EGYPTIAN
DENTAL JOURNAL

VOL. 63, 1179:1186, JULY, 2017

I.S.S.N 0070-9484

WWW.EDA-EGYPT.ORG

STUDY THE EFFECTS OF CONTACT TIME OF ANTIMICROBIAL SOLUTION ON AUTOGENOUS BONE GRAFT HEALING (AN EXPERIMENTAL STUDY)

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ABSTRACT

The purpose of this study was to evaluate the effect of contact time of autogenous bone graft with clindamycin solution on bone density around implant.

Material & Methods: This study was carried out on twelve healthy adult dogs with average weight 17kg. The animals were prepared for surgery under sterile conditions. Each animal was subjected for extraction, the right first mandibular premolar and the inter-radicular bone was harvested as a source of autogenous bone graft then the third premolar on the same side was extracted and the osteotomy site was prepared. The animals were classified into two groups. Control group (three dogs) in which the autogenous bone graft was placed in saline solution only, while study group (nine dogs) which were classified equally into three subgroups according to the contact time of clindamycin solution with bone graft for 3, 6 and 9 minutes respectively. The implants were inserted and the harvested autogenous bone grafts were placed around the implants of each group. Clinical and radiographic evaluation were done after 1,3 and 6 months postoperatively for infection, edema, looseness of implant and assessment the radiodensitometric bone changes around dental implant utilizing periapical radiographs which were analyzed using IDRISI Kilimanjaro software

Results: The clinical evaluation revealed normal healing for both groups. Radiographic evaluation revealed that the study sub-group (III) after 9 minutes has high bone density than other groups after 3 and 6 months

Conclusion: The longevity of contact time of autogenous bone graft in clindamycin solution is directly proportional with increase bone density around dental implant.

KEY WORDS: Autogenous bone graft – Contact time – Decontamination –Implant

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INTRODUCTION

The loss of one or more dental elements affect not only the psychologic aspect of the patient but also cause an atrophic condition of supporting tissues of the tooth. The highest rate of alveolar bone resportion occurs after tooth extraction within a period of six months and two years, and this will have an impact on the position and angle of dental implant. [1]

Also, alveolar ridge resorption following tooth extraction is frequently an observed phenomenon that may decrease the possibility of placing dental implants or impair the esthetic results after prosthodontic treatment. Although the degree of bone loss varies among individuals and between anatomic sites, it is well accepted that as much as 40% of the alveolar height and 60% of the alveolar width may be lost in the first six months following extraction [2].

Autogenous bone is the ideal grafting materials, for its osteogenic, osteoconductive and osteoinductive properities. Autogenous bone can be used in one piece, enbloc or in a particulated form. Autogenous bone is available either extraorally from iliac crest of the hip bone and ribs or intraorally from maxillary tuberosity, mandibular ramus and mandibular symphysis. [3]

A method of intraoral collection of autogenous bone during preparation of dental implant site with bone collector which allowed to augment small bone defects such as fenestration and dehiscence, without the need to perform a second (intra-oral or extra-oral) surgical area for obtaining autogenous bone. [4]

The harvested autogenous bone graft collected during osteotomy preparation for dental implant may be contaminated by saliva and oral flora which may affect bone formation around dental implant which represent a big challenge for the operator to find out the appropriate decontamination method to decontaminate the harvested autogenous bone graft and preserve its viability [5]

The aim of this study was to evaluate the effect of contact time of autogenous bone graft with clindamycin solution on bone density around implant.

MATERIAL AND METHODS

The study was conducted on twelve mature male Mongrel dogs (ranged from 1-2 years old), weighing about 15-20 kg, each dog received one implant in the lower jaw. All animals were subjected to the surgical procedures at the Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Suez Canal University .

Animal preparation

All animals subjected to the surgical procedures. Food and water were kept away from the animals to prepare them for anesthesia at least 6 – 8 hours before the operation. Each dog was injected with Cefotax 500 mg IV (CEFOTAXIME Epico Co., Egypt) as a prophylactic measure 24 hours before surgery.

