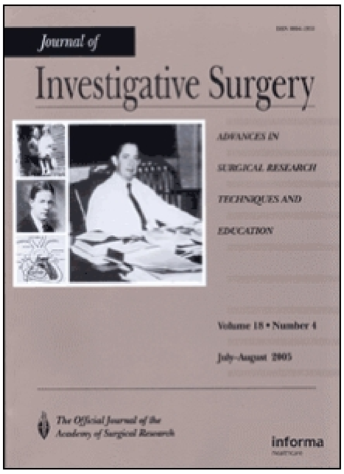
This article was downloaded by: *[Queensland University of Technology]*
On: *13 March 2009*
Access details: *Access Details: [subscription number 908224431]*
Publisher *Informa Healthcare*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Investigative Surgery
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713723105

Autogenous Greater Omentum, as a Free Nonvascularized Graft, Enhances Bone Healing: An Experimental Nonunion Model

Siamak Saifzadeh [a]; Behzad Pourreza [b]; Rahim Hobbenaghi [c]; Bahram Dalir Naghadeh [c]; Siamak Kazemi [b]
[a] Department of Clinical Sciences College of Veterinary Medicine, Urmia University, Urmia, Iran [b] Graduated from College of Veterinary Medicine, Urmia University, Urmia, Iran [c] Departments of Pathobiology and Clinical Sciences, College of Veterinary Medicine, Urmia University, Urmia, Iran

Online Publication Date: 01 April 2009

To cite this Article Saifzadeh, Siamak, Pourreza, Behzad, Hobbenaghi, Rahim, Naghadeh, Bahram Dalir and Kazemi, Siamak(2009)'Autogenous Greater Omentum, as a Free Nonvascularized Graft, Enhances Bone Healing: An Experimental Nonunion Model',Journal of Investigative Surgery,22:2,129 — 137

To link to this Article: DOI: 10.1080/08941930802566730

URL: http://dx.doi.org/10.1080/08941930802566730

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Investigative Surgery, 22, 129–137, 2009
Copyright © Informa Healthcare USA, Inc.
ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.1080/08941930802566730

SURGICAL TECHNIQUES

Autogenous Greater Omentum, as a Free Nonvascularized Graft, Enhances Bone Healing: An Experimental Nonunion Model

Siamak Saifzadeh, DVM, PhD
Department of Clinical Sciences
College of Veterinary Medicine,
Urmia University,
Urmia, Iran

Rahim Hobbenaghi and Bahram Dalir Naghadeh
Departments of Pathobiology and
Clinical Sciences, College of
Veterinary Medicine, Urmia
University, Urmia, Iran

Behzad Pourreza
Graduated from College of
Veterinary Medicine, Urmia
University, Urmia, Iran

Siamak Kazemi
Graduated from College of
Veterinary Medicine, Urmia
University, Urmia, Iran

ABSTRACT

Reconstruction of vascularity is an early event in fracture healing and upregulation of angiogenesis may therefore promote the formation of bone. We have investigated the potentiality of autogenous free nonvascularized greater omentum to stimulate the formation of bone in an experimental hypertrophic nonunion model. Twelve dogs assigned into two identical groups underwent a standard nonunion operation. In the experimental group, this was followed by application of autogenous greater omentum as a free nonvascularized graft around the osteotomy gap. Radiographic assessments were conducted time-sequentially until euthanasia 16 weeks after surgery. Histological analysis was performed on the mid-radial diaphysis containing the 4-month-old osteotomy site. Radiological and histological properties of the group treated with free transplant of the greater omentum revealed complete union. In contrast, there was no evidence indicating union in the control group. Analyses of the radiological and histological scores confirmed that osteotomies treated with free transplant of the autogenous greater omentum had united, whereas the osteotomies of the control group failed to unite. Significant differences between the mean values for radiological and histological-grading score in the control and experimental groups were detected ($p < 0.05$). We showed that free graft of autogenous greater omentum could stimulate the formation of competent bone in an environment deprived of its normal vascularization. Hence, it could be recommended to enhance healing when the fractures are at risk of nonunion.

Keywords: Omentum, Free graft, Bone healing, Osteotomy, Hypertrophic nonunion, Angiogenesis

INTRODUCTION

Normal and pathological bone physiology is inexorably tied to angiogenesis. The reestablishment of vascularity is an early event in fracture healing, and the process of bone development and repair depends

---

Received May 17, 2008; accepted July 1, 2008.
Address correspondence to Siamak Saifzadeh, DVM, PhD, College of Veterinary Medicine, P.O. Box 57155-1177, Urmia University, Urmia, Iran. E-mail: s.saifzadeh@mail.urmia.ac.ir

on the adequate formation of new capillaries from existing blood vessels [1]. Several studies showed that experimental challenges that disrupt angiogenesis can slow bone healing [2–4] and inhibition of this process can even completely prevent fracture healing [5].

It has long been recognized that the greater omentum has a remarkable capability in revascularization of tissues and encouraging angiogenesis in adjacent structures to which it is applied. The biologic mechanism responsible for these effects has not yet been completely defined. A number of polypeptide growth factors that possess potent angiogenic properties have been identified in greater omentum, amongst them vascular endothelial growth factor (VEGF) has been recognized as the major angiogenic factor produced by omentum. VEGF might underlie the mainstream mechanism of omentum-induced angiogenesis [6].

