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TUNNEL WIDENING OF ACL RECONSTRUCTION AUGMENTED BY AN PLATELET RICH OSTEOCONDUCTIVE-OSTEOINDUCTIVE ALLOGRAFT COMPOUND: A RANDOMIZED BLIND-ANALYSIS PILOT STUDY

Ruth Solomon\textsuperscript{1}, Jan Pieter Hommen\textsuperscript{2}, Francesco Travascio\textsuperscript{1,3,4,5*}

\textsuperscript{1}Department of Industrial Engineering, University of Miami, Coral Gables, FL
\textsuperscript{2}Department of Orthopedics, Florida International University, Miami, FL
\textsuperscript{3}Department of Mechanical and Aerospace Engineering, University of Miami, Coral Gables, FL
\textsuperscript{4}Department of Orthopaedic Surgery, University of Miami, Miami, FL
\textsuperscript{5}Max Biedermann Institute for Biomechanics at Mount Sinai Medical Center, Miami Beach, FL

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*Corresponding author:

Dr. Francesco Travascio, PhD
Associate Professor
College of Engineering
University of Miami
1251 Memorial Drive, MEB 276
Coral Gables, FL  33146
USA
Telephone: +1-(305)-284-2371
Fax: +1-(305)-284-4040
f.travascio@miami.edu

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ABSTRACT

Background: The anterior cruciate ligament (ACL) is a commonly injured ligament in the knee. Bone tunnel widening is a known phenomenon after soft-tissue ACL reconstruction and etiology and clinical relevance have not been fully elucidated. Osteoconductive compounds are biomaterials providing an appropriate scaffold for bone formation such as demineralized bone matrix. Osteoinductive materials contain growth factors stimulating bone lineage cells and bone growth. A possible application of osteoinductive/osteoconductive (OIC) material is in ACL surgery.

Questions/Purposes: We hypothesized that OIC placed in ACL bone tunnels: 1) reduces tunnel widening, 2) improves graft maturation and 3) reduces tunnel ganglion cyst formation. To test this hypothesis, this study evaluated the osteogenic effects of demineralized bone matrix (DBM) and platelet rich plasma (PRP) on tunnel widening, graft maturation, and ganglion cyst formation.

Study Design: Randomized controlled clinical trial pilot study.

Methods: A total of 26 patients electing ACL reconstruction surgery were randomized between OIC and control group. Measurements of tunnel expansion and graft-tunnel incorporation were conducted via quantitative image analysis of MRI scans performed at six months after surgery for both groups.

Results: No patients had adverse post-operative reactions or infections. The use of OIC significantly reduced tunnel widening (p < 0.05) and improved graft maturation (p < 0.05). Patients treated with OIC presented with a significantly lower prevalence of ganglion cyst compared to the control group (p < 0.05).

Conclusion: The use of OIC has measurable effects on the reduction of tunnel widening, improved graft maturation and decreased size of ganglion cyst after ACL reconstruction.

Clinical Relevance: This study explored the utilization of biologics to minimize bone tunnel widening in ACL reconstruction surgery.
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1. INTRODUCTION

Anterior cruciate ligament (ACL) reconstruction surgery is a standard treatment with more than 100,000 procedures performed annually in the United States [2, 4, 16, 20, 34]. The surgery aims to restore knee stability and improve the patient’s quality of life. Historically, the success rate of ACL surgery in returning patients to their previous level of sports activity ranges between 75-90% [17]. However, the process of rehabilitation after ACL injury can last for several months or even years, and represents a significant psychological and economic burden for the patient [42].