Surgical Procedures

Induction and maintenance of general anesthesia by IV administration of sodium thiopental 2.5 % solution (Egyptian International Pharmaceutical Industries Co (EIPICO) 10th of Ramadan City, Egypt) 20 – 30 mg/kg via intravenous cannula until the main reflexes were disappeared. All the animals were subjected to the same surgical procedures, under aseptic conditions, to extract the lower right first and third premolars a traumatically, the tooth was divided in buccolingual direction by tungsten carbide fissure bur so that individual roots could be extracted by means of root elevators and forceps keeping the integrity of the bony wall of the socket and preservation of the soft tissue around the socket to prevent delayed healing. Fig (1.A &C).

A total of twelve Endo-osseous, cylindrical titanium implants (4.2mm in diameter and 10mm length) were used **Dentium** (www.dentium.com). An osteotomy site was performed at 800 rpm using external irrigation with copious amount of sterile saline to avoid bone heating and subsequent necrosis. The osteotomy site was sequentially enlarged according to the standard protocol of the manufacturer using drills of sequentially increasing diameter of the drills starting from pilot drill (2.4 mm diameter), to the final drill (4.00mm in diameter) Fig (1.B).

The osteotomy depth was extended 2 mm apically beyond the apex of fresh extraction socket. Proper implant diameter and length was inserted.. However due to the differences between the roots and implants morphology, the implant may not achieve intimate contact with the crest of the bony socket and horizontal gap was present between the implant and the alveolar bony socket.

The inter-radicular bone was harvested from the extraction site of the lower first premolar using bone rongeur. The collected bone particles were placed in a glass vial containing sterile saline solution at room temperature and covered with a cap in order to minimize the risk of contamination. The dogs were divided into two main groups.

Control group (3 dogs) where the collected bone particles treated with saline only. **Study group**,(9 dogs) this group was divided into three equal sub-groups (each sub –group consisted of 3 animals) The collected bone particles were immersed in saline solution then treated with Dalacin-C solution 600mg (Clindamycin, 600mg, Pfizer Co., Egypt) with contact time 3,6 and 9 minutes for study subgroups I , II and III respectively. The bone particles were crushed to small fragments in a sterile dappen dish. The collected bone particles were placed around the inserted implants in the extraction site of the lower third premolar to fill the gap between the implant and the socket wall of alveolar bone in each dog of the experimental study.

A minimum of 1.5mm distance was maintained between each implant and the adjacent tooth. The average gap between the implant and extraction socket wall of alveolar bone was 0.75mm. The gap was filled with collected autogenous bone particles Fig (1.D). Then closure with interrupted suture was done for all animals by using 3-0 vicryl (TRUGLYDE ™ manufactured by Sutures. India, PVT,LTD.)

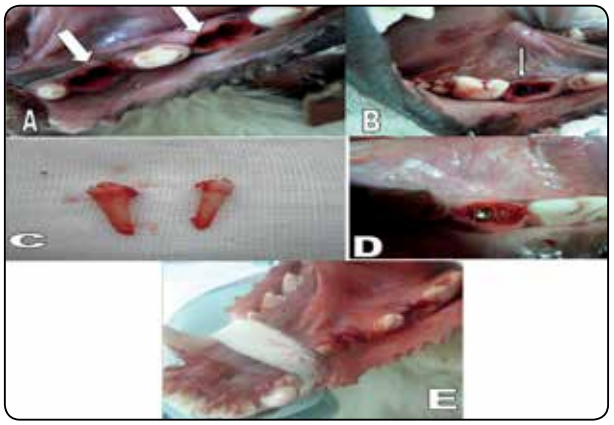

Fig (1) Photograph showing (A) the socket after atraumatic extraction of lower 1st and 3 rd premolars (B) dental implant inserted in to osteotomy site and presence of horizontal gap between the implant and the alveolar socket (C) The extracted 1st premolar roots after sectioning of the tooth, (D) collected autogenous bone particles filling the horizontal gap between the implant and socket wall and (E) closure of the extracted bony socket with interrupted sutures

Post-operative care and clinical evaluation

Clinical follow up of each animal was done looking for any postoperative complications such as infection, delayed wound healing, edema, looseness of implant, erythema around implant, loss of function, wound dehiscence and loss of graft material. The animals were fed with soft diet and were checked every day for a first week postoperatively and every week during the follow-up periods. The dogs were euthanized at 1, 3 and 6 months after surgery by administering of an over dose of thiopental sodium and the selected

area was sectioned for radiographic examination by Intraoral digital Periapical radiograph with paralleling technique at 1, 3 and 6 months to assess the radiodensitometric bone changes around dental implant**.**

Intra oral paralleling periapical direct digital radiographic procedure

Direct standardized digital radiographs were achieved using VISTA Ray charged coupled device (CCD) System* and the Rinn (XCP) periapical film holder**. The VISTA-Ray CCD is a digital mini-x-ray system taking x-ray directly with immediate image availability.