Because of this unique ability of omentum, it has been used frequently in patient surgeries. It has been used as a pedicle graft for support tracheal autograft [7], ischemic bronchial autografts [8], ischemic limbs [9], salvage in Buerger's disease [10], myocardial angiogenesis [11], and chest wall reconstruction [12]. Furthermore, transposition of free omental graft has been used in revascularization procedures involving the chronically injured spinal cord [13], head and neck deformities [14], mandibular osteoradionecrosis [15], and total ear reconstruction [16]. Both the above-mentioned techniques described for omental transfer have their own specific shortcomings. Lengthening procedures are developed to create an omental pedicle to reach more distant sites on the body [17, 18] and requires a subcutaneous tunnel over areas of motion, which raises concerns of vascular compromise. Additionally, the amount of available omental tissue at the distal end decreases with increasing pedicle length. Microvascular autotransplantation of a free omental graft is also technically difficult to develop and its failure rate is unacceptably high [19].

Considering the angiogenic factors presented in the greater omentum, we hypothesized that many of these relative disadvantages would be overcome using a free nonvascularized omental graft alternatively. If successful, this tissue would supply necessary reparative elements to the tissues in need of revascularization. In view of these considerations, we have undertaken to set up an animal model to evaluate autogenous greater omentum as a free nonvascularized graft for enhancement of bone healing in a clinically relevant model of compromised healing.

MATERIALS AND METHODS

Twelve adult male mongrel dogs weighing 255–315 N (281 ± 20.7) were entered to this study. They were determined to be healthy based on physical, orthopedic, and radiographic examination findings, normal complete blood count (CBC), and serum biochemistry results. Cranio-caudal and lateral limb radiography were performed before surgery to exclude abnormal radiographic findings and to check for skeletal maturity. The dogs randomly allocated to two groups of six, and assigned as experimental and control groups, underwent a unilateral standard operation for nonunion. In both the groups, a small piece of the greater omentum was harvested from the abdominal cavity. In the experimental group it was placed over the osteotomy gap as an autogenous free graft. The radiological and histopathological properties at the osteotomy site were assessed during the postoperative period. The study and all experimental procedures involved with the animals were approved by the ethical committee of the university. All animals received complete humane care in accordance with the requirements established by the university.

The dogs were premedicated with acepromazine (Kela Laboratoria NV., Hoogstraten, Belgium) (0.02 mg/kg intramuscularly [IM]) and butorphanol (Richter Pharma AG, Wels, Austria) (0.2 mg/kg IM), and induced with thiopental (Sandoz GmbH, Kundl, Austria) (10 mg/kg intravenously [IV]). Anesthesia was maintained with halothane (Hikma Pharmaceuticals Co., Amman, Jordan) in oxygen in a semi-closed circle system.

A validated nonunion model was used by performing a standardized transverse mid-diaphyseal radial osteotomy, leaving the ulna intact and operated limb unsplinted [20]. Using a medial approach in anaesthetized dogs, a 2-mm transverse bone defect was created at right medial radial diaphysis with a Gigli wire under saline irrigation.

In dogs of both groups, the abdominal cavity was approached through a 3-cm ventral midline incision midway between the umbilicus and pelvic inlet; then a free end of the greater omentum was located and exteriorized from the abdominal cavity. A 30 × 30 mm^2 piece of the greater omentum was isolated by two catgut ligatures and cut free from the remaining omental pedicle. The omental patch was carefully wrapped in saline-moistened laparotomy pads. In the experimental group, the resected piece of the omentum was placed as a free graft, over the osteotomy gap and secured in place with tack sutures with 3/0 polyglycolate. The surgical sites were rinsed using normal saline and closed routinely. No internal or external fixation devices were applied postsurgery. The animals were allowed unrestricted weight bearing and limb ambulation. Postoperative analgesia was provided as needed with intramuscular injections of morphine sulphate (Temad pharmaceutical Co., Tehran, Iran) (1.0 mg/kg). The animals wore Elizabethan collars and wire muzzles to prevent self-trauma to the surgical sites. Bandage changes and physical examinations were done daily under heavy sedation with acepromazine (0.05 mg/kg IV) and butorphanol (0.1 mg/kg IV), or tiletamine/zolazepam (Virbac International, Carros Cedex, France) (6.6 mg/kg IM).

In vivo X-rays were taken time-sequentially from each operated forearm immediately after surgery at 2, 4, 6, 8, 10, 12, 14, and 16 weeks postoperatively. Special attention was paid to the changes occurring at the bone-defect site; qualitative features of fracture healing including periosteal reaction, bony callus and overall callus quality, bone formation in defect, and degree of bridging callus at the osteotomy sites were evaluated. Results, scored on a 0- to 5-point scale using Itoh et al. scoring system [21], are as follows:

Grade 0: No bone formation in defect or minimal response of cut-ends.
Grade 1: Density appeared in defect but $\leq 50\%$ of total defect area and no defect bridging.
Grade 2: Density occupied $> 50\%$ of total defect area or defect bridging at least one point.
Grade 3: Density occupied almost the entire area of defect but both cut-ends are clear.
Grade 4: Dense bone occupied the entire area of defect and one of cut-ends is unclear.
Grade 5: Normal bone appearance in defect and both cut-ends not seen.

Grades 4 and 5 were regarded as union.

