Healing at the Interface Between Autologous Block Bone Grafts and Recipient Sites Using n-Butyl-2-Cyanoacrylate Adhesive as Fixation: Histomorphometric Study in Rabbits

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The aim of the present split-mouth (split-plot) study was to describe the sequential healing in the interface between autologous bone grafts and recipient parent bone, fixed using an n-butyl-2-cyanoacrylate adhesive with or without an additional titanium fixation screw. Bone grafts were collected from the calvaria and fixed to the lateral aspect of the mandible in 24 rabbits. The cortical layers of the recipient sites were perforated, and the grafts were randomly fixed using an n-butyl-2-cyanoacrylate adhesive, either alone or in conjunction with a 1.5 mm × 6.0 mm titanium fixation screw. The animals were sacrificed after 3, 7, 20, and 40 days, and histomorphometric evaluations of the interface between graft and parent bone were performed. Only 2 of 6 grafts in each group were partially incorporated to the parent bone after 40 days of healing. The remaining grafts were separated from the parent bone by adhesive and connective tissue. It was concluded that the use of n-butyl-2-cyanoacrylate as fixation of an autologous bone graft to the lateral aspect of the mandible was able to maintain the fixation over time but did not incorporate the graft to the recipient sites. Use of fixation screws did not improve the healing.

Key Words: animal study, bone formation, autologous bone graft, bone healing, histometry, morphometry, glue, adhesive, cyanoacrylate

INTRODUCTION

Several different techniques have been proposed for lateral bone augmentation, such as guided bone regeneration,¹ titanium grids,² autologous bone blocks,³ ridge splitting,⁴ and orthodontic treatment.⁵,⁶ Lateral bone augmentation has been shown to yield a high survival rate of implants installed in these regenerated sites.⁷ Autogenous bone blocks are harvested from different intraoral and extraoral donor sites, depending on the quantity of bone needed for the reconstruction. A review that analyzed morbidity, resorption and implant survival concluded that the intraoral donor sites for bone blocks should be selected from the ramus instead of the chin because the former led to lower pain, fewer skin sensory problems, and fewer wound healing complications compared with the latter.⁸ Moreover, the posterior iliac crest is preferred to the anterior as an extraoral donor site because it generates less morbidity.⁹

It has to be considered that grafts from the iliac crest undergo more resorption compared with intraoral grafts.⁹–¹¹ However, intraoral donor sites allow collection of grafts of limited dimensions, which may be not suitable for reconstructing severely atrophic jaws. In such cases, grafts collected from the calvaria may represent an alternative because their dimensions are larger, and they are associated with low resorption rate and low donor-site morbidity.¹²,¹³

Autogenous bone blocks are the most frequently used grafts for lateral bone augmentation of the alveolar crest.¹⁴ An experimental study in dogs has shown that autogenous bone blocks, secured with the use of a position screw technique, were vital and well incorporated to the parent bone after 6 months of healing.¹¹ Moreover, it was shown that implants installed in an alveolar bone crest, augmented by means of autogenous bone blocks, were well integrated after 3 more months of healing.¹⁵

The graft may be stabilized to the recipient bed by different methods, such as fixation plates or screws, and it is of the utmost importance to guarantee good integration of the graft to the parent bone.¹⁶ Another technique to fix the graft to the recipient bed is use of a cyanoacrylate adhesive, such as n-butyl-2-cyanoacrylate.¹⁷ The shear bond strength of a graft fixed to a device using n-butyl cyanoacrylate or ethyl cyanoacrylate or a titanium screw as fixation methods was measured.¹⁸ It was shown that the shear bond strengths of the screws were significantly higher than either cyanoacrylate

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adhesive. However, both adhesives presented a sufficient biomechanical potential to be used for fixation.

In experiments, n-butyl-2-cyanoacrylate (NB-Cn) has been used for bone fracture fixation. For instance, in a study in rabbits, a bone fragment was obtained from the radius and then replaced without removing the periosteum and fixed with NB-Cn at the test sites, while no adhesive was used at the control sites. It was concluded that the adhesive was able to fix the graft during the first weeks of healing without interfering with the consolidation of the graft as evaluated by radiographies.