The CCD sensor has an active surface area of 27.5 X 36.8 mm, 22x22 micron (pixel size) and 2050000 pixels.

A long cone, (sixteen inch in length) was mounted to the x-ray tube and the plastic aiming ring of XCP film holder was fixed flush ended with the round end of the long cone.

The sensor was exposed to the Trophy x-ray machine*** at 70 kilovolts and 8 milliampere for 0.10 seconds.

The exposure parameters were considered fixed for all animals. After the exposure was terminated, the readout started automatically and the image was displayed gradually on the computer screen.

The stored images of each animal were interpreted by one examiner at two different times to decrease intra and inter observer errors and the mean of the two trials was recorded.

Use of aluminum step wedge

An aluminum step wedge was used with every digital periapical radiograph for all groups for standardization. Digital radiographic systems come with software that enables image enhancement and manipulation. However, as there is no reference material included, true standardization of gray values was compromised, hence the aluminum step wedge was used to provide greater potential of standardization.

Fabrication of the Radiographic Template

Fabrication of the Radiographic Template At the time of implant placement, an individual impression was made using customized trays. A master cast was made after that grooves were made on the lingual side of the edentulous ridge (extracted side), allowing accurate positioning of the sensor mounted on an x-ray bite block. A reversible adhesive tape was used to firmly attach the template to the dog's mandible. Thus, an optimum parallel and perpendicular standardized radiographic technique was possible for minimizing errors of angulation and distortion.

Digital Image analysis and bone density calibration

The image analysis was performed using IDRISI Kilimanjaro software that facilitated image restoration, enhancement, and densitometric measurements. Image restoration allowed for retrieve of images, followed by image enhancement which allowed contrast adjustment of all images and facilitated determination of the implant edge.

This was then followed by subtracting the implant image from the background image (image of the surrounding bone). Finally, the measurements of density were calibrated by quantifying the image on 256 gray scale value where zero scale was given to totally black regions, 256 scale for totally white regions, while the values in between represented the variable shades of gray. Fig (2)

* VISTARay system by Durr-Dental, German.
** Rinn XCP holder (Rinn Corp., Elgin, IL).
*** Trophy Trex Group, France.

Fig (2) IDRISI Kilimanjaro software assessing density of the bone surrounding the implant by quantifying the image on 256 gray scales and dividing the area surrounding the implant in two zones

IDRISI assessed density of the bone surrounding the implant by dividing it into two zones. The first zone was located just adjacent to the implant and represented the osseointegration zone (implant-bone interface). The second zone was located just adjacent to first zone and represented the bone surrounding the implant. Fig (2)

Statistical analysis of data

The data were collected, tabulated, and statistically analyzed. The data were presented as minimum bone density; maximum bone density, mean bone density, standard deviation (SD), and mean percentage change. Data was calculated for zones one and two in all groups. Analytical statistics were carried out on the parametric measurements of both zones using SPSS-15 software (SPSS, Inc., Chicago, IL, USA.). Non-parametric Friedman's analysis of variance (one way ANOVA) was conducted to assess mean differences of all groups. A probability value (p-value) < 0.05 was accepted as statistically significant.

RESULTS

All dogs in the present study were recovered from the operation and healed uneventfully until the end of the experiment. No dog was missed throughout the whole study. There were no postoper complications such as edema and swelling. Extraction sockets healed normally with no signs of inflammation, infection, wound dehiscence or loss of graft material.

