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Full Length Research Paper

Evaluation of N 2-butyl cyanoacrylate adhesive material in the fixation of dentoalveolar mandibular fractures in dogs

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The prevalence of dentoalveolar fracture is usually seen among children and adolescents boys, 3 times more than girls that is due to its etiology, namely road traffic accident (minor accident), child abuse, and fall from high or epileptic seizures. It may occur as an isolated clinical entity or in conjunction with any other bone fracture. Bone adhesive to hold these fractures would be of great benefit. Recently N2 butylcyanoacrylate is being clinically used with minimal complications. The study is aimed at evaluating the efficacy of N2 butylcyanoacrylate adhesive material in the fixation of dentoalveolar mandibular fractures. 6 adults mongrel dogs 12 to 24 months old, weighting 9 to13 kg on average were used in this study. Dogs were anesthetized. A full mucoperiosteal flaps were raised on the premolar area; two vertical 1 cm osteotomies were carried out mesial and distal joined with a horizontal one. Few drops of N2-Butylcyanoacrylate (N2BCA) Histoacryl were applied to fix the fracture; on the left side the fractures were fixed conventionally with circumdental wire. Suturing is done on both sides. Despite of the slow healing rate at the N2BCA site both groups showed an uneventful healing that is free of inflammation and displacement. Both methods showed similar results; we claim that N2 butylcyanoacrylate is an alternative to circumdental wiring in fixation of dentoalvolar fractures.

Key words: Dentoalveolar fracture, adhesives, dogs.

INTRODUCTION

Dentalveolar fractures are fractures usually seen with avulsion, subluxation or fractures of the teeth with or without association with a fracture of the alveolar process, and often involve the overlying soft tissue nearby (Ippalitov et al., 2003). It may be seen as an isolated clinical entity or in conjunction with any other facial bone fractures. The prevalence of dentoalveolar fractures as posted by Andreason and Andreason (1994) is that more than three billion of the world population is dental trauma victims. Dentoalveolar fractures are seen among children and adolescents boys three times more than girls, this is due to the fact that the etiology of

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dentoalveolar fractures usually are road traffic accident (RTA) “minor accident” falls from height, epileptic seizures, and child abuse. Dentoalveolar fractures can also be iatrogenic such as during extraction of teeth, endoscopy or endotracheal intubation. Dentoalveolar trauma is a challenge to dentistry, especially in young patients, where early tooth loss can compromise oral functions, esthetics, self-esteem and physiological impairment as claimed by Saito et al. (2009) and supported by Alonge et al. (2001). Although not much of cases reported, experience suggests that dentoalveolar injury is common among patients with maxillofacial fractures. Open reduction and fixation is indicated when the fracture is significantly displaced, when the trauma is complex with damage to soft tissue or when there is missing teeth due to trauma or edentulous jaw, mini-plates or micro-plates are used. A particular problem is the bone fixation of small pieces of bone where screws and plates placing are impossible or impractical or precluded due to presence of tooth buds. Bone adhesive agents strong enough to hold these fragments in their anatomical position would be of great benefit (Zhang et al., 2003; Ganta et al., 2003).

Fracture fixation using adhesives is a promising alternative in maxillofacial surgeries, replacing the plate and screws system. The advantages include the ease of application and avoidance of drilling holes that may weaken the bone and causes additional fractures (Kandalam et al., 2013).

Surgeons have been using tissue sealants and adhesives since the early nineteenth century (Currie et al., 2001). There are presently four types of tissue adhesives: Fibrin sealants, collagen base adhesive, protein base sealant “polyurethane” and synthetic polymer – based materials “cyanoacrylates”. The fibrin gives present in the market are normally Tisseel® and hemaseel® they are used in the head and neck surgery as haemostatic agents over large surface area. Collagen base adhesive has a week adhesive bond to adjacent tissue (Streiff et al., 2002; Passage et al., 2002; Raanani et al., 2001).

Polyurethane is used in heart valves and breast implants. N2 polycyanoacrylate have been used for years as wound closure materials with good success in superficial wound. Longer chain Cyanoacrylates with properties more conductive to medical use have been developed recently, one of these approved by the US Food and Drug Administration (FDA) is N-2- butyl-cyanoacrylate (NBCA) some are colored with dye example Histoacryl. Blue, others are without. Dye is added to enhance visibility of the liquid during application (Davis and Derlet, 2013).

Cyanoacrylate is a generic name for a family that includes methyl2cyanoacrylate and ethyl 2-cyanoacrylate which are both not used in medicine. The ones used are the butyl 2-cyanoacrylate and recently developed 2-octylcyanoacrylate to reduce toxicity, skin irritation and allergic reactions. N-butyl-cyanoacrylate was used in cleft lip and palate in a comparative study with Monocryl. At the University Medical Center in the Netherland and the result showed no significant difference (Buckley and Beckman, 2010; Quatela et al., 1993; Choi et al., 2009; Elgazzar et al., 2009; Ali, 1988; Kulkarani et al., 2007; Tayfun et al., 2005).