Cyanoacrylate adhesives are used extensively in many surgical fields, including oral surgery. In a clinical report, NB-Cn was used to close wounds during different types of oral surgery, such as apicectomies, molar extractions, and mucogingival surgery. In 1 study, 130 were treated with NB-Cn, while 30 patients received sutures. It was shown that the adhesive allowed immediate hemostasis and a normal healing of the wounds.

Several studies has been performed to compare the healing of grafts fixed by means of screws or cyanoacrylates. Even though contradictory results have been reported, some limitations in the methods used in the glue groups should be considered. Among them, a possible improper fixation of the graft or the presence of a layer of glue that was too thick might have interfered with the healing process. Moreover, despite the evidence available, a description of the sequential healing processes at the graft/recipient bed interface when cyanoacrylate adhesive is used as a fixation method has not yet been provided.

Hence, the aim of the present experiment was to study the sequential healing in the interface between an autologous bone graft and the recipient parent bone fixed using an NB-Cn adhesive with or without an additional titanium fixation screw to improve the fixation and reduce the thickness of the adhesive layer.

**MATERIALS AND METHODS**

The protocol of the present experiment was submitted to and approved by the local Ethics Committee of the University of São Paulo, Ribeirão Preto, Brazil. Study diagram, variables measured, and statistical test used are presented in Figure 1.

In this experiment, 48 adult male New Zealand white rabbits, weighing between 3.5 and 4.0 kg, were divided into 2 groups; 1 group of 24 animals was used. The remaining animals were used to study other variables for graft fixation, and the results are illustrated in another report.

**Randomization**

Two different techniques were adopted to secure bone grafts collected from the calvaria to the lateral aspect of the mandibular angles: in fixation I (cyan) NB-Cn adhesive was applied to the periphery of the bone block; in fixation II (cyan-screw), NB-Cn was applied to the periphery of the bone block and a 1.5 mm x 6.0 mm titanium fixation screw (Kit Enxerto, Conexão Sistema de Prótese, Arujá, Brazil) was added at the center of the block applying a position technique.

The study had a split-mouth (split-plot) design, in that 1 fixation technique was applied on 1 side of the mandible and the other technique on the other side. The randomization, carried out electronically (http://www.randomization.com), and the treatment assignment were performed by an author not involved in the animal selection or in any of the surgical procedures (D.B.). The treatment assignment was unveiled to the surgeon before fixation of the first graft. Histologic assessments were performed by an operator not involved in any of the clinical procedures (R.F.).

**Anesthesia procedures**

All animals were first sedated with Acepromazine (1.0 mg/kg; Acepran, Vetnil, Louveira, Brazil) administered subcutaneously. Subsequently, Xylazine (3.0 mg/kg; Calier Laboratories, Barcelona, Spain) and Ketamine 10% (60 mg/kg; SA National Union Pharmaceutical Chemistry, Embu-guaçu, Brazil) were administered intramuscularly. Anesthetic was also injected locally. Before surgery, the skin at the experimental sites was shaved and disinfected.

**Graft collection**

All surgeries were performed by a well-trained surgeon (E.N.C.M.). A linear incision about 4.0 cm long was carried out anteroposteriorly in the center of the skull. Galea, fascia, and periosteum were incised and the cortical bone of the skull was exposed. A trephine of 10 mm diameter was used, under a copious irrigation with saline, to collect 2 bicalvarial bone blocks from the calvaria. Periosteum, fascia, and galea aponeurosis were then sutured with Vicryl 4-0, while the skin was sutured with simple stitches using nylon 4.0. The bone blocks were maintained in sterile gauze moistened with saline solution.
Graft fixation

Subsequently, a 3 cm long incision of the skin was performed bilaterally at the mandibular angle. Muscles and periosteum were also cut, flaps were elevated, and the bone of the lateral convex side of the mandible angle was exposed.

Nine cortical perforations of the recipient sites were performed using a manufactured 7 × 7 mm square stent made of surgical steel. Nine holes were prepared at a distance of about 1 mm from the borders and from each other (see also de Oliveira Neto et al24). A stent was used as guide to perforate the cortical layer at the recipient bed using round burs under copious irrigation with saline (Figure 2a).

The adhesive was applied at the periphery of the inner surface of both grafts (Figure 2b). The grafts were subsequently fixed in the center of the prepared recipient sites, either with adhesive alone (Figure 2c) or secured with a fixation screw (Figure 2d). No modification of the graft configuration was applied to adapt the graft to the recipient sites, so that, due to the convexity of the mandible angle, a small gap between graft and bed was expected at the most peripheral regions. Subsequently, the deeper layers of the tissues were sutured with 4-0 Vicryl, while the skin was secured with 4-0 nylon sutures.