Upon comparing the radiodensitometric values of the bone densities in the test group and control group. One month postoperatively, the mean value and standard deviation of bone density in sub group III (120.82) (±0.200) were higher than sub group II (117.75) (±0.755), sub group I (115.35) (±0.755) and control group (113.448) (±0.6844). However, this was not significant. After three months, the mean value and standard deviation of bone density in sub group III (142.71) (±0.9333) was significant than sub group II (139.73) (±0.337), sub group I (136.93) (±0.337) and control group (129.7) (±0.0042) as P value 0.004. After six months, the mean value and standard deviation of bone density in sub group III (207.93) (±0.611) was highly significant than sub group II (179.21) (±0.168), sub group I (172.21) (±0.168) and control group (156.21) (±0.390) as P value 0.000 as showing in table 1 and figure 3

TABLE (1) Showing the bone density values through study intervals (**Values considered significant when the P value < 0.05**)

	Control group (n=3)	**Sub-group I (n=3)**	**sub group II (n=3)**	**sub group III (n=3)**	**p-value**
1 month	113.448(±0.6844)	115.35(±0.755)	117.75(±0.755)	120.82(±0.200)	0.6
3 month	129.7(±0.0042)	136.93(±0.337)	139.73(±0.337)	142.71(±0.9333)	0.004
6months	156.21(±0.390)	172.21(±0.168)	179.21(±0.168)	207.93(±0.611)	0.000

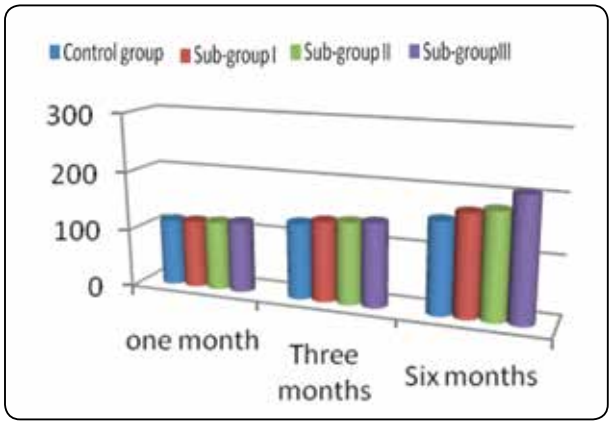

Fig. (3) Histogram showing the bone density values through study intervals

DISCUSSION

Bone particles collected in the oral cavity may be particularly susceptible to microbial contamination, because viable micro-organisms may reach up to 10^9 colony-forming units (CFU)/mL saliva. Additionally; more than 350 kinds of microbes have been identified in the human mouth.[6]

With the advent of immediate implant dentistry, implant fixtures are now regularly placed into fresh extraction sockets that the coronal aspects usually larger than the implant diameter being placed. There is usually somewhat of a gap between the circumferential aspect of the immediate implant and the extraction socket wall. The dimensions of this gap sometimes referred to as the jumping distance which will vary depending on a number of factors, such as tooth type, the particular morphology of the extraction socket and the diameter of the implant being placed.[7]

A recent review of gap management concepts and techniques asserted that the immediate peri-implant gap consists of two dimensions: the horizontal defect width (between implant circumference and socket wall) and the vertical defect height (the distance between the most coronal aspect of the socket wall and the most coronal point of macroscopic contact between the fixture and the socket wall).[8]

Peri-implant bone defects can be filled with bone particles that are collected with a bone filter during implant osteotomy; however, collected bone particles are contaminated with oral bacteria. The bacteria associated with both healthy and diseased periodontium have been shown to colonize on implants surface [9] therefore; the bacterial contaminations of collected bone particles must be reduced because the implantation of contaminated bone particles may cause infectious complications, might even reduces the efficacy of guided bone regeneration and have a negative impact on clinical outcomes.[10] This is in agreement with low bone density in the control group with less bone density values.

Bacterial contamination of bone grafts could be decreased by immersion of the graft in an antibiotic solution which has proved its efficacy for killing off

the bacteria, and bone graft healing does not seem to be inhibited or clinically influenced by the use of a topical antibiotic. Effectiveness of bone graft decontamination may vary with different contact time and various agent's concentration. [11]

Moylan (1980) [12] and **Benjamin and Volz (1984)** [13] used topical antibiotics to prevent surgical site infection and traumatic wound infection. They concluded that topical antibiotics decrease the local infection risk and the necessary of high-dose of systemic antibiotic administration.

Young et al 2001[14] Conducted the first studies to establish the extent of contamination of a bone sample collected with a bone trap during an intra-oral ostectomy performed with rotary instruments. They found that bacterial contamination of bone fragments was 9.634×10^5 colony-forming units CFU/ml per sample. Young et al., 2001 reported that bacterial contamination can be reduced to 3.168×10^5 CFU/ml by using two separate aspiration systems (one of them only for saliva and another directly applied to the drilling site, collecting only cut bone and saline solution) and to 0.72×105 CFU / ml. with a chlorhexidine-based mouthwash administered to the patient immediately prior to the operation.