Bone tunnel widening is a known phenomenon after ACL reconstruction and has a significant correlation with the utilization of all-soft tissue grafts [5]. The largest percentage of tunnel widening occurs during the first six weeks after surgery and can continue over two years after surgery [32]. The incidence of tunnel widening ranges from 25 to 100% and 29 to 100% in femoral and tibial tunnels respectively [9, 14, 28, 33]. Graft fixation implants including cortical fixation devices and bioabsorbable interference screws have been correlated with increased tunnel widening [8]. The etiology of widening is multifactorial, including mechanical factors (e.g., tunnel mal-positioning, graft motion wind-shield wiper effect, longitudinal elongation bungee-cording etc.), bone necrosis from the drilling technique, early rehabilitation, cytokine induced bone resorption, and synovial fluid ingress into the bone tunnels [5, 8]. Although the correlation of tunnel widening and clinical outcomes remains unclear, significant widening of the tunnels may complicate revision surgery [23].

Osteoinductive/osteoconductive compounds (OIC) are biomaterials characterized by bioactive properties: they provide an appropriate scaffold for bone formation (osteoconductivity) and are able to bind and concentrate endogenous bone morphogenetic proteins (BMP) (osteoinductivity) in circulation, thus promoting osteogenesis [19]. The use of demineralized bone matrix (DBM) has gained popularity in ACL reconstruction. DBM is a type I collagen matrix of allograft bone that remains after extensive
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processing of removing blood, cells, and minerals. The final result is a small particulate which can be applied to form a three-dimensional scaffold (osteconductive) with osteogenic growth factors like BMPs, a family of transforming growth factor-β (TGF-β). Moreover, autologous platelet-rich plasma (PRP) is a concentrated solution of a patient’s own platelets (or thrombocytes). By centrifuging the patient’s whole blood, the red blood cells can be separated from the serum containing the concentrated platelets. Activated platelets have demonstrated osteoinductive properties on mesenchymal stem cells [43]. A possible application of DBM and PRP is to facilitate the osseointegration of the ACL graft into the bone tunnels to minimize tunnel widening and improve the structural stability of the graft construct. This in turn may allow the knee joint to adapt to bearing physiological mechanical loads sooner, with ultimately faster recovery of the patient.

As an initial step to validate our research hypothesis, this pilot study aimed to observe the osteogenic effects of DBM and PRP on tunnel widening, graft maturation, and tunnel ganglion cyst formation relative to control groups.

2. METHODS

2.1 Patients allocation: Twenty-seven patients (twenty-eight knees) were prospectively enrolled in a randomized controlled trial from 2016 to 2019. Patients were randomized to the OIC group after electing to have surgery. There were a total of 13 patients in the OIC group and 14 in the control group. There were 14 males and 12 females with an average age of 31 y.o. One patient declined to participate to the study after undergoing treatment, and four others were lost to follow-up MRI. The Reporting Trials flow diagram is shown in figure 1. The demographic data of all participants are summarized in Table 1. The experimental protocol was approved by the Institutional Review Board at the University of Miami. All subjects provided written consent prior to participation in the study. All the surgical
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procedures were performed by JPH. Data analysis was conducted at the University of Miami and the Hommen Orthopedic Institute.

2.2 Inclusion and Exclusion Criteria: Inclusion criterion was indication for primary anterior cruciate ligament reconstruction with all-soft tissue allografts fashioned into a GraftLink® (Arthrex, Inc., Naples, FL) construct with cortical button fixation on the tibia and femur. Exclusion criteria were revision surgery, multi-ligament surgery, autografts, and patients under 18 years of age. Excluded patients were: 32 autografts, 9 ACL repairs, 7 revisions, 5 multi-ligaments, and 19 with interference screw fixation. Patients with concomitant procedures were included: 14 with meniscus repair (9 OIC, 5 control), 4 with partial meniscectomy (1 OIC, 3 control), and 3 with chondroplasty (0 OIC, 3 control).

