,
  • Rheumatoid arthritis, or history of septic or reactive arthritis,
  • Gout or a history of gout or pseudogout in the affected knee,
  • Osteochondritis dissecans of the knee with significant bone loss (greater than 6mm deep from the subchondral plate),
• Associated damage to the underlying subchondral bone requiring an osteochondral graft,
• Has HIV or other immunodeficient state including subjects on immunosuppressant therapies, or has significant illness (metastasis of any type) that decreases the probability of survival to the two year endpoint,
• Body Mass Index >35 (BMI=kg/m²),
• Has bipolar articular cartilage involvement or kissing lesions of the ipsilateral compartment, described as tibial or patellar lesions in the same compartment with greater than ICRS Grade 2 chondrosis,
• Active joint infection,
• Prior total menisectomy of either knee,
• Radiographically has >5 degrees of malalignment as measured from the hip, knee and ankle mechanical axis,
• Has received, within the past three months intra-articular hyaluronic acid therapy or cortisone injections in the index knee,
• Prior realignment surgery in the affected knee within the past 6 months,
• Failed microfracture treatment performed less than 12-months before baseline.

Preoperatively and sequentially post-op, enrolled patients completed patient reported outcome forms (PROs). In addition, the preoperative radiographs and MRIs were compared to those at different follow-up time points. Histology evaluation was planned for any elective biopsies obtained at the 12 month post-op time point.
Surgical Technique

After confirmatory arthroscopy, a limited medial or lateral arthrotomy was performed to fully visualize the lesion(s). The defect was outlined with a scalpel to create a shoulder (vertical peripheral wall) of normal or nearly normal host articular cartilage. The cartilage within the outlined area was removed carefully with a curette to the vertical wall of the host cartilage shoulder and the base of the defect. The base was cleared of all cartilage tissue including the calcified layer without entering into the subchondral bone. No marrow stimulation procedure was performed. Hemostasis, without a tourniquet, was achieved with epinephrine soaked cottonoids and fibrin glue. After measuring the defect dimensions and recording the visual findings with photographs, a thin aluminum sterile foil was pressed into the defect to create a three-dimensional mold, as a complete replica of the defect. Once formed, the foil mold was removed from the defect and placed on the back table of the operating room. Using the measured defect dimensions, the defect surface area was calculated. One package of DeNovo NT Graft was used for each 2.5 cm² defect. Larger defects require proportionally more packages of DeNovo NT Graft.

The DeNovo NT Graft, in a specially formulated nutrient preservation medium, was shipped in an aseptic temperature-controlled packaging. The medium was aspirated and the particulated cartilage pieces were transferred to the foil mold and distributed approximately 1 to 2 mm apart (potentially less separation depending upon the ratio between the implanted tissue volume and the surface area of the defect). Fibrin glued was then added to the cartilage pieces until the foil mold was filled to within approximately 1 mm of its full depth. The glue was allowed to cure (typically 3-10 minutes). At that point, the fibrin glue/cartilage tissue construct was gently separated and then lifted from the foil in one piece. Fresh fibrin glue was applied at the base of the patient’s cartilage lesion and the fibrin glue/cartilage construct was pressed into the defect and the glue allowed to cure. The fibrin glue cartilage tissue construct was thinner (average 1 mm) than the surrounding cartilage shoulders (average 2 to 3 mm), which would minimize the potential for shear or direct compressive load. The patients underwent a standard post operative protocol that mimicked the Steadman microfracture protocol (Fig. 3).

Fig 3. Surgical technique
Results

To date, 19 patients have been enrolled at 3 study sites, nine of which have been followed-up for a minimum of three months. Three, four and two of these nine patients have reached 18, 12 and three months post-op milestones, respectively. In this report, we will present the clinical data on these nine patients only (36±10 years old; 28±4 lb/in² body mass index). Of these nine patients, seven had non-traumatic cartilage lesions and two had traumatic cartilage injuries. The average lesion size was: 2.45±0.91 cm² (ranging from 1.2 to 4.62 cm²). Three subjects had defects on both femoral condyles and trochlea with total defect areas averaging 5.69±2.88 cm² (ranging from 3.4 to 7.47 cm²).

Clinical outcome scores for the seven patients who have reached the 12-month post-implantation follow-up milestone are shown in Fig. 4. Paired-t tests showed that, at 12 months post-implantation:

- The improvements in clinical outcome measured were statistically significant for KOOS Symptoms (p=0.01) and KOOS QoL (Quality of Life, p=0.04) and marginally significant for KOOS ADL (p=0.05), KOOS Pain (p=0.07), KOOS Sports and Recreation (p=0.07) and IKDC Subjective Evaluation (p=0.06).

- When Subject #7 (who had subchondral bone edema pre-op) was excluded from the analysis, six subjects have reached the 12 months follow-up milestone. The improvements in all clinical outcome measures with the exception of KOOS Sports/Recreation Subscore for these six subjects were statistically significant (p<0.05).

At 18 months post-implantation, KOOS data were not collected. IKDC Subjective Evaluation scores were collected. As there were only three patients who have reached 18 months follow-up milestone, statistical analyses are not meaningful. However, when mean and standard deviation values were plotted against follow-up time, there was a clear trend that IKDC Subjective Evaluation Score improved steadily across the 18 months follow-up period (Fig. 5).
Discussion

The initial data from the first nine patients indicate that this cartilage repair technique is capable of providing patients with improved clinical outcomes and good defect filling. Benefits of this technique include:

1. When repairing a chondral defect, there is no longer the need to surgically create an osteochondral lesion (necessary for the fresh osteochondral allograft procedure). Consequently, the biological challenge of transplant bone remodelling/incorporation to host bone can be overcome;
2. The donor site morbidity associated with osteochondral autograft transplantation is eliminated;
3. As this technique does not require the violation of the subchondral bone (necessary for marrow stimulation techniques such as microfracture), it truly represents a “burn no bridge” approach, as marrow stimulation has been shown to compromise subsequent revision surgeries;\(^{18}\)
4. The transplantation of juvenile cartilage tissue allograft is an off-the-shelf, single stage procedure, thus avoiding the need for two-stage procedures such as autologous chondrocyte implantation;
5. The fibrin-fixation surgical technique eliminates any potential hypertrophic response to a periosteal flap fixation technique often associated with autologous chondrocyte implantation. The present study provides original clinical data on this promising cartilage repair technique.

Conclusions

Patients experienced significant improvement from baseline for parameters of pain and function as demonstrated by IKDC, KOOS and VAS clinical outcome measures. MRI studies suggest good defect filling. There were no complications, no evidence of graft rejection and no reoperations to date. The outcomes suggest that particulated juvenile allograft is capable of filling chondral defects and improving symptoms, understanding the limitations of a case series without a control arm.

References
