Tr. J. of Medical Sciences
28 (1998) 285-290

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The Auricular Cartilage Graft Fixation With Butyl 2-Cyanocrylate

Received: December 24, 1996

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Abstract: Cartilage grafts are widely used in maxillo-facial reconstruction operations. There are some difficulties of conventional suture techniques in these operations such as long operation time, mobilization, shifting, and implantation or fixation of small pieces of cartilage. Therefore the advantages of a tissue adhesive over conventional suture techniques became evident in these operations. Butyl 2-Cyanoacrylate (B2-CA) is one of the least histotoxic cyanoacrylate derivatives and is used as a tissue adhesive. In this study, B2-CA was compared with conventional suture techniques in securing rabbit auricular cartilage autografts. Sutures were used in the left control ears and B2-CA was used in the right experimental ears of 24 rabbits. They were sacrificed between 15th day to 5 months. All samples were evaluated. There was no significant difference between experimental and control group with regard to histological structure, inflammation, graft migration, and graft viability. As a result, B2-CA can be used safely and effectively for fixation of cartilage grafts and has no more histotoxicity than sutures.

Key Words: Cartilage graft, tissue adhesive.

Introduction

Cartilage grafts are used in the reconstruction of frontal, nasal, maxillary, and chin depressions. Septal cartilage is usually preferred to be followed by auricular and costal cartilage. Free hand curving of cartilage to give a proper shape is not difficult, and small pieces of cartilage may be fixed together to make a larger piece. The application of cartilage grafts has several advantages such as being more easily carved and shaped to the exact form desired. It does not require functional use to retain its bulk and it does not need a vascular blood supply to survive. Post-implantation absorption rate of cartilage graft is low and its antigenicity is low. Suturing of cartilage grafts may cause difficulty in fixation and cartilage may shift and the duration of operation becames longer. Multiple suture may cause fracture of the cartilage and then may lead to resorption in postoperative period (1,2). Because of these reasons, as a cartilage adhesive Butyl 2-Cyanoacrylate is recommended. B2-CA bonds strongly and immobilizes easily cartilage grafts. B2-CA provides desirable healing characteristics of the cartilage graft results (3-5).

The ideal tissue adhesive has ability to spread readily on tissues, it should be easy to apply, nontoxic and biodegradable, and should establish a strong and flexible band (6,7). Recent interest has been focused on B2-CA which is a medium chain cyanoacrylate derivative (5). It is biodegradable resulting in formaldehyde and cyanoacetate that cause a mild inflammatory response when used in tissue (6,8). B2-CA has antibacterial and antifungal effect (9,10). B2-CA has been used in a wide variety of surgical procedures such as tracheal and bronchial closure (11), dental operation (12), augmentation rhinoplasty (13), middle ear (14), facial and plastic operations (6,15,16).

The purpose of our study was to compare the use of B2-CA with conventional suture techniques in the histological results of autolog auricular cartilage graft application.

Materials and Methods

Twenty-four adult New Zealand white rabbits weighing between 1.5 and 2 kg were used in this

study. The rabbits had similar food and environment. Feeding of each rabbit was stopped three hours before operation. All operations were performed under anesthesia by ketamine hydrochloride (30 mg/kg) and diazepam (0.5 mg/kg) which administered intramuscularly (5). Both dorsal auricles of each rabbit were shaved and cleanced with povidone-iodine. The face was draped in a sterile fashion. Supplemental doses of 1 % lidocain HCL was injected into dorsal aspect of the auricle as local anesthetic (5,17). An incision extending 1.5 cm in length was made by using a #:15 surgical blade on the dorsomedial aspect of the auricle. The incision was carried down to perichondrium The dissection of overlying subcutaneous tissue and fascia was performed. Using a template measuring 0.75 x 0.75 cm a piece of cartilage was harvested with perichondrium intact both sides by using a template,. Autograft was implanted by approximately 1.5 cm distal to the donor site.