2.3 Surgical Procedures: Patients randomized to the OIC group had blood drawn in the pre-op holding area, which was subsequently prepared utilizing the Arthrex ACP® system to yield platelet rich plasma (PRP). The PRP was mixed with 5 ml of StimuBlast® (Arthrex, Inc., Naples, FL). The graft was constructed using an allograft peroneus longus tendon with an ACL Tightrope® button (Arthrex, Inc., Naples, FL) at each end. The femoral and tibial tunnels were reamed to match the graft size. We utilized a low-profile reamer through a medial portal to create the femoral tunnel and a FlipCutter® (Arthrex, Inc., Naples, FL) for the tibia tunnel. Prior to docking the graft into the femoral and tibial sockets in the OIC group, 1 to 2 ml of mixed PRP/DBM was injected via a syringe into each tunnel with the arthroscopic irrigation turned off to avoid extravasation. The graft was then secured into the sockets utilizing the tension-slide button mechanism. Additional PRP/DBM was injected into both tunnels at the graft-bone interface after securing the graft. Concomitant meniscus pathology was addressed by meniscectomy or repaired utilizing the surgeon’s choice of an all-inside, inside-out, or outside-in technique. Chondral injuries were addressed with chondroplasty. A hinged knee brace was
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placed postoperatively for three weeks for isolated ACL reconstructions and six weeks for concomitant meniscus repairs. Immediate weight bearing as tolerated versus partial foot flat weight bearing was allowed for isolated ACL reconstructions and meniscus repairs, respectively.

2.4 MRI scanning protocol: The intra-articular graft, tunnels, and graft-tunnel interface were assessed using a 1.5T MR System (Optima MR430s; GE Healthcare) with sequences in the sagittal and coronal planes as well as axial cuts perpendicular to the femoral and tibia tunnels. A summary of the parameters used in the MRI scanning protocol is reported in Table 2.

2.5 Measurement of tunnel widening: All measurements were conducted by a researcher blinded to treatment allocation prior to statistical analysis. Measurements of both tunnel expansion and graft maturation were conducted via quantitative image analysis of MRI scans performed at six months after surgery for both groups. Measurements of the tibial and femoral tunnel widening diameters were conducted using an established approach [6]. The femoral tunnel and tibial tunnels were measured at four points: the aperture, midpoint, tunnel end, and at the greatest tunnel diameter by taking the measurement of the diameter perpendicular to the tunnel, see Figure 2A-F. The tunnel expansion was calculated in the femur and tibia by subtraction of the initial surgical tunnel diameters from the largest measurement taken in each respective region. The quantity of patients with moderate (5mm) and large (10mm+) tunnel expansion was also noted for each group.

2.6 Measurement of graft maturation: Graft maturation was quantified using a previously reported approach to evaluate the signal/noise quotient (SNQ) by taking the average intensity across three areas of the ACL graft: the proximal, central, and distal intra-articular regions [11, 22, 37], see Figure 3. The measurement of SNQ was carried out as follows: in correspondence of each region where the measurement was performed, a circular region of interest of 5-mm diameter was defined and the signal intensity from the MRI scan was measured and averaged across the region (S_{ROI}). In a similar manner,
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the signal intensity ($S_{PCL}$) was also measured in the intact PCL with the purpose of normalizing the signal intensities measured in the ACL graft. To eliminate background noise, the signal intensity ($S_{BACK}$) was also measured at a background region of interest 2 cm anterior to the patellar tendon. The SNQ was then calculated using the following equation [39]:

$$SNQ = \frac{S_{ROI} - S_{PCL}}{S_{BACK}}$$ (1)

2.7 Tunnel ganglion cyst formation: Additionally, patients with tunnel ganglion cysts were quantified for each group by the blinded radiologist. Post-surgical graft failures were also assessed.

2.8 Statistical Analysis: All the statistical analyses were conducted in Minitab® 19.2 (Minitab.LLC, State College, PA). A Mann-Whitney test was utilized to assess differences in tunnel expansion associated with OIC and Control groups in both femur and tibia tunnels. A similar approach was used to infer possible differences in tunnel diameter measurements between the femur and tibia. A quantifiable difference in proportion of ganglion cyst formation between the OIC and Control groups was determined and compared via a Chi-Squared test with Yates correction. Differences in measurements of SNQ were also investigated via Mann-Whitney test to compare OIC to Control groups within the intra-articular region. For each test conducted, a level of significance of 0.05 ($\alpha = 0.05$) was used. All the data were reported in terms of mean ± standard deviation.