Adhesives, while in early development, also show a promising chemical profile and will be of significant benefit to oral and maxillofacial surgical patients (Habib et al., 2013)

The aim of this study is to evaluate clinically and histologically the efficacy of N2-butyl cyanoacrylate adhesive material in fixation of mandibular dentoalveolar fractures in dogs.

MATERIALS AND METHODS

This is an experimental study using split mouth technique.

Experimental animals

A total of 6 adult healthy male mongrel dogs about 12 to 24 months old, weighing 9 to 13 kg on average, were kept under proper veterinary care, with free access to water, and nutritional support throughout the entire study. The experimental protocol was approved by the ethical committee in the use of animals “Alexandria University”.

Experimental groups

For each animal, the right side of the mandible was considered as the study group and the left side as the control group. N2-Butylcyanoacrylate, (B/BRAUN-ASECULAP –AG Tuttingen/ Germany) adhesive material in the form of (Histoacryl) Blue 0.5 ml liquid in a plastic vial.

Pre-operative phase

All dogs were examined by the animal house veterinarian to exclude diseases. Each animal received a dose of antibiotic “enrofloxacin”(Enrofloxacin 10% from El Nasr Pharmaceuticals chemicals Co., Egypt) 6 mg/kg body weight just before the operation.

Operative phase

Anesthesia

The animals were injected with "xylazine" hydrochloride 2% (Xyla-ject).(ADWIA S.A.E 10th of Ramadan city, Egypt.) in a dose of 1 mg/kg body weight intramuscular as a preanaesthetic medication. The animals were injected with Ketamine hydrochloride (Tekam from Hikma pharmaceuticals Co., Amman, Jordan) in a dose of 5 mg/kg body weight as anesthetic medication.

Surgical procedure

The surgical site was scrubbed with povidone iodine 10% surgical

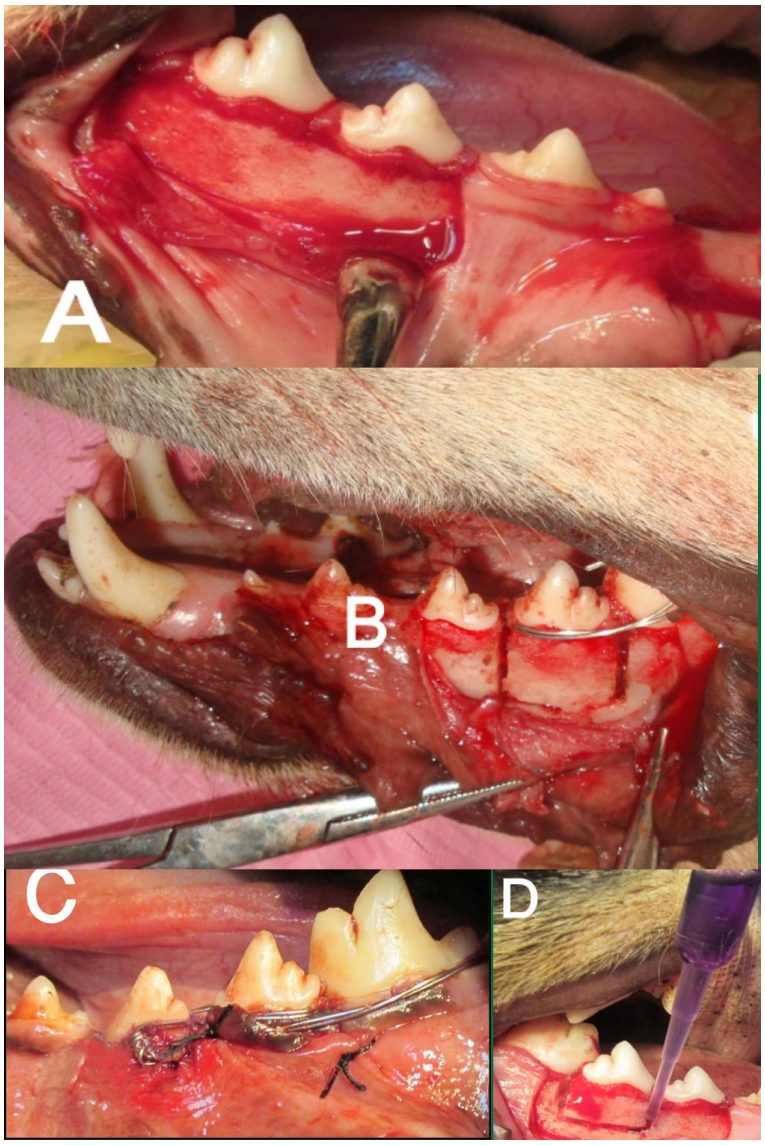

Figure 1. A: Full thickness flap; B: Bone cutting; C: Wire splint at the control site; D: Application of N2BCA adhesive Histoacryl; E: Suturing of the flap.

scrub solution and a surgical draping was performed. A full mucoperiosteal flap of about 20 mm was raised over the premolar area to expose the alveolar bone (Figure 1A). Two vertical 1 cm bicortical osteotomies were carried out 3 to 5 mm depth including tooth socket mesial and distal to the right premolar using surgical bone. A horizontal osteotomy is carried to join the two vertical cuts inferiorly the 1 cm^2 cortical fragment is displaced with an elevator (Figure 1B). The left side fractures were fixed with conventional wire (Figure 1C).