Oxytetracycline (0.2 mL/kg; Biovet, Vargem Grande Paulista, Brazil) was provided intraperitoneally the day of the surgery, and the dose was repeated the next 3 days. Additionally, buprenorphine (0.02 mg/kg; Bupaq, Richter Pharma AG, Wels, Austria) and Profenid (3.0 mg/kg; Ketojet, Agener União, São Paulo, Brazil) were administered intramuscularly.

Sacrifices and periods of healing

The animals were divided into 4 groups, each composed of 6 animals, sacrificed after 3, 7, 20, and 40 days of healing, respectively. The animals were sacrificed using an overdose of Thiopental (1.0 g, 2mL; Thiopentax, Cristália, Itapira, Brazil) administered intravenously. The experimental regions were subsequently dissected and reduced in individual blocks and maintained in 10% formaldehyde solution.

Histologic assessment

A standard preparation of the biopsies was performed. The blocks were dehydrated with increasing concentration of ethanol solutions and then embedded in resin (LR White hard grid, London Resin Co Ltd, Berkshire, UK). Subsequently, the blocks were polymerized in a buccal–lingual plane, starting from the center of the graft, using a slicing machine (Exakt,
Apparatebau, Norderstedt, Germany). Four sections were obtained, 2 representing the center and 2 the periphery of the graft. The sections were ground to a thickness of about 50–60 μm using cutting-grinding equipment (Exakt, Apparatebau, Norderstedt, Germany) and then stained with toluidine blue. The 2 central sections were used for histomorphometric measurements.

An Eclipse Ci microscope (Nikon Corporation, Tokyo, Japan), equipped with a digital video camera (Digital Sight DS-2Mv, Nikon Corporation, Tokyo, Japan) connected to a computer, was used for histologic evaluation. The inner profile of the graft and that of the parent bone were identified and the distance between them was measured, both at the most peripheral edge of the graft and in the region adjacent to the center of the graft (Figure 3). Measurements were performed at both sides of the graft at ×40 magnification.

The following regions were also identified within the interface region between the graft and the recipient site (Figure 3): (1) the zone closest to the center of the graft (internal zone), (2) a region corresponding to the osteotomies (osteotomy zone), and (3) the most peripheral region (external zone).

The composition of the interface was evaluated as the contents of: parent mineralized bone, parent bone marrow, bone graft, new mineralized bone, new marrow spaces, provisional matrix, bone debris and clot, vessels, inflammatory infiltrate tissue, connective tissue, and adhesive residues. A point counting procedure was adopted and a lattice of dimensions of about 0.4 × 0.6 mm, with squares of 50 μm in dimensions, was superposed over the tissues at a magnification of ×200 (Schroeder and Münnzel-Pedrazzoli). The lattice was centered in the middle of the gap; parts of the graft and of the parent bone were often included in the evaluated area, especially in the internal zone.

Statistical analysis
Mean values ± standard deviations were calculated for each outcome variable. The primary variables were the internal and external distances for the linear measurements and the new mineralized bone for the morphometric measurements. Secondary variables were the various tissues examined at the morphometric evaluation.

Differences between the cyan and cyan-screw groups were evaluated with the Wilcoxon signed rank test using SPSS Statistics (IBM, Chicago, Ill). The level of significance was set at α = .05.

Results
All biopsies were available for histologic processing, and no artifacts or losses of material were registered so that n = 6 was obtained for each group and for each period examined.

Table 1 reports the data on the distance between grafts and recipient bone. Table 2 reports the morphometric values of the most relevant variables of both cyan and cyan-screw groups. Values based on the means of the 3 zones evaluated are illustrated. Table 3 reports data on the mean, standard deviation, and confidence interval of the differences of the means between cyan and cyan-screw groups, with α set at .05.