3. RESULTS

No patients had adverse post-operative reactions or infections. One patient in the OIC group suffered graft failure 3 months postoperative after falling on a boat. No other participants of the study presented with graft failure.

3.1 Tunnel Widening.
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A summary of the measurements of average tunnel expansion are reported in Figure 4. A summary of the tunnel expansion and their categorical sizes are shown in Tables 3 and 4. A significant difference was determined in femoral tunnel expansion (P=0.00736) between the OIC (1.00 ± 0.64 mm) vs. Control group (2.46 ± 1.44 mm). Additionally, a significant difference in tibial tunnel expansion (P=0.0001) was found between the OIC (1.08 ± 0.63 mm) and Control group (4.13 ± 2.32 mm). For the Control group, the tibial tunnel expansion (4.13 ± 2.32 mm) was found to be larger (P=0.046) than the femoral tunnel expansion (2.46 ± 1.47 mm). However, no significant difference was found between the femoral and tibial expansion of the OIC group (P>0.05).

3.2 Graft maturation.

A 1-tailed Mann Whitney test showed a decreased SNQ in OIC group (2.28 ± 1.97 mm) when compared to the Control group (4.28 ± 2.55 mm) (P=0.04363).

3.3 Tunnel ganglion cyst formation.

Two of the ten OIC patients (20%) and eight of the eleven Control patients (72.72%) presented with ganglion cysts in the tibial tunnel. The difference between these proportions was statistically significant (P=0.048).

4. DISCUSSION

Injury at the ACL may represent a significant psychological and economic burden to patients, given the lengthy recovery process [42]. There is significant research aimed at increasing the rate of graft-to-tunnel incorporation and graft maturation [3, 38] as well as reducing the recovery time after surgery via innovative rehabilitation modalities [17]. Since OIC materials have been heralded as osteogenic [19], we hypothesized that the injection of these materials into the bone tunnel would enhance graft incorporation and graft maturation. Aimed at testing this hypothesis, we compared the use of DBM...
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combined with PRP in soft-tissue graft reconstructions to a control group, and monitored the post-
operative events of tunnel widening, graft maturation and tunnel ganglion cyst formation through a
quantitative analysis of MRI images. We adopted StimuBlast®, a DBM, due to its osteoconductive and
inductive properties. The DBM is manufactured with a reverse phase medium that allows it to be fluid-
phased during handling and more viscous at body temperature. The DBM putty was augmented with
anabolic properties of ACP®, a leukocyte-poor PRP, to promote the healing response during the phases
of inflammation, cellular proliferation, and subsequent tissue remodeling. Unlike leukocyte-rich PRP,
this does not induce the potential catabolic cytokines such as interleukin-1β, tumor necrosis factor-α,
and metalloproteinases involved in the inhibition of bone-tunnel osteointegration [36]. The intra-
operative application of DBM/PRP is relatively simple for the surgeon and assistants. The mixture is
easily mixed and can be readily injected from a syringe through a curved needle applicator into the
tunnels. Once placed, the mixture is viscous enough to remain in the tunnels and becomes more viscous
at body temperature.