On the right side a few drops of the N2-BCA (Histoacryl) adhesive were added and the fragment was hold in position and pressed by finger for two minutes and then the flap were repositioned (Figure 1D).

Both sides' flaps were sutured with 3.0 chromic catgut Absorbable sutures material. The application of the adhesive took 6 to 15 s and the polymerization took 3 to 10 s the manipulation and application wire consumed 10 to 15 min.

Post-operative phase

Follow up

1. Immediately after surgical procedure, animals received "enrofloxacin 10%" 0.6 mg/kg body weight[1M] for five days as prophylactic antibiotic.
2. Cataflam 5 mg/kg (Novartis pharma, Cairo, Egypt) was given intramuscular as analgesic for 3 days.
3. The animals were kept in cages under observation of a veterinarian to assess the presence or absence of any signs of infection, wound dehiscence or rejection.
4. Glucose water was given to all animals on the first post-operative day.
5. Soft diet milk and broth on the first day.
6. The sutures were removed 7 days later under general anesthesia.

7. Two dogs were sacrificed at different intervals 6, 9 and 12 weeks after surgery by anesthetic overdose of ketamine HCL.

Histological aspect

The surgical areas of the bone were dissected out and removed as blocks with water cooled disc all sections were prepared as decalcified sections except one section of one dogs prepared as calcified section to examine the difference in bone formation in the other sections.

Preparation of the decalcified sections

Fixation

1. All specimens were fixed in 10% buffered formalin for 2 weeks and then washed under running water to remove the formalin.
2. Specimens were decalcified with 5% Trichloracetic acid and wasted under running water for 24 h to remove the acid.
3. The specimens were gradually dehydrated by placed in ascending grades of alcohol 50, 70, ad 90% and absolute concentration of alcohol.

Clearance

Specimens were placed into xylene to wash out alcohol.

Infiltration

Specimens were infiltrated to replace xylene by placing it in a dish of melted paraffin wax under 60°C constant temperature ovens, for about 2 to 3 h.
After complete infiltration, the specimens were embedded in the center of a box of paraffin wax and were serially sectioned at 5 microns in a sagittal direction by rotary microtome.

Mounting

The section were mounted on clean slides with a thin film of egg albumin in a constant temperature drying table 56°C to adhere the sections to the slides.

Preparation for staining

Sections were deparaffinized in two changes of absolute alcohol two minutes each to ensure the removal of the xylene from section, then hydrated by descending grades of alcohol 90, 70 and 50% two minutes each and finally through distilled water.

Staining

Hematoxylin and eosin stains were used. The steps are as follows:

1. Deparaffinized sections were stained with hematoxylin for 5 min.
2. Washed in running water for 3 min and dried.
3. Counter stained in 1% eosin stain for one minute and washed with water.
4. Dehydrated in ascending grades of alcohol and cleared in xylene.
5. A cover slip was finally applied by Canada balsam to the section and left for some hours at room temperature for firm adhesion.

Resulting interpretations:
Nuclei: Deep blue.
Cytoplasm/collagen/bone: Pink.

Trichrome stain (Gomori's)

It was used for detection of collagen fibers and osteoid tissue formation. The staining steps are as follows:

1. Deparafinized sections were washed in running tap water for 3 min.
2. Nuclei were stained with hematoxylin.
3. Sections were then washed in tap water and rinsed in distilled water.
4. Sections were stained in chromotrope- green mixture for 20 min.
5. Rinsed well in 0.2% acetic acid for 2 min.
6. Finally blotted with filter paper, dehydrated in absolute alcohol, cleared in xylene and mounted with Canada balsam.

Resulting interpretations:
Collagen: Green.
Nuclei: Blue-gray.
Cytoplasm and RBCS: Red.
Evaluation of bone regeneration in the surgical was then carried out under light microscope.

Preparation of calcified sections

The specimens that include the bone graft and membranes were immediately fixed in 4% buffered formaldehyde for one week. Then the specimens were dehydrated using ascending alcohols concentrations (50, 70, 90 and 100%) in a graded series of ethanol solutions using a dehydration system under agitation and vacuum.

Specimens were then defatted in Xylene and thereafter, samples were embedded in transparent chemically polymerized Methyl Metha-acrylate resin. ((Methyl Metheacrylate 99%, sigma-Aldrich, Steinlien Germany).

After polymerization the specimens were cut using a precision cutting machine (Microcut 150 precision cutter- Metkon, Bursa, Turkey).

Serial sections were cut about 0.3 mm for conventional Stereomicroscopy. The sections were stained using Stevanl's blue and van Geison's stains. The sections were imaged and analyzed using light Stereomicroscopy equipped with a high resolution camera (Olympus BX 61, Hamburg, Germany).