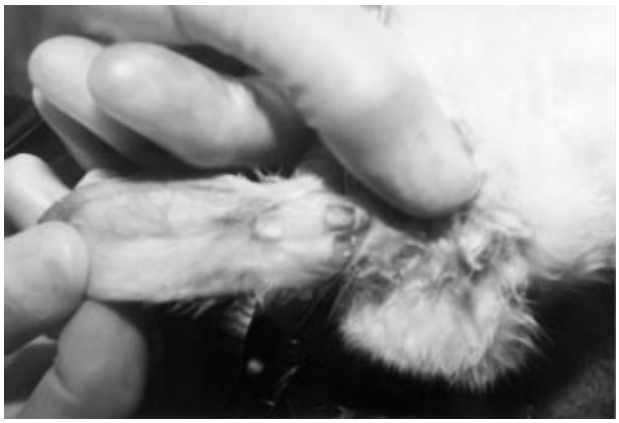

Figure 1. The cartilage graft fixed with B2-CA on the rabbit auricle.

In the right ear (experimental ear) 0.01 ml of B2-CA (Histoacryl^{R} B.Braun Malsungen AG, Germany) was dropped onto the autograft with a tuberculin needle. The graft was then fixed to the recipient site and pressure was applied for one minute over the surface of graft (Figure 1). In the left ear (control ear) was approached in a similar fashion. The graft was implanted by suturing perichondrium to perichondrium using 5/0 Polyglactin sutures (Vicryl^{R}) after harvesting the donor graft. For the purpose of graft migration measurement, the area of the graft site in both ears was tattooed with china ink, using tuberculin needle on the overlying skin. The fascia was closed with interrupted 5/0 polyglactin sutures and the skin was closed with interrupted 3/0 silk sutures in both groups. The auricles were cleansed with provide-iodine daily until skin sutures were removed on the seventh day. The auricles were examined daily during postoperative week and then weekly.

The rabbits were diveded into six groups. Each group consisted of four rabbits. They were decapitated and the migration of grafts were measured as distance from the grafts to pointed by china ink. Surgical sites were harvested and placed in 10 % buffered formalin for two days. Three specimens were excised vertically with a generous margin of normal tissue from graft sites. Slides were prepared and stained by hematoxylene and eosin, oil red O and Halle's colloidal iron strains. Histologic examination of slides was performed by light microscopy. Oil red O was used to aid in visualizing the B2-CA (5). Halle's colloidal iron selectively stains acid mucopolysaccarides. Acid mucopolysaccaride production is an indicator of chondrocyte viability (18).

Histopathologic findings are graded as described Fung et al (5). By using this grading system, each histologic slide was analysed for severity of inflammatory response (table 1), cartilaginous content of the union between the graft and the underlying cartilage (Table 2), Intensity of Halle's colloidal iron stain (Table 3), overall structural abnormalities (Table 4), cell morphology and density (Table 5). The grades resulting from histopathologic findings of the experimental and the control groups were statistically analized with Mann-whitney U test.

Results

All rabbits survived without seroma, infection, hematoma. We found that B2-CA was easier and quicker to apply. Graft migration was evaluated without one of the control group at 5th month and there was no difference between two groups. There were perichondrial damage and connective tissue developed between graft and underlying cartilage (Figure 2A). In this study, these disadvantages decreased by suturing only perichondrium.

Histopathological findings were described in tables 1-5.

We found that the perichondrial damage and connective tissue were present in sutured areas in all control slides (Figure 2B). In this study, these disadvantages were minimized by only suturing perichondrium to perichondrium. Graft migration was evaluated in all slides and there was no difference between two groups. Horizontal and vertical migration was less than 1 mm in all slides and therefore it was