Tunnel widening has several implications after ACL surgery including the possibility of
postoperative tibia stress fractures [7, 24, 35] and delays or failures of graft incorporation [31]. In the
setting of revision surgery, significant tunnel widening may require a bone grafting procedure followed
by subsequently staged ACL revision surgery [23, 32]. The presence of ganglion cysts within the tunnels
has been associated with possible incomplete graft-bone incorporation [10, 15]. Tunnel widening and
ganglion cysts formation may represent a localized osteolytic process due to a cell-mediated cytokine
response [44]. Our results indicate that the use of DBM and PRP: 1) significantly reduced tunnel
widening, 2) had a positive effect on graft maturation, and 3) reduced presence of ganglion cyst
formation within the tibial tunnel.
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It should be noted that the present contribution is a pilot study, and its findings do not allow establishing causal relationships among the DBM/PRP injected and the postoperative outcomes. Nevertheless, we postulate that the use of DBM/PRP may have enhanced the organization of the fibrocartilage insertion and mineralization, as well as reduced the cell-mediated osteolysis at the graft-bone interface. In animal studies, the use of DBM has been shown to create a 4-zone fibrocartilage tendon-to-bone healing [12]. In a rabbit model, Anderson et al. wrapped a BMP and TGF-β soaked collagen sponge around the graft inside the tunnel. Histologically, they observed more consistent bone-to-graft apposition and a fibrocartilaginous interface relative to controls at 2, 4 and 8 weeks. Biomechanically, the grafts also demonstrated significantly increased ultimate tensile strength at 2 and 8 weeks [1]. It has been proposed that synovial cytokines, such as TNF-α, interleukin 1β, IL-6, BMPs, and nitric oxide may mediate ACL tunnel widening [44]. DBM/PRP may have served as a grout, limiting the ingress of synovial fluid into at the interface between the graft and tunnel wall. The use of DBM/PRP may also have improved the location of graft fixation within the tunnel. In the control group, the graft relied solely on cortical suspensory fixation which may have led to increased graft motion due windshield wiper and bungee cord effects [30]. Instead, in the treatment group, the grafts were “potted” into a DBM/PRP putty which may have resulted in more aperture fixation and less micromotion. If DBM/PRP can enhance osteointegration of the graft within the tunnels, this may lead to earlier graft “ligamentization”, or maturation, due to increased revascularization of the implanted graft, as may have been demonstrated by the MRI signal intensity findings in our study [25, 39].

Several studies have investigated the effects of injectable OICs in tendon-to-bone healing. Animal studies conducted on rats [40], dogs [13], rabbits [18, 41] and goats [27] have found accelerated graft-to-bone healing rates with calcium phosphate. In fact, Ma et al. demonstrated that calcium phosphate could deliver increased local BMPs in tendon-to-bone healing [21]. Accordingly, in a
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randomized controlled human trial, Mutsuzaki et al. used calcium phosphate on 64 patients with follow-ups at 1 and 2 years [26]. At 1 year, tunnel expansion in patients treated with calcium phosphate was reduced by approximately 7% when compared to the control group. The use of calcium phosphate remains controversial due to its potentially slow degradation rate, which may slow osteointegration [29].

As a pilot study, we used a convenience sample of 26 patients performed by a single surgeon. A multicenter study, including a larger sample size and more surgeons will be needed to confirm the preliminary evidence reported in this contribution. Another limitation of this study is the short observation period of less than 1 year as a longer-term analysis may show larger differences in graft incorporation and maturation between OIC and Control groups.

In conclusion, an in vivo study was conducted to evaluate the effects of an injectable OIC compound on the graft incorporation and tunnel widening after ACL reconstructive surgery. The results of this investigation indicate that the injection of OIC has measurable effects on the reduction of tunnel widening and ganglion formation and enhanced graft maturation after ACL reconstruction. A comparison of our findings to those of previous studies suggests that the benefits of augmenting ACL reconstruction with the injection of OIC may be observed within one year after surgery. The use of osteogenic compounds may enhance ACL reconstruction graft incorporation and thereby promote earlier rehabilitation and return to sports.
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LIST OF TABLES

Table 1. Summary of the demographic data of participants

|                        | OIC (StimuBlast® /PRP) | Control |
|------------------------|------------------------|---------|
| Male                   | 8                      | 7       |
| Female                 | 5                      | 7       |
| Age at Time of Surgery | 32.7±10.5              | 32.7 ± 12.7 y.o. |
| Age Range              | 16 to 50 y.o.          | 15 to 55 y.o.     |