Witsø et al 2000[15] carried out study in vitro and in vivo in an animal model to evaluate the effect of reducing the bacterial load by the use of bone particles impregnated with antibiotic solutions. They reported that cancellous bone may act as a successful antimicrobial carrier. This in agreement with the radiographic results of study groups (3,6and 9 minutes) as the bone density measures were higher than that recorded in control group through the research intervals.

No histological damage to harversian canals was found after 15 minutes of exposure to 10% povidone-iodine and osteocytes and osteoblasts seemed viable. [16] This is in agreement with our study as study sub group (III)after 9 min contact time showed more bone density and osseointegration than study sub group (I) and (II) 3 and 6 min contact time respectively

The use of broad-spectrum antibiotics as irrigating solutions to treat harvested bone has been proposed. Some authors have reported that irrigation with antibiotic solutions provides no advantage because the short contact time with the specimen would not allow the antibiotic to be effective.[17] Other researchers[16] have obtained positive decontamination benefits for the microorganisms tested using rifampicin solution for 10 and 30 minutes. This is in agreement with our study as study sub group (III) with 9 min contact time showed more bone density values than study sub group with 3 and 6 min contact time respectively.

CONCLUSION

The longevity of contact time of autogenous bone graft in clindamycin solution is directly proportional with increase bone density around dental implant.

REFERENCE

1- Adell R., Lekholm U., Branemark PI.: A 15-year-old study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981; 6:387-396.

2- Goldberg V., Stevenson S.: Natural history of autografts and allografts. Clin Orthop Related Res 1987: 22:7-16

3- Atwood D.: Reduction of residual ridges . A major oral disease entity. J Prosthet Dent, 1971; 26: 266- 279.

4- Savant TD., Smith KS., Sullivan SM., Owen WL.: Bone volume collected from dental implant sites during osteotomy. J Oral Maxillofac Surg 2001; 59:905—907.

5- Egbert M., Stoelinga PJW., Blijdorp PA., Koomen HA.: The "three piece" osteotomy and inter-positional bone graft for augmentation of the atrophic mandible. J Oral Maxillofac Surg, 1986; 44:680-687.

6- Buckner RY., Kayrouz GA., Briner W.: Reduction of oral microbes by a single chlorhexidine rinse. Compendium1994;15:512-520.

7- Rosenquist B., Grenthe B.: Immediate placement of implants into extraction sockets: Implant survival. Int J Oral Maxillofac Impl 1996;2:205-209.

8- Woolfe SN., Kenney EB., Keye G., Taylor D., O'Brien M.: Effect of implantation of titanium implants into fresh extraction sockets. J Dent Res 1989;68:962-965.

9- Papaioannou W., Quirynen M., van Steenberghe D.: The influence of periodontitis on the subgingival flora around implants in partially edentulous patients. Clin Oral Implants Res 1996; 7:405-409.

10- Quirynen M., Papaioannou W., Van Steenberghe D.: Intraoral transmission and the colonization of oral hard surfaces. J Periodontol 1996; 67:986-993.

11- Tezulas E., Dilek O.: Decontamination of autogenous bone grafts collected from dental implant sites via osteotomy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:679-684.

12- Moylan JA.: The proper use of local antimicrobial agents in wounds. World J Surg 1980;4:433–447.

13- Benjamin JB., Volz RG.: General orthopedics efficacy of a topical antibiotic irrigant in decreasing or eliminating bacterial contamination in surgical wounds. Bacterial Contam Surg 1984;184:114–117.

14- Young MP., Carter DH., Worthington H., Korachi M., Drucker DB.: Microbial analysis of bone collected during implant surgery : A clinical and laboratory study. Clin Oral Impl Res,2001;12:95-103.

15- Witsø E., Persen L., Løseth K,. Benum P., Bergh K.:Cancellous bone as an antibiotic carrier. Acta Orthop Scand 2000;71:80-84.

16- Yaman F., Unlu¨G., Atilgan S., Celik Y., Ozekinci T., Yaldiz M.: Microbiologic and histologic assessment of intentional bacterial contamination of bone grafts. J Oral Maxillofac Surg2007; 65:1490-1494.

17- Hooe W., Steinberg B.: Management of contaminated bone grafts: An experimental in- vitro study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:34-37.