At 16 weeks postsurgery, the animals were euthanized and their operated limbs were harvested for histological evaluation. The operated radii were removed, cleaned of soft tissue, and samples of repaired tissue from grafted bone defects were fixed in 10% neutral buffered formalin. Specimens were decalcified in 10% formic acid for 2 weeks and embedded in paraffin. Paraffin sections of 5 μm were stained with hematoxylin-eosin. Histological findings of the fracture healing were evaluated and scored according to the criteria defined by Allen et al. [22]. A 5-point scale (grades 0–4) was used to determine the stage of fracture healing:

Grade 0: Described the presence of large cavity containing blood or other fluid in the cartilaginous plate.
Grade 1: Described retention of fibrous elements in the cartilaginous plate.
Grade 2: Described complete cartilaginous union.
Grade 3: Indicated incomplete bony union because a small amount of cartilage was present in the callus.
Grade 4: Indicated that bony union was complete.

Nonparametric analyses were used for comparison of groups because the dependent variables were classified as ordinal data. Dependent variables included the radiographic and histological scores of bone healing in treatment and control groups. Mann–Whitney rank sum test was used for radiographic comparison of bone healing between two groups in each occasion. To evaluate the radiographic changes during experiment, the Friedman repeated measures analysis of variance on ranks was used followed by Tukey test for multiple comparisons among periods. Histological scores assigned for two groups were compared using the Mann–Whitney rank sum test. For all analyses, $p < 0.05$ was considered significant. Results were expressed as median and interquartile range (25 to 75%). The analyses were done by statistical software (SigmaStat, Version 3 for Windows, SPSS INC, California).

RESULTS

Of the 12 operated dogs, 10 went through the period of investigation. In two dogs, fracture of the ulna occurred 1 and 2 weeks, respectively, after the omental transfer. The 10 remaining dogs all had uneventful healing of their surgical incisions. All of the dogs were moderately lame on the operated limb after surgery. No significant morbidity was associated with the celiotomy.

Radiographic examination identified disturbed healing with abundant callus formation in dogs of control group (Figure 1). At the first 4 weeks, resorption cavities at the osteotomy line and the widening of osteotomy gap were evident (Figure 1a). During the second month, the gap became wider and concurrently a scanty young callus appeared on the external surface of the fragments (Figure 1b). At the third month, sclerosis of the osteotomy ends and appearance of the great amount of external callus were noticed (Figure 1c). During the last 4 weeks, bone extremities became broader and denser. Furthermore, the osteotomy gap became narrower and the medullary canal sealed. During this period, a well-developed external callus was also formed and completely incorporated to the adjacent bone. Notwithstanding the formation of considerable amount of callus, it was unable to bridge the osteotomy and radiolucent gaps without restoration of continuity that was demarcated between the bone fragments (Figure 1d). These features indicated to the so-called hypertrophic nonunion.

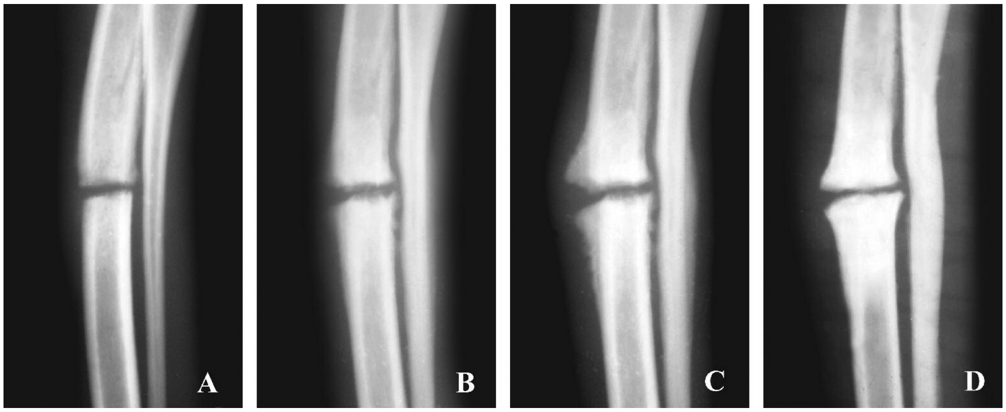


Figure 1. Radiological sequences showing the development of hypertrophic nonunion in the control group. (A) Week 4: A scattered bone rarefaction around the osteotomy fragments and widening of the gap occurred. (B) Week 8: A scanty young callus is seen on the external surface of the fragments. (C) Week 12: Bone sclerosis and the external callus are evident. (D) Week 16: The osteotomy ends are denser, broader, and enlarged by abundant external callus that do not bridge the gap. The osteotomy gap is narrower with a clear-cut radiolucent line of demarcation between the bone fragments.

In the experimental group, complete bony union was diagnosed by serial radiographs during 4 months of postoperative period (Figure 2). In the first month, there was some initial resorption at the fracture line with apparent widening of the fracture gap. During this time, mild periosteal reaction was also noticed (Figure 2a). At the second month, bone formation in defect resulted in narrowing of the osteotomy gap, and concurrently external callus appeared and bridged the osteotomy gap (Figure 2b). While these radiographic alterations had tendency to increase in dogs of the experiment group, at the third month, the size of the external callus and its opacity increased, defect bridging occurred at several points, and osteotomy gap got sealed (Figure 2c). Large amount of external callus with bone opacity was seen on radiographs in the fourth month. Density occupied the entire area of defect, both cut-ends were not found, and osteotomy gap was completely sealed (Figure 2d). These features indicated to complete bony union.