Table 2. Summary of MRI scanning parameters by image type. Parameters shown are repetition time (TR), echo time (TE), inversion time (TI), echo train length (ETL), matrix size, field of view (FOV), and slice thickness (with 0 mm gaps)

| Image Type | TR/TE/TI (msec) | ETL | Matrix Size | FOV (mm) | Slice thickness (mm) |
|------------|-----------------|-----|-------------|----------|---------------------|
| FSE Sagittal PD | 3200/30          | 10  | 512 × 256   | 140      | 4                   |
| FSE Sagittal STIR | 4000/36/130    | 10  | 256 × 192   | 160      | 4                   |
| FSE Sagittal (meniscus) | 1500/19         | 10  | 256 × 224   | 140      | 3                   |
| FSE Coronal PD      | 3200/30          | 10  | 512 × 256   | 120      | 4                   |
| FSE Coronal STIR    | 4000/36/130      | 10  | 256 × 192   | 140      | 4                   |
| FSE Axial PD        | 3200/30          | 10  | 512 × 256   | 140      | 4                   |
| FSE Axial PD (Additional-perpendicular to graft tunnels) | 3000/34-40 | 10  | 256 × 224   | 140      | 3                   |
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Table 3. Summary of femoral tunnel expansion by categorical size

| Expansion Size               | OIC (StimuBlast®/ACP®) | Control |
|-----------------------------|------------------------|---------|
| Minimal Expansion (<1mm)    | 5                      | 0       |
| Mild Expansion (<5mm)       | 6                      | 11      |
| Moderate Expansion (5-10mm) | 0                      | 1       |
| Large Expansion (10+ mm)    | 0                      | 0       |

Table 4. Summary of tibial tunnel expansion by categorical size

| Expansion Size               | OIC (StimuBlast®/ACP®) | Control |
|-----------------------------|------------------------|---------|
| Minimal Expansion (<1mm)    | 4                      | 0       |
| Mild Expansion (<5mm)       | 8                      | 9       |
| Moderate Expansion (5-10mm) | 0                      | 2       |
| Large Expansion (10+ mm)    | 0                      | 1       |
Effect of Platelet OIC on ACL Surgery

FIGURES LIST

**Figure 1:** The chart shows the Consolidated Standards of Reporting Trials flow diagram for this randomized controlled trial with intention to treat analysis.

**Figure 2A-F:** Measurement of tunnel expansion are shown in diagrams A-F: the femoral tunnel diameter was determined in each region of interest by taking measurement of the diameter perpendicular to the greatest diameter in the (A) sagittal (C) coronal, and (E) axial MRI images; the tibial tunnel diameter was measured in each region of interest by taking the measurement of the diameter perpendicular to the tunnel in the (B) sagittal, (D) coronal, and (F) axial MRI images.

**Figure 3:** The locations of each region of interest used for the calculation of the SNQ are shown, including $S_{PCL}(x)$, $S_{BACK}(y)$ and the proximal (a), central (b), and distal (c) intra-articular regions, $S_{ROI}$.

**Figure 4:** The figure shows the tunnel diameters measured from the post-operative MRI scans. Values for StimuBlast®/ACP® (OIC) (white) and Control (grey) groups are reported for both the femur and tibia.
Figure 4
CONSORT 2010 Flow Diagram

Enrollment
Assessed for eligibility (n=113)
- Excluded (n=87)
  - Not meeting inclusion criteria (n=72)
  - Declined to participate (n=15)
  - Other reasons (n=0)

Randomized (n=26)

Allocation
- Allocated to control (n=14)
  - Received allocated intervention (n=14)
  - Did not receive allocated intervention (n=0)
- Allocated to intervention (n=12)
  - Received allocated intervention (n=10)
  - Did not receive allocated intervention (give reasons) (n=2)

Follow-Up
- Lost to follow-up (n=0)
- Discontinued intervention (n=0)
- Lost to follow-up (refused participation) (n=1)
- Discontinued intervention (n=0)

Analysis
- Analysed (n=11)
  - Excluded from analysis (No MRI Scan) (n=3)
- Analysed (n=10)
  - Excluded from analysis (No MRI Scan) (n=2)