Study of Outcome of an Implanted Autologous Auricular Cartilage: A Preliminary Experimental Research in Rabbits

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Abstract To investigate the viability of the implanted crushed and uncrushed auricular cartilage graft with intact perichondrium with respect to macroscopic and microscopic parameters. Cartilage grafts from 8 white New Zealand rabbits were harvested from the right auricle, with intact perichondrial layers. There were two categories Pre implant and Post-implant and two types, mildly crushed and uncrushed cartilage graft. The cartilage grafts were implanted into the subcutaneous pockets over the right upper paraspinal area. At the end of 2 months, implanted grafts were retrieved and examined histopathologically. There was a difference among the both types of cartilages in both the categories with respect to chronic inflammation, fibrosis, cartilage mass viability and vascularization. The mildly crushed auricular autologous cartilage with intact perichondrium does not lose the viability and maintains the structural integrity and thus increasing the clinical predictability for cosmetic correction of nose in rhinoplasty.

Keywords Autologous auricular cartilage · Animal model · Histopathology · Perichondrium · Rhinoplasty

Introduction

Amongst the other grafts, cartilage grafts are widely being used in rhinoplasty to overcome certain irregularities on the nasal dorsum and to achieve a smoother surface after correction. But ironically these grafts tend to have memory and revert to their original physique even after years of implantation. This leads to postoperative distortion and deformities [1, 2]. Erol's [3] modification of diced cartilage for dorsal nasal augmentation popularized the use of these types of Turkish delight grafts. Nevertheless, clinical studies reported that the long term follow up, crushed or diced cartilage pieces wrapped in surgical resorb after few months resulting in irregularities of the nasal dorsum [4].

The comparison of diced and crushed cartilage grafts without perichondrium and some with being wrapped in surgical or hyaluronidase have been widely studied in previous papers [5, 6]. But the fate of implanted crushed and uncrushed autologous auricular cartilage graft with intact perichondrium on both the sides has not been evaluated as per our literature review. In the present study, an attempt has been made to compare the fate of the crushed and uncrushed autologous auricular cartilage with intact perichondrium with respect to macroscopic and microscopic variances.

Materials and Methods

Animal Care

The experimental design was reviewed and approved by the institutional animal ethical committee (IAEC) for Ethics in Animal Experiments (No. 2/1, dated 20-11-2011) and approved to conduct the research at central

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experimental animal house at KLE University's J.N. Medical College, Belgaum, Karnataka, India. The study was carried out in compliance with the guidelines for the animal experimentation of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The adult male white New Zealand rabbits aged between 12 and 15 months were procured from the registered animal breeder (627/02/a/CPCSEA). All animals used for this experiment received care according to the guidelines for use of laboratory animals in medical colleges—ICMR-2001 (Indian Council of Medical Research). Animals were housed individually in a controlled environment at a room temperature of $18 \pm 1^\circ\text{C}$ and with 50–70 % relative humidity, with a 12 h light–dark cycle and were taken care with food and water as per their body weight. A total of 8 New Zealand rabbits, each weighing approximately 1250 to 1500 gms, were acquired for the study. Each rabbit was anaesthetized with intramuscular route with mixture of freshly drawn injection of Ketamine hydrochloride of 35 mg/kg (Aneket-Neon Lab) and Xylazine Hydrochloride of 5 mg/kg (Xylaxin-Indian Immunologicals LTD). Local anesthesia was given at operating site with Lidocaine Hydrochloride with adrenaline (Xylocaine 2 % with adrenaline, 1:200,000, Astra Zeneca Pharma, India Ltd).

Experimental Protocol

Animals were put into the individual cages a week before the proposed surgery for the adaptation and the hair of the operative site from the right auricle and the right upper para-spinal area were shaved on the day of surgery with the fresh blade and mild antiseptic solution (Savlon) was applied. After the animal was anaesthetized, operative sites were cleaned with antiseptic solution and draped with sterile drapes. The operative sites were infiltrated with local anesthetic agent with 26 gauge dental needle. Approximately 40 mm × 10 mm cartilage graft was harvested from each animal through the ventral surface of right auricle (Fig. 1). The obtained cartilage with intact ventral and dorsal perichondrial layers was sectioned with fresh 11 no. blade into four pieces of each 10 × 10 mm size. Two pieces were mildly crushed in the cartilage crusher. One crushed and another uncrushed cartilage pieces as pre-implant grafts were sent for histopathological analysis.

Then two separate stab incisions about 4 cm apart were made in the skin of the upper paraspinal region and a subcutaneous pocket was created at each site (Fig. 2). Then one crushed and another uncrushed cartilage grafts were implanted into the pockets separately. The incisions of both donor and the recipient sites were sutured with 3–0 Vicryl (Centisorb 910 Violet-Centennial Surgical Suture Ltd) and the grafts were left at the recipient sites for 2 months.