RESULTS

Clinical

All dogs remained active, alert and survived the experimental protocol in good health; they started to eat on the first post-operative day normally. The absence of abnormal immunological response at the operative sites confirmed the biological safety of the N2BCA. Healing of the incision was uneventfully without any signs of infection, inflammation nor wound dehiscence (Table 1). The mucosa over the operative sites appeared normal and all fractured showed stability without displacements.

Table 1. Distribution of the samples in the N2BCA and control sides at weeks 3, 9 and 12 by means of the degree of inflammations.

Degree of inflammations	N2BCA (%)	Control (%)
Week 3		
None	1 15	1 20
Low	4 70	3 50
Moderate	1 15	2 30
Extensive	0	0
Total	6	6
Week 9		
None	1 20	1 20
Low	3 50	3 50
Moderate	2 30	2 20
Extensive	0	0
Total	6	6
Week 12		
None	4 70	3 50
Low	2 30	2 30
Moderate	0	1 20
Extensive	0	0
Total	6	6

The classification used to evaluate inflammation was graded 0, non; 1, low; 2, moderate; 3, extreme.

Table 2. Distribution of the samples in the N2BCA and wire sides at 3, 9, 12 by means of bone necrosis.

Level of necrosis	N2BCA	Control
Week 3		
None	4	5
Limited necrosis	2	1
Total necrosis	0	0
Total	6	6
Week 9		
None	3	4
Limited necrosis	3	2
Total necrosis	0	0
Total	6	6
Week 12		
None	2	3
Limited necrosis	3	3
Total necrosis	1	0
Total	6	6

Bone resorption and necrosis of tissue varies from mild to moderate (Table 2). Specimens were prepared by longitudinal serial section and stained with Hematoxylin and Trichrome for histological evaluation (H & E) stain was used to evaluate granulation tissue, new bone formation “woven bone” and lamellar bone formation. Trichrome stain was used to evaluate collagen formation and to better differentiation between bone and collagen.