Distance between graft and recipient bone site
After 3 days, the distance between the graft and the recipient bed was smaller for the cyan-screw group (internal zone 0.07 ± 0.06 mm; external zone 0.51 ± 0.27 mm) compared with the cyan group (internal zone 0.21 ± 0.11 mm; external zone 0.63 ± 0.35 mm) (Table 1). The difference between the 2 groups was statistically significant only for the internal zone. The mean difference for the internal zone was 0.14 ± 0.13, 95% CI 0.04, 0.25 mm, and for the external zone it was 0.12 ± 0.43, 95% CI –0.22, 0.46 mm.

The mean distance between graft and recipient bed was not reduced to zero during the healing period examined. In fact, no grafts were found to be incorporated to the recipient sites after 3, 7, and 20 days of healing. After 40 days, only 2 of 6 grafts in each group were partially incorporated to the recipient bed.

Morphometric evaluation (Tables 2 and 3)
The first evidence of new bone within the interface region was seen after 7 days of healing, in a very small amount (0.2 ± 0.6% for the cyan-screw group), limited to the internal zone close to the screw. After 20 days of healing, new mineralized bone was found, however, again at small percentages, reaching 2.3% ± 2.7% and 3.7% ± 4.8% at the cyan and cyan-screw sites, respectively. After the 40-day healing period, a total amount of new bone of 9.3% ± 6.6% in the cyan (Figure 4a through d) and 15.4% ± 9.8% in the cyan-screw (Figure 5a through d) groups was found. Concomitantly to the formation of new mineralized bone, marrow spaces were developing, reaching percentages of 3.3% ± 3.6% and 7.0% ± 11.2% at the cyan and cyan-screw sites, respectively.

In all cases, new bone appeared to be formed from the parent bone of the recipient sites. The highest proportion of new bone was found in the cyan-screw group in the osteotomy zone. In some cases, new bone was connecting the graft to the parent bone in the peripheral zones subjacent the periosteum (Figure 6). Only 2 of 6 grafts were partially in contact with the recipient bed in both groups (Figure 7). The remaining regions of these grafts, as well as the other grafts, were separated by adhesive residue (Figure 8) or by connective tissue, either from the parent bone (Figure 9a), or from bridges of new bone generated from the recipient bed (Figure 9b and c). The presence of adhesive clearly appeared to be an obstacle to a proper integration of the graft to the recipient bed.

Bone resorption activity at the grafts was low in all periods of healing and already visible after 7 days, but it was limited to areas around the screw. After 40 days, the few grafts that were partly incorporated to the parent bone showed zones of remodeling, while other grafts appeared to be partly resorbed, especially toward the parent bone. However, most grafts showed minimum changes of the peripheral contour.

The adhesive was still found after 40 days of healing at a high percentage in both groups and was mostly interposed between grafts and the parent/newly formed bone (Figures 8 and 9c).
FIGURES 3–5. **FIGURE 3.** Linear measurements (red lines) at the peripheral edge of the graft and in the region adjacent to the center of the graft. Internal, osteotomy, and external zones evaluated morphometrically (yellow squares). (a) Cyan group. (b) Cyan-screw group. **FIGURE 4.** Cyan sites at the (a) 3-day, (b) 7-day, (c) 20-day, and (d) 40-day healing periods. Toluidine blue stain; images originally merged at magnification ×20. **FIGURE 5.** Cyan-screw sites at the (a) 3-day, (b) 7-day, (c) 20-day, and (d) 40-day healing periods. Toluidine blue stain; images originally captured and merged at magnification ×20.
**DISCUSSION**

The aim of the present experiment was to study the sequential healing at the interface between the recipient parent bone and autologous bone grafts, fixed using cyanoacrylate adhesive, with or without the placement of an additional titanium fixation screw to improve the fixation and reduce the thickness of the adhesive layer. For this aim, autologous bone grafts were harvested from the calvaria and fixed laterally to the angle of the mandible using NB-Cn, either alone or in conjunction with a position screw. It was shown that NB-Cn interferes with the healing processes. In fact, after 40 days of healing, 8 of 12 grafts in the 2 groups were not incorporated at all to the parent bone, despite new bone forming from the parent bone. The remaining 4 grafts presented sparse incorporation to the recipient site, especially in correspondence of the peripheral areas, close to the periosteum or in proximity of the osteotomies.

The low success of integration of the grafts to the recipient sites registered in the present study does not seem related to the possible cytotoxicity of the cyanoacrylate adhesives, as shown in an in vitro study. Osteoblastic cells derived from human alveolar bone of the mandible were cultured with methyl-2-cyanoacrylate, with ethyl-2-cyanoacrylate, or without cyanoacrylates. It was concluded that ethyl-2-cyanoacrylate was biocompatible and could be used for graft fixation.