Changes in mean radiographic grade in each group are shown in Figure 3. All defects in the experimental group were scored as grades 4 and 5 (radiographic union) by 16 postoperative weeks, whereas no remarkable changes in scores were observed in the control group throughout postoperative period. In the experimental group, defect bridging, at least one point, was observed in one out of five defects

Figure 2. Radiological sequences showing the development of complete bony union in the experimental group. (A) Week 4: The osteotomy gap widened as a result of resorption of the bone extremities. Shadows of subperiosteal callus can be seen on the cortical surfaces. (B) Week 8: Bone formations in defect, appearance of the external callus, defect bridging and narrowing of the osteotomy gap are seen. (C) Week 12: Abundant external callus with bone opacity bridges both cut-ends at several points. Bone defect and cut-ends are not clearly seen. (D) Week 16: Density occupies the entire area of defect and makes defect and the line of the cut-ends unclear.

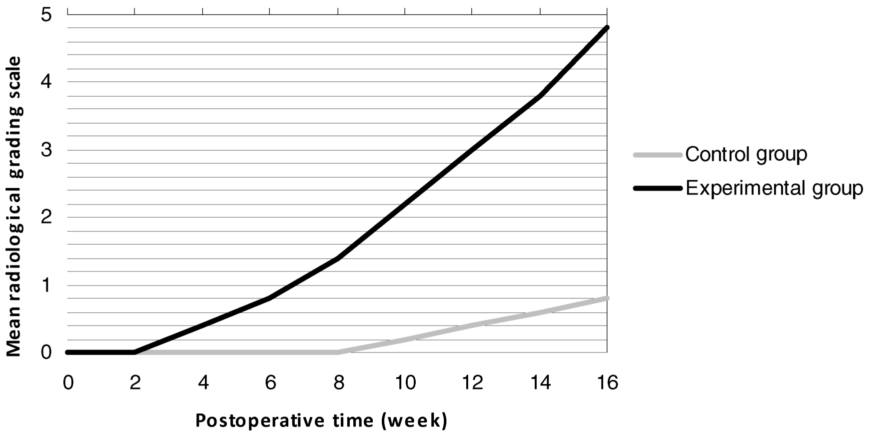


Figure 3. Changes in mean radiographic grade in control and experimental groups during 16 weeks of postoperative period. After week 14, the mean values for radiological-grading score in the experimental group pass the point 4, indicating the occurrence of union at this time, whereas no remarkable changes in the mean values were seen in the control group throughout postoperative period, and the scores at last weeks couldn't even reach the point 1, which indicated the occurrence nonunion in this group. Significant differences ($p < 0.05$) were observed for control *vs* experimental groups (6–16 weeks).

by 6 weeks and thereafter until week 16 all defects in this group were completely bridged by callus, whereas no bony bridging was seen at the fracture site in control group and the fracture gap remained for 16 weeks, indicating nonunion. Dramatic differences between the two groups in mean value of radiographic-grading scores were obvious from sixth postoperative week.

Histological study of 4-month-old osteotomies of the control group revealed development of the nonunion (Figure 4). The histological sections showed that the

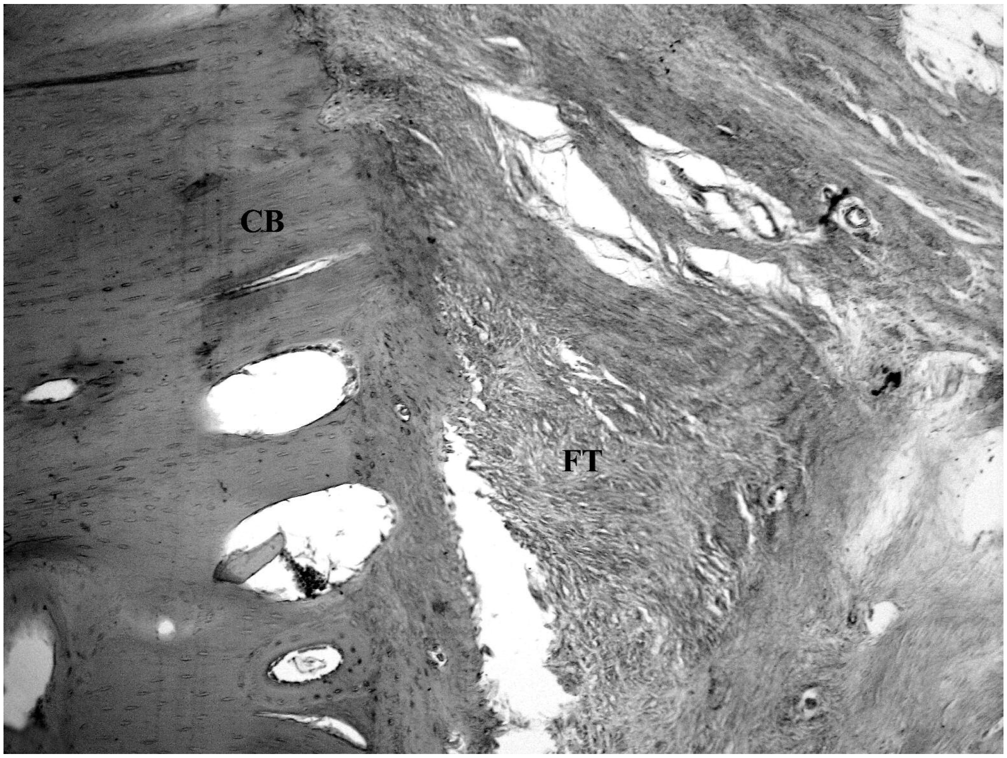


Figure 4. Representative histological image of osteotomy site in the control group at 16 weeks postoperatively. Tissue formation consisted of fibrotic tissue (FT) between the borders of the native compact bone (CB) (hematoxylin-eosin stain, original magnification ×100).