6th week postoperatively

Control group: The alveolus was filled with moderate

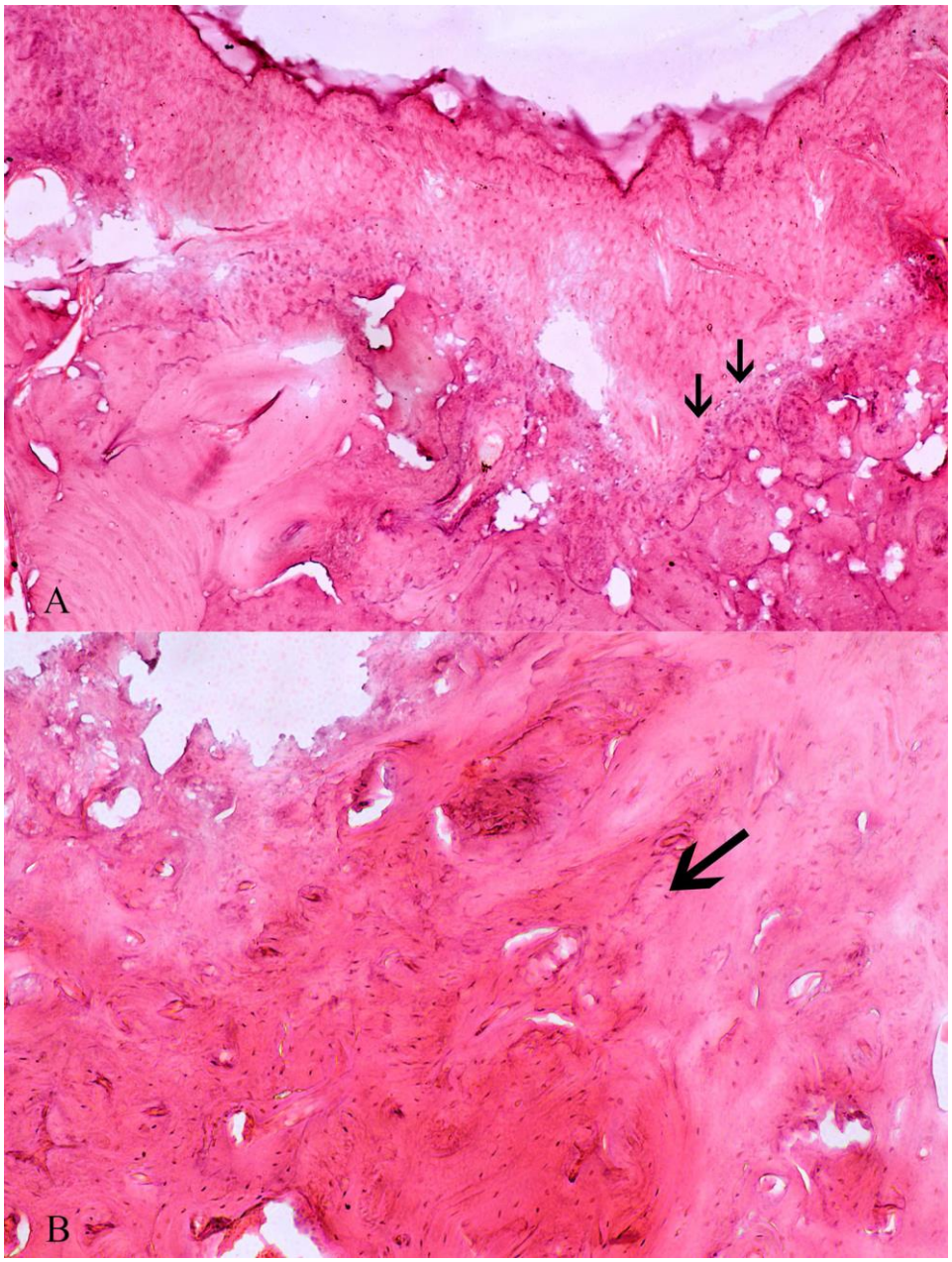

Figure 2. A: Light micrograph showing formation of granulation tissue. The arrows show the newly formed bone. B: Increased granulation tissue and bone formation towards the center of the socket.

density of granulation tissue, organization of newly formed woven bone along the lateral walls of the socket and confined within the apical region (Figure 2a). A line of osteointegration between the newly formed bone and the old bone is seen at the apical part of the socket.Large number of ostocytes is enclosed within the newly formed trabeculae (Figure 2a).

Study group: The socket was filled with dense aggregation of granulation tissues and more woven bone was seen extending along the lateral wall of socket and to a higher level in the healing socket (Figure 2b). The newly formed woven bone was more organized with greater thickness of bony trabeacule. A greater number of enclosed osteocyte and osteoblast cells lining was detected along the line of osteointegration between the new and old bone interface.

9 weeks postoperatively

Control group: The socket was filled with newly formed woven bone. Newly organized Haversian system is seen