In addition, the strength of cyanoacrylate adhesives was tested in an in vitro study in which 4 adhesives, namely octyl cyanoacrylate, n-butyl cyanoacrylate, a novel methyl methacrylate, and a novel cyanoacrylate derivative, were tested for their microtensile and shear bond strengths. It was shown that n-butyl cyanoacrylate had the greatest potential for fixation of fractured bones in craniofacial surgical applications.

In the present study, the grafts seemed to be well fixed to the recipient sites after all periods of observations. However, adhesive and connective tissue were mainly interposed between grafts and recipient sites. The results from the present study are in agreement with another study in rabbits. In that study, bone blocks were harvested from the calvaria, grafted anteriorly to osteotomies, and fixed to the cortical bone layer of the calvaria with either a screw or a cyanoacrylate adhesive. The healing was studied after 5, 15, 30, 60, and 120 days. The grafts of the screw-retained group were well incorporated to the recipient sites after 120 days, while the grafts of the glue-retained group were only partially integrated.

In another experiment in rabbits, autogenous bone blocks were harvested from the tibia and fixed to the lateral aspect of the angle of the mandible with n-butyl cyanoacrylate or a mini screw. The animals were sacrificed after 1 and 3 months of healing. A higher rate of graft necrosis was found in the cyanoacrylate group compared with the screw group after 1 and 3 months of healing. Bone formation between graft and

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Cyan Group</th>
<th>Cyan-Screw Group</th>
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<tbody>
<tr>
<td></td>
<td>Internal Zone</td>
<td>External Zone</td>
</tr>
<tr>
<td>3 days, mean ± SD</td>
<td>0.21*± 0.11</td>
<td>0.631 ± 0.35</td>
</tr>
<tr>
<td>7 days, mean ± SD</td>
<td>0.211 ± 0.11</td>
<td>0.561 ± 0.19</td>
</tr>
<tr>
<td>20 days, mean ± SD</td>
<td>0.211 ± 0.20</td>
<td>0.491 ± 0.22</td>
</tr>
<tr>
<td>40 days, mean ± SD</td>
<td>0.23 ± 0.12</td>
<td>0.30 ± 0.21</td>
</tr>
</tbody>
</table>

*P < .05 between cyan and cyan-screw groups.

†P < .05 between internal and external zones.

**Table 2**

Morphometric evaluation of the tissues of interest in the cyan and cyan-screw groups; means ± standard deviations; n = 6 for each period

<table>
<thead>
<tr>
<th></th>
<th>New Bone</th>
<th>Primitive Marrow</th>
<th>Provisional Matrix</th>
<th>Clot Debris</th>
<th>Inflammatory Infiltrate Tissue</th>
<th>Connective Tissue</th>
<th>Adhesive</th>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyan</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>3.7 ± 4.2</td>
<td>4.4 ± 4.4</td>
<td>0.4 ± 1.0</td>
<td>63.9 ± 12.7</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>Cyan-screw</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>8.8 ± 5.8</td>
<td>3.5 ± 5.7</td>
<td>0.4 ± 1.0</td>
<td>44.1 ± 11.8</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyan</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0*± 0.0</td>
<td>4.2 ± 3.0</td>
<td>8.3 ± 5.0</td>
<td>54.6 ± 12.3</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cyan-screw</td>
<td>0.2 ± 0.6</td>
<td>0.1 ± 0.2</td>
<td>1.4 ± 3.5</td>
<td>3.2 *± 2.7</td>
<td>6.8 ± 7.8</td>
<td>9.8 ± 8.7</td>
<td>49.5 ± 20.5</td>
<td>0.8 ± 0.9</td>
</tr>
<tr>
<td>20 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyan</td>
<td>2.3 ± 2.7</td>
<td>2.9 ± 4.5</td>
<td>5.6 ± 7.1</td>
<td>0.0 ± 0.0</td>
<td>7.7 ± 6.8</td>
<td>9.8 ± 9.6</td>
<td>36.7 ± 11.4</td>
<td>1.3 ± 2.2</td>
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<td>Cyan-screw</td>
<td>3.7 ± 4.8</td>
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<td>0.3 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>7.2 ± 7.7</td>
<td>22.6 ± 26.7</td>
<td>29.1 ± 18.5</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>40 days</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyan</td>
<td>9.3 ± 6.6</td>
<td>3.3 ± 3.6</td>
<td>2.4 ± 5.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>12.8* ± 6.6</td>
<td>43.0 ± 17.0</td>
<td>1.3 ± 1.5</td>
</tr>
<tr>
<td>Cyan-screw</td>
<td>15.4 ± 9.8</td>
<td>7.0 ± 11.2</td>
<td>0.2 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>5.4 ± 13.1</td>
<td>29.6* ± 6.9</td>
<td>28.9 ± 16.7</td>
<td>1.9 ± 1.5</td>
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</table>

