Viability of Cultured Human Nasal Septum Chondrocytes After Crushing

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Objective: To investigate how different degrees of crushing affect the viability of human nasal septum chondrocytes in adherent cell cultures.

Methods: Cartilage grafts were harvested from the nasal septa of 15 patients who underwent submucosal resection. Five cartilage pieces were prepared from each specimen as follows: the cartilage was left intact, slightly crushed, moderately crushed, significantly crushed, or severely crushed. Chondrocytes were isolated for trypan blue dye exclusion testing, and the numbers of viable cells were determined at 1, 2, 3, and 10 days after culturing. Comparisons were made among the groups.

Results: The day 1 viability rates for the intact, slightly crushed, moderately crushed, significantly crushed, and severely crushed cartilage preparations were 96%, 92%, 82%, 72%, and 54%, respectively. The corresponding rates on day 10 were 93%, 90%, 84%, 75%, and 68%.

Conclusions: The viability and proliferative capacity of crushed human septal cartilage depend on the degree of crushing sustained. Slightly or moderately crushed cartilage grafts show good chondrocyte viability and proliferation and are valuable for fashioning soft nasal contours, filling defects, and concealing dorsal irregularities. However, significant or severe crushing reduces chondrocyte viability and proliferation and may result in unpredictable degrees of graft volume loss.

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ing a cartilage crusher (Cottle cartilage crusher model 523900; Karl Storz GmbH & Co, Tutlingen, Germany), and 5 cartilage pieces from each patient were prepared as follows: the cartilage was (1) left intact, (2) slightly crushed, (3) moderately crushed, (4) significantly crushed, or (5) severely crushed (Figure). The different grades of crushing were defined as follows: slightly crushed, 1 moderate-force hit to soften the surface without reducing the elastic strength of the cartilage; moderately crushed, 2 moderate-force hits to soften the surface and reduce the elastic strength; significantly crushed, 3 or 4 moderate-force hits, enough to cause the graft to bend with gravity; and severely crushed, 5 or 6 forceful hits to totally destroy the integrity of the cartilage. After preparation, each piece of cartilage was placed in a separate tube containing 5 mL of sterile transfer medium and was immediately sent to the genetics laboratory.

In the genetics laboratory, each cartilage sample was first washed with RPMI 1640 medium (with 25mM HEPES and L-glutamine; Biological Industries Ltd, Kibbutz Beit Haemek, Israel) and then mechanically minced using a sterile lancet. A volume of 1.5 mL of RPMI 1640 medium, containing 2 mg/mL of type II collagenase (Gibco, Invitrogen Corp, Karlsruhe, Germany) was added to each tube, and the specimens were left overnight at 37°C. The next day, each collagenase-treated cell suspension was placed in a sterile tube containing 3 mL of RPMI 1640 medium and centrifuged at 1200 rpm for 10 minutes. After the supernatant was discarded, the pellet was homogenized with 3 mL of RPMI 1640 medium, and the specimens were left overnight at 37°C. The next day, the medium in each flask was transferred to a tube using a glass Pasteur pipette. The cells were detached from the surface of the flask by applying a trypsin-EDTA solution (0.05% trypsin and EDTA at a 1:3000 ratio, Biological Industries Ltd) and were added to the same tube. The tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded, 10 µL of trypan blue (Gibco) was added to the pellet, and the mixture was homogenized. This mixture was incubated at room temperature for 2 minutes, and living cells (those that excreted trypan blue) were added to the same tube. The tube was centrifuged at 1000 rpm for 10 minutes. After the supernatant was discarded, the pellet was homogenized with 3 mL of RPMI 1640 medium, and the specimens were left overnight at 37°C. The next day, the medium in each flask was transferred to a tube using a glass Pasteur pipette. The cells were detached from the surface of the flask by applying a trypsin-EDTA solution (0.05% trypsin and EDTA at a 1:3000 ratio, Biological Industries Ltd) and were added to the same tube. The tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded, 10 µL of trypan blue (Gibco) was added to the pellet, and the mixture was homogenized. This mixture was incubated at room temperature for 2 minutes, and living cells (those that excreted trypan blue) and dead cells were counted under a light microscope (Nikon E600; Nikon Corp, Tokyo, Japan) (Table 1).

Statistical analysis was performed using the software package SPSS for Windows version 10.0 (SPSS Inc, Chicago, Ill). A mean chondrocyte viability rate was calculated for each group at each time point studied. One-way analysis of variance (ANOVA) was used to assess differences in the viability rates among the different cartilage groups (intact and slightly, moderately, significantly, or severely crushed) at each evaluation time point (1 day, 2 days, 3 days, and 10 days) (Table 1). If this step revealed a significant difference, the Tukey test was applied to identify where differences occurred (Table 2).

RESULTS

Chondrocyte viability data for the 5 cartilage groups are summarized in Table 1. For the intact cartilage group, the mean viability rates on days 1, 2, 3, and 10 were 96%, 93%, 93%, and 93%, respectively. The corresponding rates for the slightly crushed cartilage were 92%, 88%, 86%, and 90%; those for the moderately crushed cartilage were 82%, 75%, 76%, and 84%; those for the significantly crushed cartilage were 72%, 65%, 67%, and 75%; and those for the severely crushed cartilage were 54%, 50%, 55%, and 68%, respectively.

