INCREASED INTERFACIAL STRENGTH OF TRANSPLANTED CARTILAGE IN VIVO FOLLOWING ENZYMATIC TREATMENT OF WOUND EDGES

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INTRODUCTION

Articular cartilage possesses very little intrinsic repair capacity. Therefore, one of the main problems in cartilage transplantation is poor integration of the wound interface area. One of the factors contributing to this limited integrative capacity seems to be the loss of viable chondrocytes from the wound edges [1, 2]. This prohibits any significant matrix deposition at the wound interface area, which seems to be a prerequisite for cartilage integration. We recently developed a method which increases chondrocyte density at the wound interface area using enzymatic treatment with hyaluronidase and collagenase [3]. We now present the first in vivo study using this enzymatic treatment technique on articular cartilage explants implanted subcutaneously in a nude mouse. Effect on wound edges and cartilage integration was evaluated histologically. The effect of an improved histological integration on interface bonding strength was measured using a mechanical push-out test.

MATERIALS AND METHODS

Articular cartilage samples were harvested from the metacarpophalangeal joints of 6 months old calves. Full-thickness cartilage explants of 8mm diameter were prepared with a thickness of 0.9-1.2mm. The explants were randomly divided into 2 groups. From the center of the explants 3mm cores were punched out. Specimens from group 1 (controls) were put in medium supplemented with 2% FCS and incubated at 37°C for 48hrs. Group 2 were incubated for 24hrs. in 0.1% hyaluronidase followed by 24hrs. in 10U/ml highly purified collagenase VII, both in medium with 2% FCS. After 48hrs the samples were thoroughly washed in medium and the 3mm inner-cores were reimplanted in their accompanying 8mm outer ring. Constructs were then implanted subcutaneously in nude mice. After 5 weeks the constructs were harvested. Constructs for histological analyses (n=5) were fixed in 4% phosphate-buffered formalin and embedded in paraffin. Sections were stained for HE, proteoglycans (thionin) or collagen type II (MAB, II-II-6B3). Cell-counts for surface-, middle- and deep zone were performed on HE-slides. Constructs for mechanical push-out tests (n=5 for controls and n=6 for the treated group), assessing the mechanical integrity of the cartilage matrix deposition at the wound interface area, were prepared with a thickness of 0.9-1.2mm. Before the start of the test the thickness of the sample was measured to an accuracy of 50µm using calipers. For each specimen the peak load-to-failure was used to calculate the interface stress-to-failure (load/interface area), representing interfacial strength. Statistical analyses were done using the Student’s t-test for independent samples. Values presented are average ± sem.

RESULTS

Cell counts in the integration area showed a significant increase in the number of cells in the enzyme treated group at all depths (Table 2). The interfacial tissue was almost acellular in the control-group, whereas many cells were located in the interfacial zone in the treated-group (fig.2). No differences were found in either thionin or collagen type II stains. Mechanical assessment of the cartilage interface between inner core and outer ring by push-out test showed a 58% increase in stress-to-failure from controls to enzyme-treated group (Table 1). This is still approximately 7-fold less than the values observed for intact cartilage, which has an interfacial strength of around 9 MPa in our setup (unpublished data). A typical force-displacement curve is shown in fig.1B.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Control (n=5)</th>
<th>Treated (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>1151±133</td>
<td>2316±209 *</td>
</tr>
<tr>
<td>Middle</td>
<td>866±27</td>
<td>1097±59 *</td>
</tr>
<tr>
<td>Deep</td>
<td>589±16</td>
<td>962±45 *</td>
</tr>
</tbody>
</table>

Table 1: *p<0.05

DISCUSSION

Successful and durable cartilage repair will depend on the integration between transplanted and native tissue. This study shows that the enzymatic treatment of wound edges improves chondrocyte density at the wound edges in vivo, similar to what we have shown before with cultured explants [3]. This results in a better integrated construct as shown by the increased interfacial strength in the push-out tests. These data correspond with a previous report by Obradovic et.al.[4], which indicated that interfacial strength was dependent on cell-density at the interface. This effect could be enhanced by tryptic treatment. As a result of the observed high cellularity at the wound edge the deposition of collagen fibers across the wound gap of adjacent cartilage surfaces probably increases. This cross-gap deposition of collagen would correlate with increased interfacial strength, as shown by DiMicco et.al. in integration experiments using living and dead cartilage explants [5].

Our study shows a histologically and biomechanically improved integration of transplanted cartilage with surrounding cartilage following a combination of hyaluronidase and collagenase treatment. The increased chondrocyte density and subsequent matrix deposition in the interface area are probably responsible for this effect. Further studies need to be undertaken to learn more about the mechanisms involved, such as cell-migration to the wound area and matrix deposition and to further improve mechanical interface strength to the levels of intact cartilage. Because cartilage has a relatively low metabolic activity, long-term studies will be required to judge the success of different integration-enhancing techniques against the mechanical strength of intact cartilage.


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