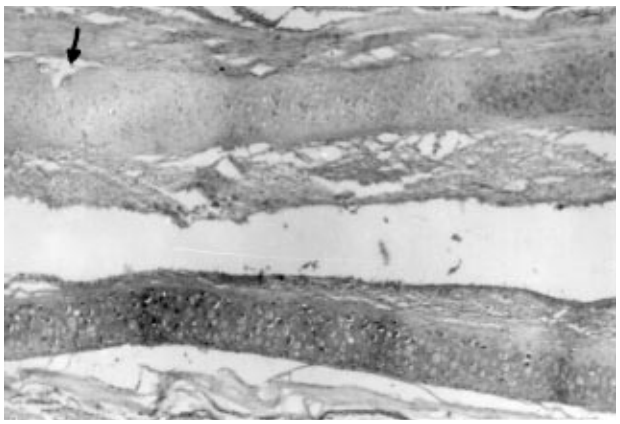

Figure 2A. The experimental graft in 1st month. Disruption of perichondrium and hypocellularity (arrow) (HEX40).

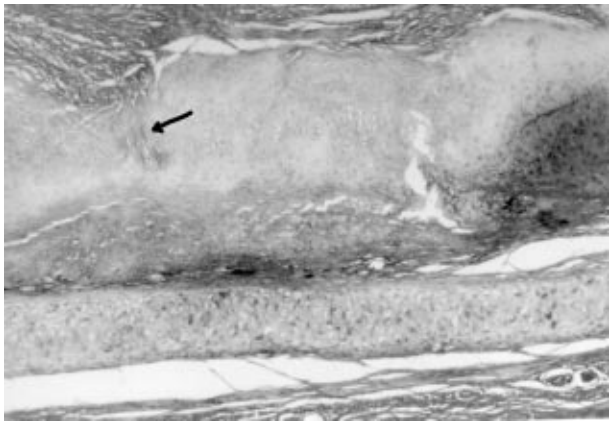

Figure 2B. The control groft in lst month. Superficial infiltration of connective tissue (arrow), the union between graft and underlying cartilage was fibrocartilaginous (Halle's colloidal iron dyeX40).

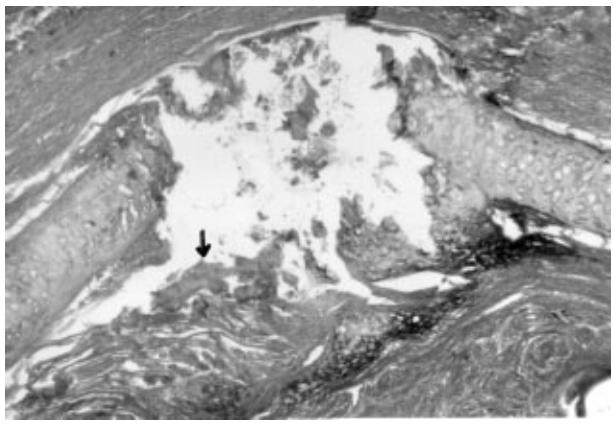

Figure 3A. The experimental graft in 5th month. Hypocellularity was present in the graft cells (above) and the bony gap (arrow) was shown in union between graft and underlying cartilage (Halle's colloidal iron dyeX40).

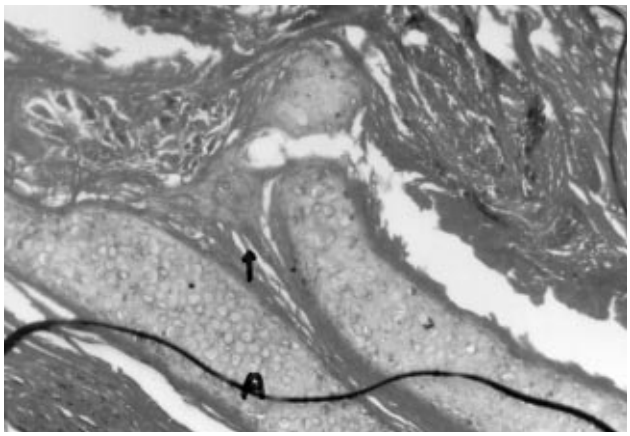

Figure 3B. The control graft in 5th month. Similar findings were shown to be found in the control slide (A:artefact) (Halle's colloidal iron dyeX40).

not evaluated statistically. The borders of grafts are established where slightly stained of Halle's colloidal dye and empty lacunae and local infiltration of connective tissue were seen (5,18). The evidence of neoplasia was not detected in all slides during 5 months.