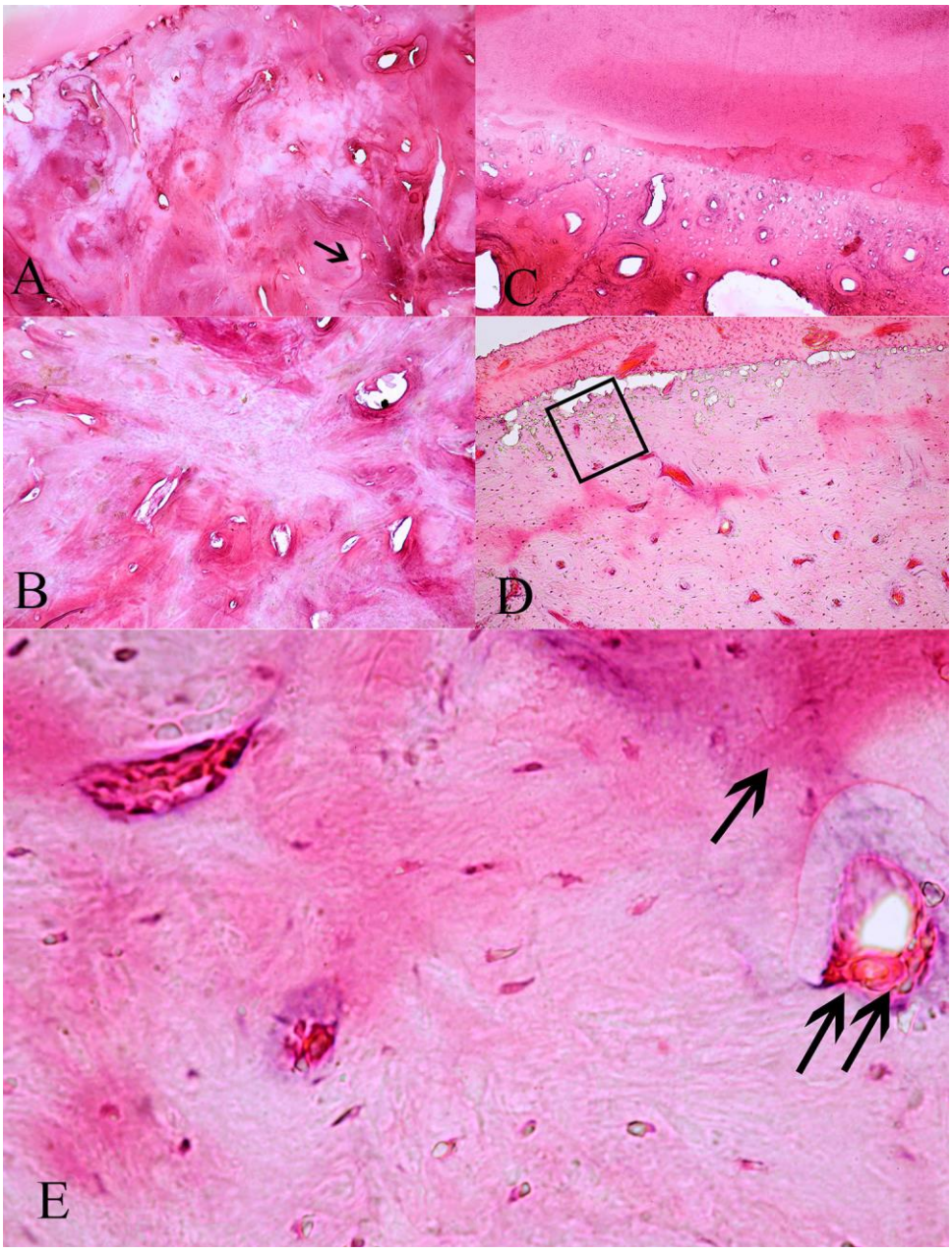

Figure 3. A: Control group, 6 weeks newly formed woven bone with some organizedosdteones and psteointegration, B: Higher magnification of the newly organizedosteones enclosing osteoblast; C: study group, 6 weeks showing dense osteocytes content, osteoblast lining and enclosed blodd supply; D: Higher magnification of the osteocytes and blood vessels.

within dense connective tissue lining (Figure 3a). Also, the line of osteointegration between the new bone and the boundary of the old bone was evident at the lateral surface of the healing socket (Figure 3b). The healing features of the group showed dense fibrous tissue with enclosed osteocytes and blood supply within the new bone (Figure 3c).

Study group: A control mass of newly formed bone was seen lining the lateral wall of the healing socket extending towards its apical part. Well organized primary osteons formations were seen enclosing large number of osteocytes and more blood supply (Figure 3d). The line of fusion between the newly formed bone and the old bone was obvious and easily detected (Figure 3e). The lateral part of the socket exhibited newly formed bone contained high density of osteocytes, lined by continuous layer of osteoblasts and enclosed bone marrow spaces containing high blood supply (Figure 3f). At the stage the line of fusion between the old bone and the newly formed

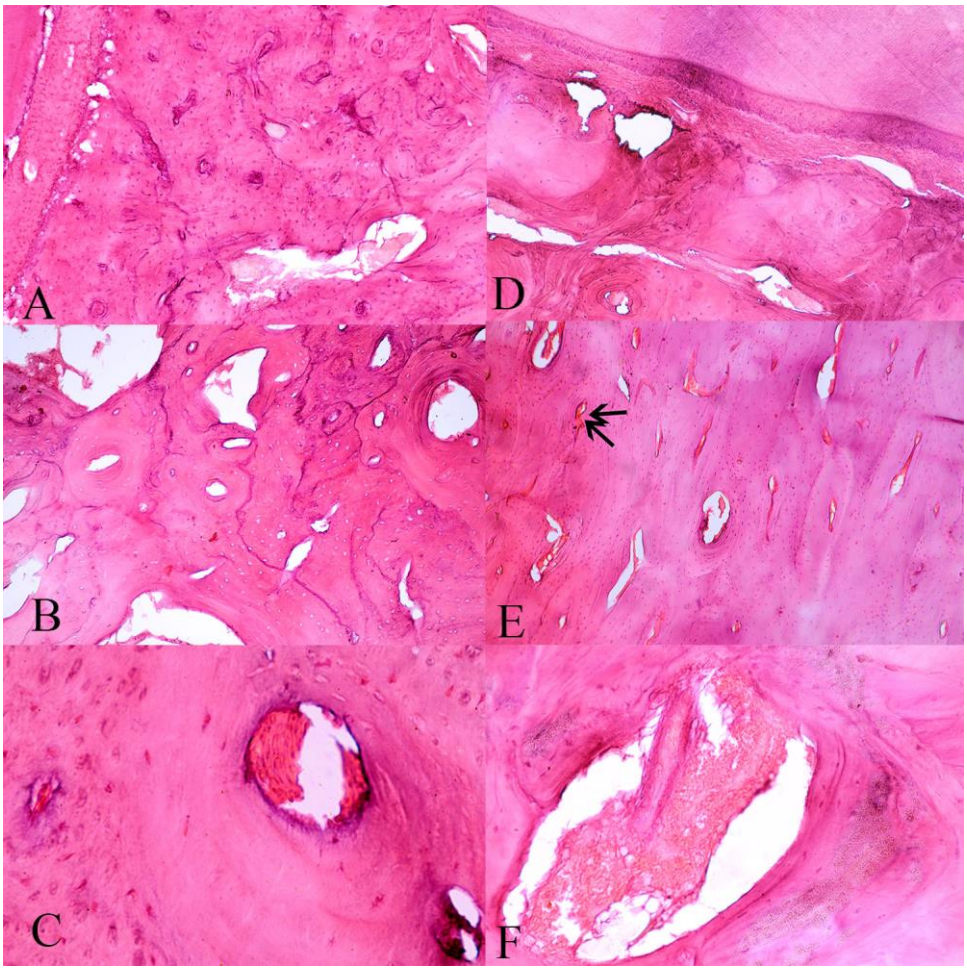

Figure 4. A: Light micrograph (Control, 12 weeks) showing a part of compact bone with Haversian system. B: Higher magnification showing complete osteointegration between the old and newly formed bone; C: Higher magnification showing vascularity of the newly formed osteons; D: Light micrograph (study group, 12 weeks) showing dense compact bone filling the healing socket. Complete osteointegration between the newly formed and the old bone; E: Higher magnification showing complete mature compact bone and rich blood vessels in the Volkimann's canal.

bone was evident.