DAY 1

One-way ANOVA revealed a significant difference in the chondrocyte viability rates among the 5 cartilage groups on day 1 (P<.001) (Table 1). The Tukey test results revealed that the day 1 viability rate for the intact cartilage group was significantly higher than the corresponding rates for all the other groups except the slightly crushed cartilage group (Table 2). The day 1 viability rate for the slightly crushed cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups. The day 1 viability rate for the severely crushed cartilage group was significantly lower than the corresponding rates for all the other groups.

DAY 2

One-way ANOVA also revealed a significant difference in the chondrocyte viability rates among the 5 cartilage groups on day 2 (P<.001) (Table 1). As observed on day 1, the Tukey test results showed that the day 2 viability rate for the intact cartilage group was significantly higher than the corresponding rates for all the other groups except the slightly crushed cartilage group (Table 2). The day 2 viability rate for the slightly crushed cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups. The day 2 viability rate for the severely crushed cartilage group was significantly lower than the corresponding rates for all the other groups except the significantly crushed cartilage group.

DAY 3

As on days 1 and 2, the 1-way ANOVA showed a significant difference in the chondrocyte viability rates among the 5 cartilage groups on day 3 (P<.001) (Table 1). The Tukey test results revealed that the day 3 viability rate for the intact cartilage group was significantly higher than the corresponding rates for all the other groups except the slightly crushed cartilage group (Table 2). The day
Table 1. Chondrocyte Viability Rates for the Cartilage Groups at the Different Time Points*  

<table>
<thead>
<tr>
<th>Day</th>
<th>Cartilage</th>
<th>Slightly Crushed</th>
<th>Moderately Crushed</th>
<th>Significantly Crushed</th>
<th>Severely Crushed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96 ± 3</td>
<td>93 ± 4</td>
<td>88 ± 7</td>
<td>86 ± 8</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>93 ± 4</td>
<td>88 ± 7</td>
<td>75 ± 12</td>
<td>76 ± 9</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>93 ± 3</td>
<td>88 ± 7</td>
<td>65 ± 17</td>
<td>67 ± 10</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>93 ± 3</td>
<td>90 ± 4</td>
<td>84 ± 4</td>
<td>75 ± 11</td>
<td>68 ± 14</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD percentages. P < .001 for all time points, 1-way analysis of variance.

Table 2. Tukey Test Results for the Cartilage Group Comparisons at the Different Time Points*  

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact vs slightly crushed</td>
<td>.89</td>
<td>.83</td>
<td>.41</td>
<td>.87</td>
</tr>
<tr>
<td>Intact vs moderately crushed</td>
<td>.01</td>
<td>.01</td>
<td>&lt;.001</td>
<td>.06</td>
</tr>
<tr>
<td>Intact vs significantly crushed</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intact vs severely crushed</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Slightly vs moderately crushed</td>
<td>.11</td>
<td>.15</td>
<td>.07</td>
<td>.39</td>
</tr>
<tr>
<td>Slightly vs significantly crushed</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Slightly vs severely crushed</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Moderately vs significantly crushed</td>
<td>.09</td>
<td>.26</td>
<td>.14</td>
<td>.048</td>
</tr>
<tr>
<td>Moderately vs severely crushed</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Significantly vs severely crushed</td>
<td>&lt;.01</td>
<td>.67</td>
<td>.03</td>
<td>.21</td>
</tr>
</tbody>
</table>

*Data are given as P values.

3 viability rate for the slightly crushed cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups. The day 3 viability rate for the severely crushed cartilage group was significantly lower than the corresponding rates for all the other groups.

**DAY 10**

One-way ANOVA on day 10 also showed a significant difference in chondrocyte viability rates among the 5 cartilage groups (P < .001) (Table 1). The Tukey test results showed that the day 10 viability rate for the intact cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups (Table 2). The day 3 viability rate for the slightly crushed cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups. The day 3 viability rate for the moderately crushed cartilage group was significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups.

**COMMENT**

Autogenous cartilage is an ideal graft material for rhinoplasty operations. However, when solid carved pieces of this material are used to conceal residual deformities, the edges of the onlay graft tend to become more noticeable as years pass, especially in patients with thin skin on the nose. Some authors have recommended the crushed cartilage graft as an option for concealing dorsal irregularities. However, there is limited information about the viability of crushed cartilage and the predictability of clinical outcome with these grafts. Moreover, the results in the literature are conflicting.