The inflammation had subsided considerably earlier in the experimental slides than the control slides. The inflammation appeared during 1 month in the experimental group while maintaining for 3 months in the control group (Table 1). Union between the cartilage grafts and underlying cartilage commonly was fibrous and similar in both groups (Table 2). Halle's colloidal iron dye staining was similar in both groups (Table 3) (Figure 3A-B). Dead cells and hypocellularity in the cartilage graft were much more in the control group than the experimental group (Table 5). In the experimental group, connective tissue was less than the control group and segmental replacement was absent in both groups in graft structure (Table 4). But, statistical significance was not found between the two groups with regard to inflammation, union between graft and underlying cartilage, graft structure and graft cells.

Table 1. The inflamation on the cartilage grafts.

	The experimental group	The control group
15th day	2,0,2,1	0,2,2,2
1st month	2,0,0,0	1,1,1,0
2nd month	0,0,0,0	0,0,0,1
3th month	0,0,0,0	2,0,1,0
4th month	0,0,0,0	0,0,0,0
5th month	0,0,0,0	0,0,0,0

U:233, Z:1.44,P:0.14 SD:0.29±0.69 0.54±0.78
0=None, 1=Mild, 2=Moderate, 3=Severe, 4=Severe with tissue necrosis.

Table 2. Union between the cartilage grafts and underlying cartilages.

	The experimental group	The control group
15th day	2,2,2,2	2,2,2,2
1st month	2,2,2,2	1,2,2,2
2nd month	2,2,3,2	2,2,2,3
3th month	2,3,2,1	2,3,2,0
4th month	1,2,1,2	1,2,2,2
5th month	1,1,2,2	1,2,2,2

U:279, Z:0.224P:0.8 SD:1.87±0.54 SD:1.87±0.61

0=Caltilaginous, 1=Fibrocartilaginous, 2=Fibrous, 3=Bony.

Table 3. Halle's colloidal yron dye staining of the cartilage grafts.

	The experimental group	The control group
15th day	1,2,2,0	2,1,2,0
1st month	1,1,0,2	2,3,0,1
2nd month	1,1,2,1	1,3,3,1
3th month	3,0,2,2	0,3,3,0
4th month	1,3,0,0	0,0,1,1
5th month	2,3,0,0	2,1,0,0

U:238 Z:0.92 P:0.35 SD:1.25±1.03 1.25±1.15

0=Normal, 1=Sligt reduction, 2=Moderate reduction, 3=Severe reduction,

4=No dye noted.

Table 4. The structure of cartilage grafts.

	The experimental group	The control group
15th day	0,0,1,2	0,2,0,2
1st month	0,0,0,1	2,1,0,1
2nd month	0,2,2,1	2,2,1,3
3th month	0,2,3,2	2,3,2,1
4th month	1,2,3,1	1,2,2,1
5th month	1,3,2,1	1,2,2,1

U:244, Z:0.9P:0.34 SD:1.25±1.03 SD:1.5±0.83

0=Normal, 1=Disruption of perichondrium,

2=Superficial infitration of connective tissue,

3=Deep penetration of connective tissue,

4=segmental replacement by connective tissue.

Table 5. The cartilage graft cells.

	The experimental group	The control group
15th day	0,0,3,0	0,3,1,0
1st month	0,2,0,4	4,0,2,1
2nd month	3,0,0,2	4,0,4,3
3th month	2,3,1,3	4,2,4,1
4th month	2,0,2,0	0,1,2,0
5th month	1,2,2,1	1,2,2,1

U:249, Z:0.8P:0.4 SD:1.36±1.27 SD:1.75±1.48

0=Normal mature or prolipeferating cartilage,

1=Foci of empty lacunae,

2=Segment of empty lacunae and hypocellularity,

3=Diffuse hypocellularity

4=Cell death with only matrix remaining.