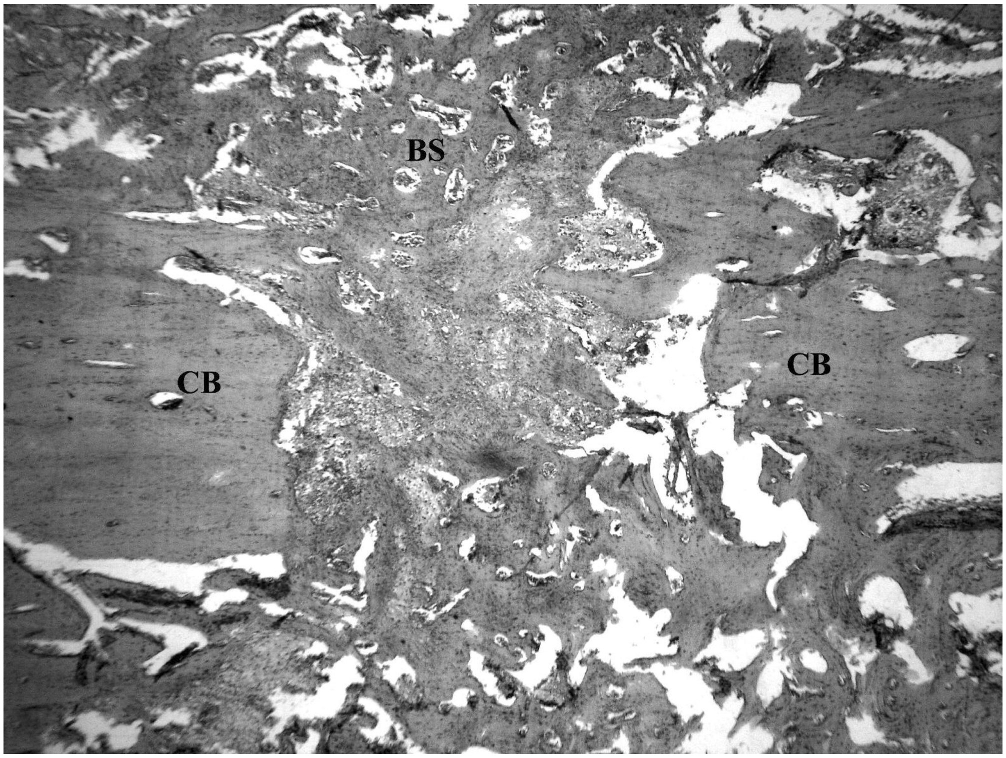


Figure 5. Free-omentum autografted bone defects in the experimental group at 16 weeks postoperatively. The new bony spicules (BS) are seen to completely bridge the defect and to some extent indistinguishable at the margins from native compact bone (CB). Bone marrow in the lumen of the medullary canal was formed by the mesenchymal tissue (hematoxylin-eosin stain, original magnification ×32).

osteotomy was encompassed by a fibrous tissue envelope that isolated the inner environment from the neighboring tissue. Periosteum was present and very active in forming the external callus. Most of the osteotomy gap was filled with young connective tissue. The osteotomy line was irregular and the transitional area between connective tissue and bone ends was undergoing active ossification. In the experimental group, all dogs presented a bridged bone gap (Figure 5). At the fracture site, periosteal and endosteal reactions gave rise to callus with some cartilaginous and no fibrous connective tissue. The periosteal reaction often produced a cuff of bone which surrounded the fracture. The endosteal reaction produced internal callus composed of bone lamellae, which are randomly distributed. The lamellae that appeared to be produced by endochondral ossification traversed the fracture gap. They were well attached to the bone ends and bridged the osteotomy. Some remarkable foci of endochondral ossification were seen within the periosteal and endosteal callus (Figure 6).

Histological assessments are summarized in Figure 7. There were findings of union in the sections prepared from the dogs of experimental group sacrificed on week 16. In this group complete bony union and incomplete bony union occurred in two and three dogs respectively. There was no finding of nonunion (grade 0 and 1) in any of the sections taken from the dogs of experimental group whereas two to three sections taken from the dogs of control group were scored as grade 0 and 1, respectively. Average grades in experimental group were significantly higher ($p < 0.05$) than those in control group.

DISCUSSION

In this study, we have shown that autogenous free omental graft inserted in the vicinity of transverse mid-diaphyseal osteotomy with a 2-mm gap, without microvascular anastomosis to the recipient bed, could have survived and potentially enhanced bone healing.

The radial osteotomy gap model we used in this study is a hypertrophic nonunion model [23, 24]. The present model yields a consistent pattern of a disturbed reparative process that mimics human cases of atrophic or hypertrophic nonunion [25].This model has been well studied, and our results in the control group confirmed previously reported findings concerning the radiological aspect and histological picture of hypertrophic

