Soft Tissue Healing in an In Vivo ACL Allograft Model: Comparison to Autograft and Effects of Gamma Irradiation

Introduction
ACL reconstruction is one of the most frequent surgical procedures within orthopedics. Further, the use of allografts for ACL reconstruction is increasing, primarily due to decreased donor site morbidity. Common options for allograft ACL reconstruction include bone-patellar tendon (BPTB) constructs or soft-tissue grafts such as hamstring (semimembranosus, gracilis) and Achilles tendons. Allograft BPTB has demonstrated high initial fixation strength, but availability and graft tunnel length mismatch continue to be of concern. For these reasons, soft-tissue allografts have become increasingly popular. Although the safety profile of allografts is well known, an inherent risk of blood borne disease transmission continues to exist. Such risks are minimized by donor and graft screening, as well as secondary graft sterilization commonly using gamma irradiation. Initially, human tissue allografts were irradiated with 5 Mrad (50 Gy)—“high dose radiation”—which compromised the structural integrity of the graft and led to high failure rates. Newer sterilization protocols have used lower doses of irradiation, 1 to 1.5 Mrad (10 to 15 Gy), and have demonstrated efficacy against most microorganisms. To date, several basic science studies have investigated allograft healing following irradiation treatment. These studies, however, have examined allograft healing in situations with bone-to-bone interfaces (e.g., BPTB ACL allograft reconstruction). To our knowledge, there are no published reports on soft tissue ACL allograft healing (i.e., tendon-to-bone healing) following low-dose irradiation treatment. The primary aim of this study was to compare soft tissue healing in a bone tunnel using three types of ACL grafts—non-irradiated allografts, low-level (1.2 Mrad) gamma irradiated allografts, and autograft controls.

Methods
Surgery: Under an IACUC approved protocol, forty skeletally mature New Zealand White male rabbits underwent bilateral ACL reconstruction receiving semitendinous tendon grafts using a previously published surgical technique. Sixteen of the rabbits received autografts while the remaining rabbits received allografts. The allograft treated rabbits received one irradiated allograft with the contralateral leg receiving a non-irradiated allograft. Animals were not immobilized after surgery. Animals were euthanized at 2 weeks or 8 weeks post-operatively and both legs were harvested for biomechanical testing or histological evaluation.

Biomechanics: Following animal sacrifice, knees were dissected exposing the ACL graft while preserving fixation points. Using custom designed grips, each specimen was mounted on an electromechanical materials testing system (MTS Insight 5, Eden Prairie, MN). The femur and tibia were aligned at an approximate knee flexion angle of 60° in order to orient the graft vertically, in line with the test actuator. Each tendon graft was pre-loaded to 1N for 2 minutes and then preconditioned between 0 and 0.5 mm at a rate of 0.1 mm/sec for 10 cycles. A load-to-failure test was conducted at a rate of 0.1 mm/sec. The following structural properties were evaluated: linear stiffness, maximum load, and actuator displacement at maximum load. These parameters were used to statistically compare the biomechanical properties among experimental groups.

Histomorphometry: Bone tunnel samples were placed in paraformaldehyde for fixation, decalcified using 30% formic acid, and embedded in paraffin (tibial and femoral portions separately embedded). Histological sections were taken perpendicular to the bone tunnel axes approximately 10 mm from the knee capsule and stained with either Hematoxylin and Eosin or Masson’s Trichrome. Digital pictures were captured from the cross-sectional sections of the femoral and tibial tunnels. The difference in the tunnel area compared to the size of the tendon was measured using Image J (NIH) to determine the amount of new growth. Percentage of new growth was calculated normalized to the size of the tunnel.

Statistical Analysis: Statistical comparisons between all the groups were performed using a 1-way ANOVA within a time point followed by post-hoc pairwise comparisons with Tukey’s test. The p-values were compared within treatment using a two-tailed, unpaired Student’s-t test.

The statistical significance level was set to p < 0.05.

Results
There were no significant differences in the biomechanical results except at the 2 week time point where maximum load for the nonirradiated allograft group exceeded that of the autograft group (Table 1). The location of failure was significantly different between 2 weeks and 8 weeks with the 2 week grafts failing 82% of the time within the tunnel whereas the 8 week grafts only failed 90% of the time at mid-substance. Histomorphometry results revealed no significant differences between any of the groups when comparing autograft to irradiated allograft at all time points (Table 1).

Discussion
The purpose of this study was to determine whether low dose irradiation adversely affects the quality of bone-tendon healing responses of soft-tissue allografts following ACL reconstruction. We have shown that, with the exception of the surprising result of decreased maximum load for autografts at the 2 week time point, no significant differences were noted among graft types or treatments. We believe that this result was due to the autograft surgeries being performed early in the study during our learning curve of the surgical technique.

Low-dose irradiation has demonstrated efficacy in the neutralization of most microorganisms. The results of the current study suggest that there are few deleterious effects on soft tissue healing biomechanically or histologically and are consistent with a recent study which showed that time zero biomechanical properties of ovine bone-patellar tendon-bone constructs are not altered following irradiation doses of 1.5 Mrad (15 Gy). Such findings afford surgeons flexibility in choosing the proper graft and tissue bank, while preserving confidence in biosafety profiles.

References

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Table 1- Matching symbols indicate p < 0.05.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Max Load (N)</th>
<th>Disp to Max Load (mm)</th>
<th>Stiffness (N/mm)</th>
<th>% of New Growth</th>
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</thead>
<tbody>
<tr>
<td>2 Week Auto</td>
<td>18 ± 8*</td>
<td>4.9 ± 2.9</td>
<td>9.0 ± 5.2</td>
<td>44 ± 12</td>
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<tr>
<td>2 Week Irrad</td>
<td>28 ± 10</td>
<td>7.0 ± 4.8</td>
<td>12.6 ± 5.4</td>
<td>43 ± 18</td>
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<tr>
<td>2 Week Non-Irrad</td>
<td>37 ± 11*</td>
<td>5.1 ± 2.6</td>
<td>14.6 ± 6.3</td>
<td>37 ± 10</td>
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<tr>
<td>8 Week Auto</td>
<td>28 ± 18</td>
<td>2.3 ± 0.7</td>
<td>17.7 ± 15.2</td>
<td>43 ± 18</td>
</tr>
<tr>
<td>8 Week Irrad</td>
<td>29 ± 20</td>
<td>3.1 ± 1.5</td>
<td>14.9 ± 7.9</td>
<td>42 ± 14</td>
</tr>
<tr>
<td>8 Week Non-Irrad</td>
<td>31 ± 16</td>
<td>3.3 ± 2.2</td>
<td>14.3 ± 8.7</td>
<td>44 ± 21</td>
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