Discussion

Cartilage graft is used for a wide variety of otolaryngology procedures. Attainments of immobilization, the ideal graft shape, graft viability and stability are important when cartilage graft is used in facial reconstruction The cartilage grafts have been fixed with suturing. But, cartilage defects were produced by surgical trauma. Suturing the cartilage entirely by multiple sutures causes severe damages in cartilage including fractures of grafts. This damage leads to graft resorbtion (4,13,15). Moreover, conventional suture techniques are not easy to perform and duration of operation is longer in graft fixation with suturing. It has been reported that it was not possible to suture small pieces of cartilage graft and using tissue adhesives such as cyanoacrylate derivatives may be alternative method for fixation of small pieces of cartilage graft (5,19). Quentala et all (4) have reported that B 2-CA was the only cyanoacrylate adhesive by achieving with minimal adverse effects on cartilage while maintaining the advantages on a fastacting, strong and reliable adhesive.

Achieving of immobilization and graft stability is important when using cartilage graft in reconstructive surgery. Instability, mobility, suboptimal graft and increased operative time occur when suture techniques are used to fix the cartilage graft. B2-CA holds the cartilage grafts tightly, immobilizes easily (5,15). Sachs

(13) has used B2-CA as a cartilage adhesive in nasal augmentation and achieved excellent results by immobilizing graft during the healing period. In present study, the mobilization of grafts were investigated and any difference was not found between two groups. B2-CA was recommended in more difficult situation such as augmentation rhinoplasty (13). Silverstein et all (19) reported that B 2-CA has been used to fix small pieces of the cartilage graft to the head of the ear ossicle prosthesis for improved extrusion. In this study, small piece cartilage grafts were fixed more easily with B2-CA than suturing.

In our study, B2-CA was quicker and easier than suturing in graft fixation. Suturing cartilage caused focal damage in perichondrium and superficial connective tissue infiltration were placed in all slides of the control (suture) group. But there was no statistically significant difference between the structures of grafts in two groups.

Although there was no statistically difference between two groups, the inflammation in samples of experimental group was less than the control group in first 3 month period. It was occurred only at 15th day and 1st month in the experimental group while maintaining for 3th month in the control group. The foreign body reaction was not present in both groups. Thus, B2-CA was not different from suturing regarding to foreign body reaction and foreign body cells were not observed in slides of experimental group. These findings may be explained by slow degradation of B2-CA and well tolerated the toxic products (5). Toriumi et all (8) reported that B2-CA was safe with minimal inflammation when used in small quantities between poorly vascularized tissue such as cartilage. Kamer (15) reported that B2-CA could be used beneath the skin securely.

In this study, when B2-CA was used, union of graft with underlying cartilage, and the structure of grafts were not significantly different from that of suturing. The fibroblast and other connective tissue cells were infiltrated around the B2-CA polymers with causing partial breaks. These findings may be explained by B2-CA did not cause tissue necrosis. It was reported that B2-CA was well tolerated by repairing cells in wound area and they could continue their normal functions by attaching to B2-CA monomers (5,20).

Halle's colloidal iron dye straining as an indicator of controcyte viability was also similar in two groups. All grafts were viable in two groups. Although graft cells were not different in two groups, hypocellularity and number of death cell were lower in the B2-CA used grafts than the suturing grafts. It may be explained by focal damage to pericondrium due to suturing and using small amount of B2-CA without well-vascularized tissue contact. Also, it may be explained by decreasing seroma formation and rejection because B2-CA fixed graft strongly. Thus, we observed that B2-CA had no toxic effect on cartilage graft viability compared with suture technique (3-5,15). Ronis et all (17) reported that the healing process was similar to conventional suturing technique when used B2-CA for fixation of the cartilage grafts without any cartilage toxicity and damage. Our findings were similar to literature.

As a result, the cartilage graft healing process of B2-CA was similar to that of conventional suturing techniques and B2-CA has the advantages such as ease of use, strong and reliable adhesive effect. We concluded that B2-CA would provide an alternative and even superior method for cartilage graft fixation, especially on facial and plastic operations in which cartilage graft placement and fixation are difficult.

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