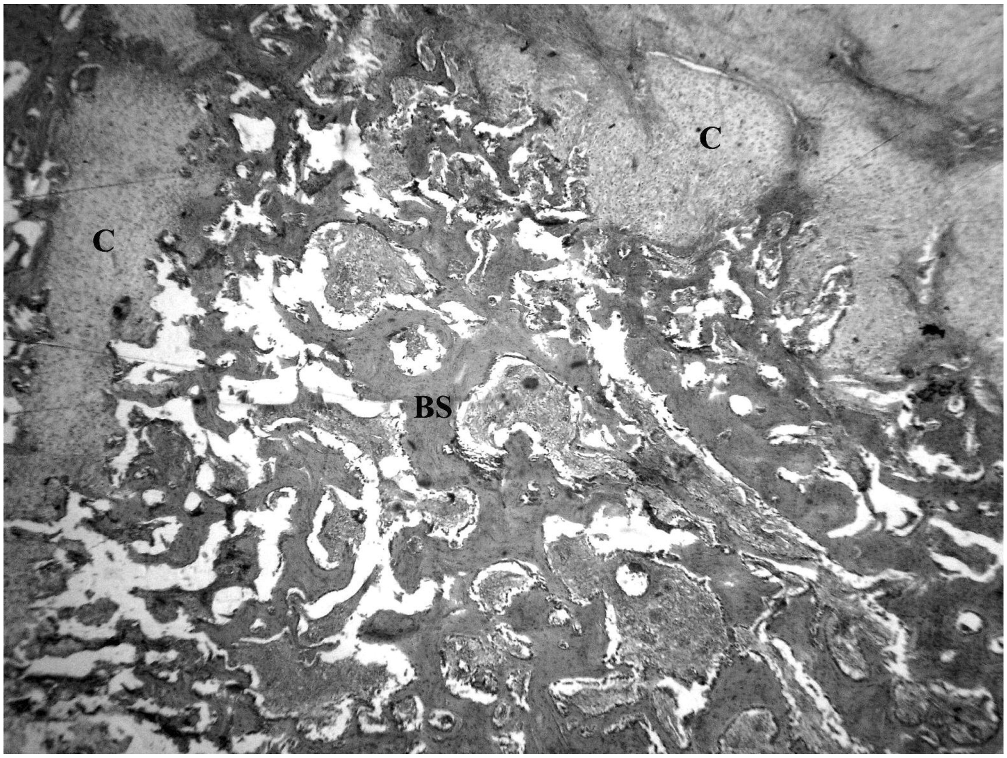


Figure 6. Cross-section of photomicrograph of the osteoctomy site in experiment group, 16 weeks after surgery. Note the foci of cartilage islands (C) and endochondral ossification adjacent to the newly formed bony spiculs (BS) (hematoxylin-eosin stain, original magnification ×100).

nonunions [26]. While stability and rigidity of the bone defect fixation has been found to be a critical factor in achieving consistent osteotomy gap healing [24], in this model the ulna acts as an internal support, but micro movement at the osteotomy gap prevented any bone union. In reality, while interfragmentary movement does favor the formation of callus, it has deleterious effects on angiogenesis that resulted in hypertrophic nonunion [27]. Clinically, this type of nonunion is usually secondary to premature weight bearing or inadequate fixation [28, 29].

Bone fracture results in the disruption of the marrow architecture and blood vessels within and around the fracture site [30–32]. Since increased metabolic demands during bone repair require an increased blood flow rate (BFR) and functional vascular density (VD) [33], reconstruction of the circulation is one of the earliest and most important events during bone repair [30, 34]. Recruitment process, and also the activation of osteoclasts, osteoblasts, and their precursor cells, depends on new vessel formation and properties of the microcirculation, which are also involved in the regulation of the metabolic microenvironment [35]. Thus, microcirculatory properties may play an essential role during osteogenesis [36] and in this regard vascular reorganization and blood supply at the fracture site can have an important role in the healing of fracture. Establishment of a functionally intact vascular network appears not only to precede the event of bone formation,

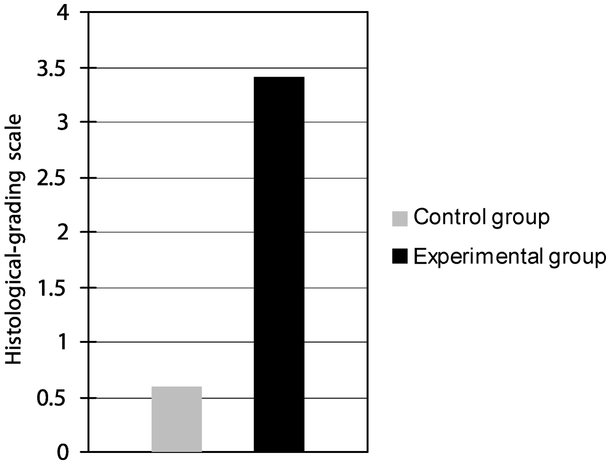


Figure 7. Mean histological grades of the sections prepared from the dogs in control and experimental groups sacrificed on week 16. Significant difference between the control and experimental groups in the mean values for histological-grading score was detected.

but also to have a substantial influence on the result [37, 38].

It should be noted that during the healing of fractures signal peptides are released from the initial hematoma; the temporal and spatial expression patterns of these factors suggest that they have a pivotal role in bone repair. High levels of angiogenic growth factors have been recorded in the fracture hematoma early in the healing sequence which, probably, stimulate interfragmentary vascular invasion [39]. Vascular invasion of the fracture hematoma is a crucial step in the progress to union, as Chidgey et al. (1986) [40] demonstrated that the return of mechanical integrity of the fractured bone is in direct relation to the vascular reorganization at the fracture site.