In a rabbit model of fresh crushed cartilage grafts, Ruderman et al documented a retained graft volume of 70% and a chondrocyte viability rate of approximately 70% to 90%. Based on their findings, these authors concluded that crushed cartilage could be used to attain aesthetic goals with a fair degree of predictability, and they recommended overcorrection to achieve optimal final results. In work done approximately 20 years earlier, Huising found that crushing of human cartilage led to diminished viability and partial transformation to connective tissue, slightly reducing graft volume. Like Ruderman et al, Huising recommended slight overcorrection during placement of crushed cartilage grafts. In contrast, in experimental work in rabbits by Breadon et al, all the uncushed and crushed cartilage grafts remained viable, and the authors identified crushed autogenous cartilage as the preferred material for filling small nasal-facial defects. In line with the findings of Breadon et al, a clinical evaluation in humans by Guyuron and Friedman revealed a surgical success rate of 87.3% with fresh crushed cartilage grafts. Similarly, Yilmaz et al found that crushed cartilage grafts in the rabbit model were viable after extensive crushing. In contrast to many of these findings, rabbit experiments by Verwoerd-Verhoef et al showed that crushed cartilage grafts led to extensive necrosis and that only 10% to 30% of cells survived the process, depending on the crushing technique used.

In an attempt to understand the inconsistent results with crushed cartilage grafts, a recent study investigated how different degrees of crushing affect cartilage from rabbit ears. This work revealed that the severity of crushing has an important effect on cartilage viability and the clinical predictability of autogenous cartilage grafts. Specifically, graft cartilage viability, cartilage proliferation, and predictability of clinical outcome decreased as more intense crushing was applied. The mean proportion of viable cartilage after crushing decreased as the degree of crushing increased (70% for slightly crushed, 50% for moderately crushed, 30% for significantly crushed, and 10% for severely crushed cartilage grafts), whereas the viability rate for intact cartilage grafts was 90%. This earlier work also showed that slight crushing of rabbit ear cartilage induces variable amounts of chondrocyte proliferation and metaplastic bone formation, while preserving viability. The findings suggest that the use of slightly crushed cartilage grafts in rhinoplasty might help soften the contours of the nasal dorsum and balance any graft resorption that occurs.

Autogenous septal cartilage is the first-line graft choice for rhinoplasty when this material is available. However, most of the animal studies summarized herein involved viability assessments after crushing of auricular cartilage. Lattyak et al prepared cartilage grafts from rabbit nasal septa, ears, and costal cartilage. They implanted these specimens into the nasal dorsum of rabbits and then compared resorption rates after 3 months. They documented resorption rates of 30.8%, 12.6%, and
7.6% for septal, auricular, and costal cartilage, respectively. The results suggest that cartilage obtained from different sources might have different biological characteristics. It is logical to assume that the properties of human cartilage probably differ from those of animal cartilage. However, except for one study, the published experimental studies on cartilage crushing have investigated the viability of animal cartilage only.

Similar to the present study, in research published in 1994, Bujia examined the viability of crushed human septal cartilage using cell cultures. The author documented viability rates of 94% for intact cartilage, 30% for mildly crushed cartilage, and 4% for severely crushed cartilage. In our study, the day 1 viability rates (1 day after crushing and culturing) for the intact, slightly crushed, moderately crushed, significantly crushed, and severely crushed cartilage groups were 96%, 92%, 82%, 72%, and 54%, respectively. These high viability rates for crushed human septal cartilage are similar to those reported in most animal experiments and in human clinical observations. However, most previous studies did not define the force or severity of crushing that was applied. This lack of standardization of crushing intensity might be a major reason for the conflicting results noted among the different studies.

Bujia examined chondrocyte viability at only one time point (24–48 hours after crushing), and this manner of assessment may not be sufficient to determine the preservation of chondrocyte viability or the ability of chondrocytes to regenerate. In our study, we counted viable and nonviable cells on days 1, 2, 3, and 10 after culturing and made comparisons among the groups at each time point. The intact and slightly and moderately crushed cartilage groups had high chondrocyte viability rates in the early days after culturing and at 10 days. These groups showed similar frequencies of mitoses and colony formation at all 4 time points evaluated. On day 10, all of these groups showed large numbers of mitotic figures before harvesting. At all time points assessed, the viability rates for the slightly crushed cartilage group were significantly higher than the corresponding rates for the significantly crushed and severely crushed cartilage groups. The cells from the significantly crushed and severely crushed cartilage specimens had to adapt to the culture medium in the first 2 days, and we subsequently noted cell adhesion and colonization. At all time points assessed, the viability rate for the severely crushed cartilage group was significantly lower than the corresponding rates for the intact, slightly crushed, and moderately crushed cartilage groups.

Our study was an in vitro experiment, and cellular behavior in the culture microenvironment may differ from that under normal conditions. In the in vivo setting, the viability of crushed cells may also be affected by circulation and cellular interactions, factors that are unrelated to culture medium ingredients. However, our results suggest that the viability of crushed human septal cartilage and the ability of these chondrocytes to proliferate depend on the degree of crushing that is applied. As crushing intensity increases, the viability of chondrocytes, their ability to proliferate, and the clinical predictability of the graft decrease. Slightly and moderately crushed tissues show good chondrocyte viability and proliferation at levels comparable to those of intact cartilage. Slight or moderate crushing of human septal cartilage creates excellent graft material for filling defects, concealing dorsal irregularities and creating a smooth surface in rhinoplasty operations. In contrast, significant or severe crushing reduces chondrocyte viability and proliferation and may result in unpredictable degrees of graft volume loss.

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REFERENCES