Fig. 1 Cartilage graft harvested through the ventral surface of right auricle

Fig. 2 Two incision sites for graft implant (right upper paraspinal area)

Histopathological Staining

The grafts were explanted after 2 months, adhering to the similar implantation operative protocol without sacrificing the animals (Fig. 3) and the samples were fixed in 10 % neutral buffered formalin for 24 h in separate coded containers. The entire specimen was examined histopathologically. The histopathologist was blinded to the specimens. Two groups were categorized as pre-implant and post-implant. Each group had two types of grafts, one crushed and another uncrushed. Specimens included in the study were processed, embedded in paraffin and cut into 5 μm sections. The sections were stained with Haematoxylin–Eosin (H & E), Masson Trichrome and Van Gieson Verchhoff stain and were examined under light microscopy. The H & E stained sections were used to evaluate chondrocyte viability and the status of the chondroid tissue. Masson trichrome tissue stain is an important indicator of collagen content of the matrix. With this stain, a blue color in the matrix marks collagen fibrils and was used to assess connective tissue. Cartilage graft viability was determined

Fig. 3 Area of graft explantation and area of healed donor site of right auricle

based on the state of the chondroid matrix, specifically the loss of matrix chondromasia and the loss of chondrocyte nuclei from the lacunae. Absence of matrix chondromasia and chondrocyte nuclei was considered to indicate nonviable chondroid tissue. The level of cartilage mass, fibrosis, chronic inflammation and vascularization for each specimen was expressed as a percentage of the total tissue present. The cartilage mass in the specimens were quantified as 1+ when 0–25 % of the analyzed material was recorded, 2+ when 26–50 %, as 3+ for 51–75 % and as 4+ when 76 % and above. When evaluating the chronic inflammation, presence of the lymphocytes were quantified as absent, mild, moderate and severe depending on the number of chronic inflammatory cells observed. For analyzing the fibrosis and vascularization density representing 0–25 % of the analyzed material were recorded as minimal (1+), 26–50 % as moderate (2+), 51–75 % as moderate to severe (3+) and 76 % and above as severe (4+). In addition to chondrocyte proliferation, viability and new cartilage cell formation were investigated. Histopathologic findings for each group were recorded.

Results

Variances of histopathological data were analyzed statistically with Wilcoxon signed ranks test and Fischer's exact test. Comparisons were made among and within the groups. Fischer's exact test was used in the comparison of the qualitative data in the study. Results were evaluated at a $P < .05$ significance level.

Macroscopic Observation

It was observed that the grafts were firmly embedded into the deep fascia and were greyish and shining in appearance. Both the grafts retained their cartilaginous

characteristics. Relatively the crushed cartilage was pliable, softer with intact perichondrium on both sides and more vascularity was observed around the implanted graft.

Microscopic Examination

Uncrushed Cartilage

Pre implant H and E stain of the uncrushed bare cartilage revealed intact perichondrial covering both the sides and showed no changes in any histopathological variances where as in post-implant grafts, though all the variances were statistically significant except chondrocyte proliferation but were only mild from 1+ to 2+ histopathologically.

Crushed Cartilage

Crushed cartilage showed few sparse islands of bare areas without the perichondrium in both pre-implant and post-implant specimens indicating abraded areas because of crushing but there were no damaged or fragmented areas. All the other comparative parameters were statistically significant and showed moderate—severe fibrosis, minimal vascularization and moderate to severe inflammation (Table 1).

Comparing to the crushed or uncrushed cartilages to their counter parts in pre-implant and post-implants, the P value was not significant (Table 2).

Discussion

We the human beings are after the pursue of beauty of face in general and the nose in particular. But ailing or ailed noses may get deformed and are rendered free of ailment before embarking on any corrections. Thus the maintenance of the harmony between the nose and the face is obviously the aim of any corrective rhinoplasty [7]. Addressing the certain subtle irregularities over the dorsum especially in thin skinned individuals during corrective rhinoplasty is more challenging and at the same time tedious because of various limitations. In cases of secondary rhinoplasty or a primary complicated rhinoplasty, establishing a symmetric and smooth nasal dorsum is a challenging part of the procedure. Especially in cases of post traumatic deformity or asymmetric nasal bones, establishment of the symmetric nasal dorsum is the most difficult aspect of the surgery [8]. Another aim of the surgery is to prevent an operated—on look by means of camouflaging the graft. Many grafting techniques have been described and used for this purpose in the past, but none have been ideal [9–11].

Table 1 *P* value for pre implant and post-implant comparison of types CR and UCR cartilage

Sl. no.	Parameters	<i>P</i> value	
		CR	UCR
1.	Chronic inflammation	.046*	.046*
2.	Fibrosis-collagen content	.005*	.005*
3.	Vascular proliferation	.005*	.005*
4.	Cartilage mass/collagen content	.007*	.007*
5.	Viability chondrocyte-loss of chondrocyte nuclei	.046*	.046*
6.	Perichondral proliferation/chondrocyte proliferation	.058*	.058*

Wilcoxon signed ranks test

* Significant

Table 2 *P* value for pre implant and post-implant comparison of types CR with UCR cartilage