*P < .05 between cyan and cyan-screw groups.
recipient site was found to be significantly higher in the screw group compared with the cyanoacrylate group.

However, positive results were obtained in another animal study in which n-2-butyl cyanoacrylate was used. Calvarial grafts were harvested from 6 New Zealand rabbits and fixed at the lateral aspect of the mandible using either NB-Cn in one side or a titanium screw (TiS) in the other. The rabbits were sacrificed after 4 and 8 days. Microcomputed tomography analysis and molecular analysis were performed to assess gene expression of interleukin-6, interleukin-10, and tumor necrosis factor-α. The NB-Cn promoted a higher bone volume and density preservation. Genes from the NB-Cn group were upregulated compared with those in the TiS group at 4 days, while in the 8-day group, the osteoclastogenesis-related genes were upregulated in the screw group.

In a study in guinea pigs, bone grafts were harvested from the calvarial bone and placed adjacent to the osteotomies. The recipient bed was demineralized with 50% citric acid (pH 1.0) for 3 minutes in the test group, while the control was left untreated. The blocks were secured with a resorbable membrane that was fixed to the parental bone with NB-Cn in the peripheral zone. No new bone was seen after 7 days. However, after 30 days, new bone increased to 17% and 34% at the control and test sites, respectively. Mineralized bone increased after 90 days to 39% and 54% at the control and test sites, respectively.

In the present study, a negligible amount of new mineralized bone was found after 7 and 20 days of healing. After 40 days, the percentage was still lower (9%–15%) than the previously cited paper. The difference could be related to the fact that, in the present study, only a selected region of interface between graft and recipient parent bone was evaluated.