12 weeks postoperatively

Control group: In the group, the socket was entirely filled with compact bone. A higher magnification revealed the structural organization of the Haversian system with high rate of bone remodeling. Large number of osteocytes cells entrapped and enriched vascular spaces at the lateral wall of the socket (Figure 4a). The socket was filled with mature compact bone and surrounded by narrow spaces of rich blood supply (Figure 4b).

Study group: In this group, the socket was filled with mature compact bone at lateral wall of the socket; the compact bone was of higher density than the previous group enclosing central Haversian canals (Figure 4c). Complete osteointegration between the newly formed and the old bone was seen at the walls of the socket. Areas of profound blood supply were abundant near the area of osteointegration. A great density of enclosed osteocytes entrapped within the bony osteons (Figure 4d). The compact bone was of higher density than previous weeks. It consisted of enriched blood supply within the Haversian system marrow spaces (Figure 4e).

Trichrome stain

6 weeks

Control group: Collagen fibers, dilated blood vessels inflammatory cells, granulation tissue and new bone

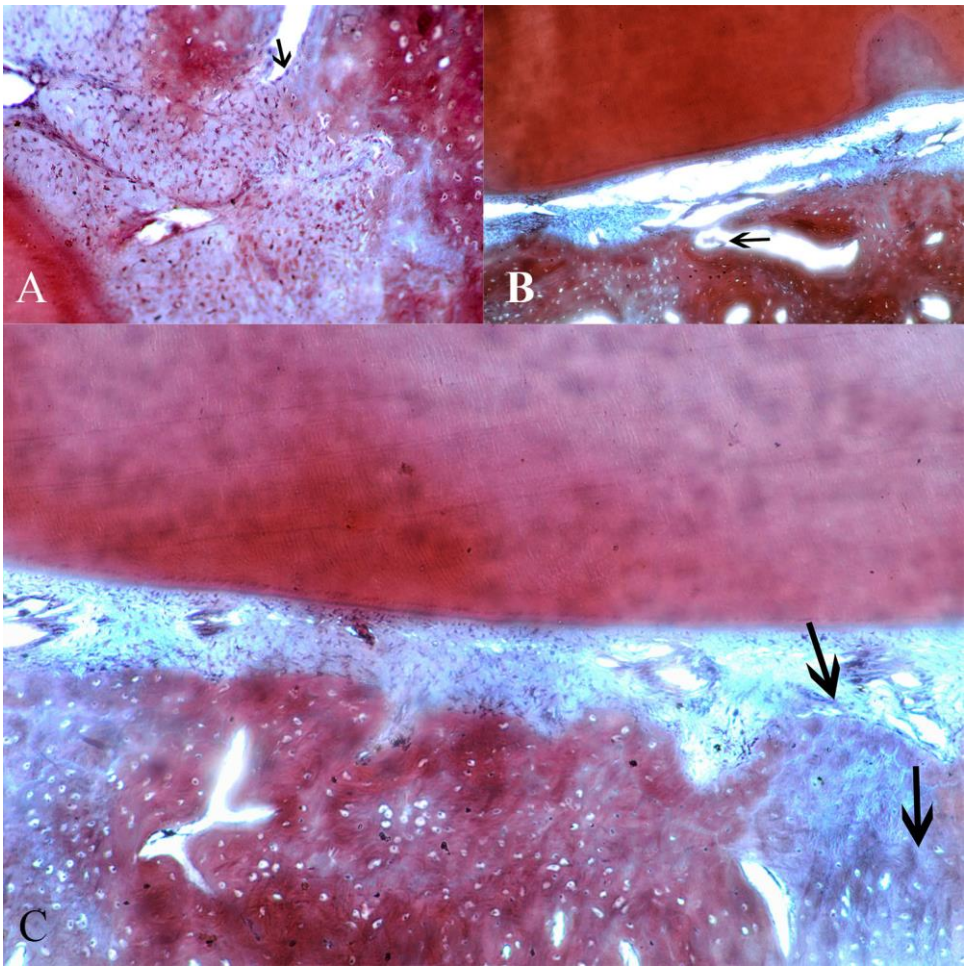

Figure 5. A: Light micrograph (study groupm 6 weeks) showing, the granulation tissue and the newly formed woven bone; B: Light micrograph (study group, 9 weeks) showing the newly formed bone trabeculae surrounded by fibrous tissue. Blood are supply in their bone marrow spaces; C: Study group, 12 weeks showing the density of the fibrous tissue surrounding the newly formed bone trabeculae.

formation were seen.