The great ability of omentum in revascularization of tissues has been well documented in the medical literature. In this regard, several studies have shown the angiogenic activity of omental fat [41, 42]. The process of neovascularization allows the omentum to provide vascular support, and promote function and healing in ischemic or inflamed tissue [43, 44]. A number of polypeptide growth factors that possess potent angiogenic properties have recently been identified in greater omentum [45]. In this regard, Zhang and coworkers demonstrated that VEGF is the major angiogenic factor produced by omentum and possibly underlies the mechanism of omentum-induced angiogenesis [6]. They also demonstrated that omental adipocytes are the primary source of VEGF protein [6]. Street and coworkers showed that VEGF can stimulate bone repair not only by promoting angiogenesis but also by accelerating and enhancing bone turnover [46]. They demonstrated that VEGF directly promotes the differentiation of primary osteoblasts and play an important role in callus formation, conversion of the soft, cartilaginous callus to a hard, bony callus and mineralization in response to bone injury during fracture repair [46].

We found that all the radii treated with free transplant of the greater omentum got united, whereas those of the dogs in the control group went on to nonunion. Time-sequential radiological study identified a good potential for bone formation in experimental group, as indicated by the well-developed external callus that invaded the osteotomy gap and resulted in complete bony bridging, but although the same amount of callus was formed in control group, it was unable to bridge the defect. This finding was in accordance with the observation of the histological sections of radii in this group, which showed interfragmentary gap filled with connective tissue notwithstanding the presence of active periosteum and ossification at bone ends. In the experimental group, periosteal reaction resulted in formation of the exuberant callus that sealed the gap and prevented the local infiltration of fibrous tissue. In this group, endochondral ossification produced the bone lamellae that attached to the bone ends and resulted in bridging the osteotomy gap. In this study, the radiological grading provided quantitative measure for follow-up of fracture healing during a 16-week postoperative period. Dramatic increase in the radiological grade (4 and 5, which were regarded as union) was seen in the dogs that received the omentum graft. On the contrary, no remarkable changes in radiological grading were seen in the control group throughout the experimental period, which could indicate nonunion. Although we couldn't sort out any histological grading during the follow-up period but analysis of data obtained from the sections of radii of dogs sacrificed on week 16 confirmed previous radiological grading findings and provided insight into the degree of agreement between the two observers. Moreover, statistical analysis of the radiological as well as histological-grading scores established significant difference between the mean values in the control and experimental groups.

CONCLUSION

In summary, our findings showed an unequivocal difference between the experimental and control groups in this study. Since the only variable between the two groups was the presence of greater omentum used as a free autograft in the treatment group, it is plausible that it acted through its major angiogenic factors to induce angiogenesis, reconstruct circulation, enhance blood supply to the osteotomy site, and accordingly hasten the process of bone healing. One should remember that use of autogenous greater omentum as a free graft instead of pedicled omentum may be associated with decline in the rate of complications like morbidity caused by major laparotomy, hemorrhage, infarction causing peritonitis, intestinal obstruction, and perineal hernias. Based on the results of this study, autogenous greater omentum as a free graft might have clinical application in the treatment of fractures in patients at risk of developing delayed or nonunion fractures.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support for this study by the Urmia University College of Veterinary Medicine. We would like to extend our special gratitude to Dr. Tanja Eindorf, Postdoctoral Fellow, Adelaide University for her critical review of the manuscript.