In the present study, the new bone that generated from the

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>New Bone</th>
<th>Primitive Marrow</th>
<th>Provisional Matrix</th>
<th>Clot/Debris</th>
<th>Inflammatory Infiltrate Tissue</th>
<th>Connective Tissue</th>
<th>Adhesive</th>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>Mean ± SD</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 1.6</td>
<td>19.8 ± 21.6</td>
<td>–0.1 ± 0.2</td>
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<tr>
<td>Lower, Upper 95%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>–12.6, 2.4</td>
<td>–5.3, 7.0</td>
<td>–0.2, 0.1</td>
<td></td>
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<tr>
<td>P value</td>
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<td>1.000</td>
<td>1.000</td>
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</tr>
<tr>
<td>7 days</td>
<td>Mean</td>
<td>–0.2 ± 0.6</td>
<td>–0.1 ± 0.2</td>
<td>–1.4 ± 3.5</td>
<td>–3.2 ± 2.7</td>
<td>–2.7 ± 8.9</td>
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<tr>
<td>Lower, Upper 95%</td>
<td>0.2, 0.2</td>
<td>0.3, 0.1</td>
<td>4.3, 1.4</td>
<td>5.3, –1.1</td>
<td>9.8, 4.5</td>
<td>12.2, 9.2</td>
<td>15.3, 25.4</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.317</td>
<td>.317</td>
<td>.317</td>
<td>.042</td>
<td>.600</td>
<td>.753</td>
<td>.463</td>
<td></td>
</tr>
<tr>
<td>20 days</td>
<td>Mean</td>
<td>–1.4 ± 5.5</td>
<td>0.6 ± 7.3</td>
<td>5.3 ± 7.5</td>
<td>0.0 ± 0.0</td>
<td>0.5 ± 6.1</td>
<td>–12.9 ± 28.2</td>
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<tr>
<td>Lower, Upper 95%</td>
<td>–5.7, 3.0</td>
<td>–5.2, 6.4</td>
<td>–0.7, 11.3</td>
<td>NA</td>
<td>–4.4, 5.4</td>
<td>–35.4, 9.7</td>
<td>–10.4, 25.6</td>
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</tr>
<tr>
<td>P value</td>
<td>.600</td>
<td>1.000</td>
<td>.144</td>
<td>1.000</td>
<td>.581</td>
<td>.345</td>
<td>.752</td>
<td></td>
</tr>
<tr>
<td>40 days</td>
<td>Mean</td>
<td>–6.2 ± 14.8</td>
<td>–3.7 ± 12.6</td>
<td>2.1 ± 5.6</td>
<td>0.0 ± 0.0</td>
<td>–5.4 ± 13.1</td>
<td>–16.8 ± 12.5</td>
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</tr>
<tr>
<td>Lower, Upper 95%</td>
<td>–18.0, 5.7</td>
<td>–13.8, 6.4</td>
<td>–2.3, 6.6</td>
<td>NA</td>
<td>–15.9, 5.1</td>
<td>–26.7, 6.8</td>
<td>–4.0, 32.2</td>
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<tr>
<td>P value</td>
<td>.345</td>
<td>.345</td>
<td>.593</td>
<td>1.000</td>
<td>.317</td>
<td>.028</td>
<td>.116</td>
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**Figures 6–8.** **Figure 6.** Bone was found in all zones, including the peripheral zones, where it was in close contact with the periosteum and, in some case, was integrating the graft to the parent bone (yellow arrows). Toluidine blue stain; image originally captured and merged at magnification ×40. **Figure 7.** Few grafts were partially in contact with the recipient bed in both groups. Toluidine blue stain; image originally captured and merged at magnification ×100. **Figure 8.** Most grafts were separated from the parent bone by adhesive residues. Toluidine blue stain; image originally captured and merged at magnification ×40.
recipient sites could not properly reach the graft because of the presence of high amounts of unresorbed adhesive and connective tissue interposed between graft and parent bone. This, in turn, means that the adhesive appeared to interfere with the healing processes and consequently with the integration of the graft to the new bone forming from the parent bone of the recipient site.

It might be argued that a longer period of healing (eg, 90 days) may have improved the results. However, the adhesive was found to be devoid of cells and vessels, and connective tissue often separated the graft from the recipient site. Moreover, the percentage of adhesive and connective tissue together only decreased from 64% to 56% in the cyan group while increasing from 45% to 59% in the cyan-screw group. This, in turn, means that the potential for bone formation to incorporate the grafts seemed to be very limited. Nevertheless, longer periods of healing than those used in the present experiment should be studied.

It has to be considered that, in a similar experiment in rabbits in which the grafts were also collected from the calvaria but fixed to the lateral wall of the mandible using 2 different methods of screw fixation without adhesive, all grafts were well incorporated to the recipient sites after 40 days of healing.23

In the present study, some grafts were integrated with the parent bone in the peripheral zone, in close contact with the periosteum. This phenomenon was described previously in another similar study in which grafts from the calvaria were fixed to the lateral aspect of the mandible.24

It should be emphasized that, even though efforts were made to keep the adhesive in the periphery of the graft, the

**Figure 9.** Connective tissue surrounding parts of the graft from the parent bone (a) as well from bridges of newly formed bone (b). Toluidine blue stain; (a) original magnification ×100, (b) original magnification ×40, (c) originally captured and merged at magnification ×100.
adhesive spread around the inner surface of the small graft. In humans, the larger dimensions of the bone block grafts may allow better control during placement of the adhesive in the periphery of the graft. Furthermore, it has to be considered that the results from the present study were from phylogenetically lower animals than humans. The differences illustrated previously may suggest the need to perform human randomized clinical trials to compare the use of NB-CN adhesive with different types of graft fixation in order to discover different possible outcomes. Even though the use of such adhesive may simplify the fixation of a graft to the recipient site, clinicians should take into account the results from the present experiment and use this adhesive with caution in similar clinical conditions.

In conclusion, the use of NB-CN to fix an autologous bone graft to the lateral aspect of the mandible was maintained but did not incorporate the graft to the recipient sites. The additional use of a fixation screw did not improve the results of healing.

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NOTE

All the authors declare that they have no conflict of interest with regards to the materials used in the present study.

REFERENCES