Study group: Dens collagen fibers, dilated blood vessels and connective tissue cells filled the area. Newly bone formation started to appear. Osteoblast were also seen (Figure 5A).

9 weeks

Control group: Fractured area was filled by the calcified material, large narrow spaces surrounded with collagen fibers, osteocytes and osteoblasts were also seen.

Study group: Healing of the fracture site by active woven bone, new bone filling the fracture area, numerous osteocytes, osteoblasts, were seen, collagen fibers and area of complete fusion were seen (Figure 5B).

12 weeks

Control group: Complete healing with dens bone, restoration of the Haversian system, osteocytes trapped in bone, osteoblasts were seen traced on bone surface, lines of demarcation were seen between old and new bone.

Study group: Complete obliteration of the fractured area, with slight demarcation between the old and new bone. Haversian system showed few fibrous tissues. Large bone narrow spaces were seen (Figure 5C).

DISCUSSION

For over 50 years, N2 butylcyanoacrylate was investigated and was thought to be significantly altering the initial

tensile strength of primary wound closure, when compared to other conventional methods (Khalil et al., 2009). Some of the results that we obtained in this study showed that the initial tensile strength was enhanced, where other obtained results did not support that.

In this study, we demonstrated that the time which is needed for the application of the adhesive material N2BCA is only 3 to 10 s, which is significantly less than what has been demonstrated in previous study (El-rewainy et al., 2016). This is attributed to the easy application, and the rapid polymerization time of N2BCA, which is 3 to 10 s, this result is in agreement with a previous study (Khalil et al., 2009) and supported by Gonzalez et al. (2000) who concluded that N2BCA fixation method is a reliable and easy applicable. However Coulthard et al. (2006) found that in real practice, reduction in times is not adequate enough to allow more surgeries to be done in a given period of time.

In this study, both studied groups showed postoperative edema. We attribute this, in addition, to the lack of drainage, which usually allows both fluid and blood to escape, to the lack of good oral hygiene. Two previous studies concur this finding (Ghoreishian et al., 2009; Gulalp et al., 2009).In one study, the efficacy of N2BCA was evaluated by monitoring post-operative pain and bleeding, and no significant difference was found in the first and second day indicating that N2BCA resulted in a better homeostasis after the removal of the third molar impacted in the mandible (Ghoreishian et al., 2009). In a second study, the N2BCA was found to cause fixation of the muco-periosteal flap to the hard palate (Gulalp et al., 2009).

In our study, none of the animals showed marked signs of inflammation. N2BCA was thought to cause minimal inflammatory reaction, and to have possible antibacterial effect this was in agreement with Vastani and Maria (2013).

On the 3rd week postoperatively there were no infections, or signs of inflammation on the N2BCA treated group. We believe that this is likely due to the bacteriostic effect of N2BCA. On the other hand; mild inflammatory cells were noticed on the control group, We refer this to the plaque induced gingivitis that developed from the food accumulation (El-rewainy et al., 2016). Regarding inflammatory reaction in the intramedullary space, there was no significant difference between the two groups. This can be explained by the histocompatibility of the adhesive material. Saska et al. (2009) compared the fixation of autogenousonlay bone graft with cyanoacrylate, and reported a delay in graft interpretation at the N2BCA site; they attributed that to the material entrapped beneath the graft.

In this study, histological examination revealed numerous osteoblast, and osteoclasticcells, that were observed at the borders of the fractured lines in both groups. We described a newly formed woven bone in the interface indicating bone remodeling. The adhesive material was found to accumulate the space of the fracture with scanty fibrovasculartissue, leading to the slow healing pattern that was noticed on the study group. This finding was compatible with previous studies (Jardini et al., 2005; Dadaş et al., 2007).

Using N2BCA in dentoalveolar fracture fixation is advocated to be superior to the conventional methods, due to lack of requirement of patients to remove it after healing, and for their risk - free of needle - prick of the wire to the tissues and/or to the operator's hand (Barbosa et al., 2009; Kulkarni et al., 2007).

Drawbacks, generally tissue adhesive cost more than wires. This can compensate for the time saved in application of the adhesive material. Adhesives, while in early development, also show a promising chemical profile and will be of significant benefit to oral and maxillofacial surgery patients (Brothers et al., 1989; Dalvi and Faria, 1986).

N2BCA is the newest for edges approximation; it forms a solid film that bridges and holds the edges together (Boymuradov, 2011).

While this study was in agreement with a previous study done by Knott et al. (2007) who used N-butylcyanoacrylate in treating congenital cleft lip and palate, and claimed that N2BCA is bacteriostatic as it minimize the exposure to the nasal secretions; it is against the findings of another study conducted in 2007 which reported toxicity of N2BCA in sealing corneal incision (Chen et al., 2007).

Conclusion

By this study, we conclude that the use of the N2BCA tissue adhesive is a promising material in oral and maxillofacial surgery for its ease of application and time saving, and bone fixation. This study supports the use of N2BCA as an alternative to fractured bone fixation methods.

Conflict of Interests

The authors declare that they have no conflicts of interests.

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