REFERENCES

1. Streeten EA, Brandi ML. Biology of bone endothelial cells. *Bone Miner*. 1990;10:85–94.
2. Rhinelander FW. The normal microcirculation of diaphyseal cortex and its response to fracture. *J Bone Joint Surg Am.* 1968;50:784–800.
3. Macey LR, Kana SM, Jingushi S, et al. Defects of early fracture healing in experimental diabetes. *J Bone Joint Surg Am.* 1989;71:722–733.
4. Pelker RR, Friedlaender GE. Fracture healing: radiation induced alterations. *Clin Orthop Relat Res.* 1997;341:267–282.
5. Hausman MR, Schaffler MB, Mjeska RJ. Prevention of fracture healing in rats by an inhibitor of angiogenesis. *Bone*. 2001;29:560–564.
6. Zhang QX, Magovern CJ, Mack CA, et al. Vascular endothelial growth factor is the major angiogenic factor in omentum: mechanism of the omentum mediated angiogenesis. *J Surg Res*. 1997;67:147–154.
7. Nakanishi R, Shirakusa T, Takachi T. Omentopexy for tracheal autografts. *Ann Thorac Surg*. 1994;57:841–845.
8. Morgan E, Lima O, Goldberg M, et al. Successful revascularization of totally ischemic bronchial autografts with omental pedicle flaps in dogs. *J Thorac Cardiovasc Surg*. 1982;84:204–210.
9. Bhargava JS, Makker A, Bhargava K, et al. Pedicled omental transfer for ischaemic limbs. *J Indian Med Assoc.* 1997;95:100–102.
10. Talwar S, Jain S, Porwal R, et al. Pedicled omental transfer for limb salvage in Buerger's disease. *Int J Cardiol.* 2000;72:127–132.
11. Kanamori T, Watanabe G, Yasuda T, et al. Hybrid surgical angiogenesis: omentopexy can enhance myocardial angiogenesis induced by cell therapy. *Ann Thorac Surg*. 2006;81:160–167.
12. Politi L, Scanagatta P, Salani A, et al. Intrathoracic omentoplasty in the treatment of pleural cavity secondary to stabilized bronchial fistula. *Minerva Chir.* 2004;59:495–499.
13. Zhan MS, Ji QY, Xu Z, et al. The effect of transposition of pedicled omentum to spinal cord ischemia segments. An animal experiment. *Chin Med J (Engl).* 1989;102:214–218.
14. Losken A, Carison GW, Culbertson JH, et al. Omental free flap reconstruction in complex head and neck deformities. *Head Neck*. 2002;24:326–331.
15. Moran WJ, Panje WR. The free greater omental flap for treatment of mandibular osteoradionecrosis. *Arch Otolaryngol Head Neck Surg.* 1987;113:425–427.
16. Park C, Roh TS, Chi HS. Total ear reconstruction in the devascularized temporoparietal region: II. Use of the omental free flap. *Plast Reconstr Surg*. 2003;111:1391–1397.
17. Alday ES, Goldsmith HS. Surgical technique for omental lengthening based on arterial anatomy. *Surg Gynecol Obstet.* 1972;135:103–107.
18. Das SK. The size of the human omentum and methods of lengthening it for transplantation. *Br J Plast Surg*. 1976;29:170–174.
19. Roa DM, Bright RM, Daniel GB, et al. Microvascular transplantation of a free omental graft to the distal extremity in dogs. *Vet Surg.* 1999;28:456–465.
20. Müller J, Schenk R, Willenegger H. Experimental studies on the development of reactive pseudarthroses on the canine radius. *Helv Chir Acta*. 1968;35:301–308.
21. Itoh T, Mochizuki M, Nishimura R, et al. Repair of ulnar segmental defect by recombinant human bone morphogenetic protein-2 in dogs. *J Vet Med Sci.* 1998;60:451–458.
22. Allen HL, Wase A, Bear WT. Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. *Acta Orthop Scand.* 1980;51:595–600.
23. Millis DL, Wilkens BE, Daniel GB, et al. Radiographic, densitometric, and biomechanical effects of recombinant canine somatotropin in an unstable ostectomy gap model of bone healing in dogs. *Vet Surg.* 1998;27:85–93.
24. Inoue N, Ohnishi I, Chen D, et al. Effect of pulsed electromagnetic fields (PEMF) on late-phase osteotomy gap healing in a canine tibial model. *J Orthop Res.* 2002;20:1106–1114.
25. Garcia P, Holstein JH, Maier S, et al. Development of a reliable nonunion model in mice. *J Surg Res.* 2008;147(1):84–91.
26. Santos Neto FL, Volpon JB. Experimental nonunion in dogs. *Clin Orthop Relat Res.* 1984;187:260–271.
27. Wallace AL, Draper ER, Strachan RK, et al. The vascular response to fracture micromovement. *Clin Orthop Relat Res.* 1994;301:281–290.
28. DeAngelis MP. Causes of delayed union and nonunion of fractures. *Vet Clin North Am.* 1975;5:251–258.
29. Robello GT, Aron DN. Delayed and nonunion fractures. *Semin Vet Med Surg (Small Anim)*. 1992;7:98–104.
30. Glowacki J. Angiogenesis in fracture repair. *Clin Orthop Relat Res*. 1998;355:S82–S89.
31. Rhinelander FW. Tibial blood supply in relation to fracture healing. *Clin Orthop Relat Res.* 1974;105:34–81.
32. Brighton CT, Hunt RM. Early histological and ultrastructural changes in medullary fracture callus. *J Bone Joint Surg Am.* 1991;73:832–847.
33. Rhee JG, Eddy HA, Hong JJ, et al. Divergent changes of flow through individual blood vessels upon localized heating. *Int J Hyperthermia*. 1996;12:757–769.
34. Wang JS. Basic fibroblast growth factor for stimulation of bone formation in osteoinductive or conductive implants. *Acta Orthop Scand Suppl.* 1996;269:1–33.
35. Helmlinger G, Yuan F, Dellian M, et al. Interstitial pH and PO_2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat Med.* 1997;3:177–182.
36. Leunig M, Yuan F, Berk DA, et al. Angiogenesis and growth of isografted bone: quantitative in vivo assay in nude mice. *Lab Invest.* 1994;71:300–307.
37. Wallace AL, McLaughlin B, Weiss JB, et al. Increased endothelial cell stimulating angiogenesis factor in patients with tibial fractures. *Injury*. 1991;22:375–376.
38. Wallace AL, Makki R, Weiss JB, et al. Measurement of serum angiogenic factor in devascularized experimental tibial fractures. *J Orthop Trauma.* 1995;9:324–332.
39. Street J, Winter D, Wang JH, et al. Is human fracture hematoma inherently angiogenic? *Clin Orthop Relat Res.* 2000;378:224–237.
40. Chidgey L, Chakkalakal D, Blotcky A, et al. Vascular reorganization and return of rigidity in fracture healing. *J Orthop Res.* 1986;4:173–179.
41. Levy Y, Miko I, Hauck M, et al. Effect of omental angiogenic lipid factor on revascularization of autotransplanted spleen in dogs. *Eur Surg Res.* 1998;30:138–143.
42. Silverman KJ, Lund DP, Zetter BR, et al. Angiogenic activity of adipose tissue. *Biochem Biophys Res Commun.* 1988;153:347–352.
43. Konturek SJ, Brzozowski T, Majka I, et al. Omentum and basic fibroblast growth factor in healing of chronic gastric ulcerations in rats. *Dig Dis Sci.* 1994;39:1064–1071.
44. Williams JK, Carlson GW, Austin GE, et al. Short gut syndrome: treatment by neovascularization of the small intestine. *Ann Plast Surg.* 1996;37:84–89.
45. Goldsmith HS, Griffith AL, Kupferman A, et al. Lipid angiogenic factor from omentum. *JAMA.* 1984;252:2034–2036.
46. Street J, Bao M, deGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci USA.* 2002;99:9656–9661.