Sl. no.	Parameters	<i>P</i> value pre implant	<i>P</i> value post-implant
1.	Chronic inflammation	NA	.608
2.	Fibrosis	NA	1
3.	Vascular proliferation	NA	1
4.	Cartilage mass/collagen content	.007*	1
5.	Viability chondrocyte-loss of chondrocyte nuclei	1	1
6.	Perichondral proliferation/chondrocyte proliferation	1	1

Fischer's exact test

NA not applicable

* Significant

There are numerous clinical and experimental studies in the literature comparing the viability of crushed and diced cartilage either wrapped in surgicel or fascia. The application of diced or crushed cartilage grafts on the nasal dorsum were abandoned for a long period of time until they were made popular again at the beginning of this century by Erol [3]. As delineated in the 'Turkish Delight' technique, Erol prefers to harvest the cartilage from septal, alar, conchal and costal origins and dice the cartilage into pieces of 0.5–1 mm. This fine textured cartilage mass is then wrapped in Surgicel to obtain a cylindrical form and is moistened with rifampicin and parenteral blood. The surgicel wrapped diced cartilage is then inserted under the nasal dorsal skin, into the nasal tip or into the lateral side wall. Overcorrection is desirable as he states, because of partial resorption.

Erol's Turkish Delight technique applied to 2365 patients over 10 years demonstrated stable, long term results [3]. However experimental studies conducted afterward to evaluate the viability of the diced crushed

cartilage showed discrepant results [12, 13]. The studies showed that the use of oxidized regenerated cellulose to wrap diced or crushed cartilage grafts demonstrated no evidence of proliferation thus reducing their clinical predictability because of negative effects on cartilage viability and regeneration. This might be related to the finding that surgical cellulose fibers persist for long periods of time after insertion in humans and that surgicel is degraded by foreign body reaction rather than by hydrolyzed absorption [1]. Humans do not possess a hydrolytic enzyme for metabolizing the cellulose [14]. The wrapping procedure may arrest the cartilage tissue oxygenation for up to 48 h, which may give rise to a relative hypoxia in the early hours after implantation. This relative hypoxia may result in loss of regenerative potential. Daniel conducted a clinical study using diced cartilage graft wrapped in surgicel, but this study had to be halted abruptly at 4 months because of unexpected absorption and clinical failure of all diced cartilage grafts wrapped in surgical. However, Daniel and Calvert used these grafts for contour to produce distinct changes in the nasal profile. Their histological studies from human specimens also indicated that a significant foreign body reaction occurred in the diced cartilage grafts wrapped in surgicel but not in those grafts wrapped in fascia [4].

We have attempted a different and practical research on animal model, comparing pre-implant and post-implant crushed and uncrushed autologous auricular cartilage grafts with intact perichondrium to know and understand the behavior of the intended implanted autologous auricular cartilage graft and to ascertain, whether the results are extrapolatable in human beings in corrective rhinoplasty. With this we were able to observe the histopathological results in the same animal. We did not find any foreign body reaction in any groups. Our findings are consistent with the fact that surgicel wraps appear to incite an inflammatory response and subsequent absorption of the cartilage, whether diced or crushed [15]. The degree of crushing has been shown to be an important factor in cartilage viability and clinical predictability of autogenous cartilage grafts. As the severity of crushing increases, cartilage viability, chondrocyte proliferation and predictability of clinical outcome decreases [12]. Thus, we used mildly crushed intact cartilage grafts in our experimental study and found no significant difference among them. To note, all types of cartilage grafts showed viability, mild chondrogenesis especially at the periphery just beneath the perichondrium.

We are earnestly convinced that this study is of first of its kind in the literature to compare the results of histopathological changes of crushed and uncrushed cartilages grafts with both sides intact perichondrium. The perichondrium acts like smooth sheath over the cartilage and crushing the graft mildly makes it more adaptable over

nasal dorsum to place it properly as a fascia and this type of graft can be easily trimmed to allow for insertion into any surgical site. Thus, chondrocytes have little capacity to regenerate when injured and cartilage tissue that is not supplied nutritionally is replaced by fibrous tissue [16]. In the present study, this phenomenon has been evaluated once again, especially in crushed cartilage grafts with intact perichondrium.

Conclusion

In this study, we aimed to demonstrate the viability of crushed and uncrushed autologous auricular cartilage grafts with intact perichondrium in regard to histopathological results. Thus we propose that with intact perichondrium there would not be much difference between mild crushing and non crushing and but to the advantage mild crushing helps relative pliability and softening of the graft so that it can be moldable over the dorsum and also promotes cartilage integrity and survival, thus increasing clinical predictability and avoiding the need for over correction. It is important to keep in mind that this is an experimental model in rabbits and similar procedures might result in different findings in humans. Thus further studies are needed to evaluate long-term effects and clinical applications in rhinoplasty procedures and authors intend to continue the research further for long term results.

Compliance with Ethical Standards

Conflict of interest None declared.

Ethical Approval The experimental design was reviewed and approved by the institutional animal ethical committee (IAEC) for Ethics in Animal Experiments (No. 2/1, dated 20-11-2011) and approved to conduct the research at central experimental animal house at KLE University's J.N. Medical College, Belgaum, Karnataka, India. The study was carried out in compliance with the guidelines for the animal experimentation of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

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