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Effect of Autogenous Omental Free Graft on the Biomechanical Properties of Fracture Healing in Dog

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Abstract

Objective- To evaluate greater omentum as an autogenous free graft in enhancing bone healing.
Design- Prospective descriptive trial.
Animals- Ten mongrel adult dogs, 3 to 4 years old and weighing 25.2 to 31 kg, assigned into two equal experimental and control groups (5dogs/group), were used.
Procedures- Mid-shaft osteotomy on right radius was followed by a partial omentectomy in all dogs. A 30x30 mm^2 piece of greater omentum was prepared as a free graft. Then, it was positioned over the osteotomy site in the experimental dogs. After 8 weeks, the dogs were euthanized and both operated and intact radii were harvested in each dog and subjected to a three-point bending test.
Results- The values of biomechanical parameters in operated bones of both groups were lower than those in intact bones. The percent of reduction in biomechanical values of the operated bones in experimental group was significantly less than control group
Conclusions and Clinical Relevance- The results of this study suggested that autogenous greater omentum as a free graft could enhance the biomechanical aspects of bone healing in dogs. Application of autogenous greater omentum as a free graft might advantageously promote fracture healing process in the dogs at risk of developing delayed or nonunion fractures.
Key words- Omentum, Angiogenesis, Bone healing, Biomechanics.

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Introduction

Normal and pathological bone physiology is inexorably tied to angiogenesis. The re-establishment of vascularity is an early event in fracture healing; and the process of bone development and repair depends on the adequate formation of new capillaries from existing blood vessels.[1] Several studies showed that experimental challenges that disrupt angiogenesis can slow fracture 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 great ability in revascularization of tissues and promote angiogenic activity in adjacent structures to which it is applied. Indeed the angiogenic material obtained from the omentum is abundant in supply.[6] Because of this unique ability of omentum, it has been used in many frequent surgeries; e.g. it has been used as a pedicle flap for support tracheal autograft,[7] ischemic bronchial autografts,[8] ischemic limbs[9] and limb salvage in Buerger's disease.[10] Furthermore transposition of the nonpedicled omentum has been used in revascularization procedures involving the chronically injured spinal cord,[11] head and neck deformities,[12] and mandibular osteoradionecrosis.[13]
In this study, in view of the fact that upregulation of angiogenesis may promote the formation of bone and accelerate the process of bone healing, the capability of greater omentum in dog, as a free graft, to stimulate and accelerate bone healing was evaluated biomechanically.

Materials and Methods

Ten male adult mongrel dogs, 3 to 4 years old and weighing between 25.2 and 31 kg, have been entered to this study. They were determined to be healthy on the basis of physical, orthopedic and radiographic examination findings and normal CBC and serum biochemistry results. The dogs were randomly assigned to either an experimental or a control group; each consisting of 5 animals. The experimental protocol was approved by the Veterinary Clinical Sciences Committee at Urmia University.
The operative procedure was done under general anesthesia. In all dogs, right forelimb from olectranon region to metacarpal region and ventral abdomen from umbilicus to the pelvic inlet were prepared for aseptic surgery. Radius was exposed via a medial approach and a 2-mm transverse bone defect was created at mid-diaphysis with a gigli wire. In both groups, the abdominal cavity was approached through a 3-cm ventral midline incision midway between the umbilicus and pelvic inlet; then free end of the greater omentum was located and exteriorized from the abdominal cavity. A 30x30-mm^2 piece of the omentum was isolated by two catgut ligatures and cut free from the remaining. In experimental group, the resected piece of the omentum was placed as a free graft, over the radial bone gap and secured in place with tack sutures with no. 3/0 polyglycolate. The surgical sites were rinsed with normal saline immediately before closure. All dogs received sodium ampicillin (Zakaria Pharmecutical Co., Tabriz, Iran) (25 mg/kg, intravenously, every 6 hours), and gentamicin sulfate (Darou pakhsh, Tehran, Iran) (5 mg/kg, intravenously, every 24 hours) for five consecutive post-operative days. Tramadol (KRKA, d. d., Novo mesto, Slovenia) (0.2 mg/kg, IM) was administered every 6 hours after surgery for 24 hours and as needed thereafter to control pain and discomfort. The animals were euthanized 8 weeks after the surgery by intravenous administration of sodium thiopental solution. The radius of both right (operated) and left (intact) forelimbs of each dog were dissected off the surrounding soft tissues, wrapped in saline-soaked gauze and subjected to mechanical testing,

immediately. Bone stiffness was evaluated by 3-point bending test for ultimate strength, maximum load, and Young's modulus of elasticity with a manual custom-made three-point bending device (Rezazadeh *et al*., Urmia University, Iran). Data derived from mechanical testing were expressed as the mean (± SD) for each group.

Statistical Analysis

A one-way between-groups analysis of covariance was conducted to compare the effectiveness of intervention (free omental graft application) on bone healing. The independent variable was the type of intervention (treatment or control group= with or without application of free omental graft, respectively); and the dependent variable consisted of scores on radial bone strength measures (ultimate strength, maximum load, and Young's modulus of elasticity) after the intervention was completed. The contralateral intact radial bone strength measures were used as the covariate in the analysis. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances homogeneity of regression slops, and reliable measurement of the covariate. Differences were considered significant when $P < 0.05$. The statistical software, SPSS Version 9.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis.

Results

The 10 operations on the dogs did not lead to any major complications, and all the animals survived the operations and interventions. All dogs had some degree of incisional swellings, which resolved in a few days. All dogs remained healthy throughout the study.

Results of the biomechanical evaluation by 3-point bending test for maximum load, ultimate strength, and Young's modulus of elasticity, which were expressed as mean (± SD) for each group are summarized in table 1. After adjusting for intact bone scores, there was a significant difference between the two groups on all post-intervention scores on radial bone strength ($P<0.05$).

Table1. Statistical comparison of biomechanical parameters of radii in control and experimental groups. Data were expressed as mean±SD.

		Maximum load (N)	Ultimate strength (MPa)	Young's modulus of elasticity (MPa)
Control	Intact limb	796.93±56.29	10.76±1.7	836.86±66.38
	Operated limb	204.82±51.14	2.68±0.24	234.01±29.58
Experiment	Intact limb	809.72±53.77	13.81±1.10	1187.33±70.41
	Operated limb	399.35±30.16	6.76±0.81	621.96±30.75

N=Newton
MPa= Mega Pascal

In both groups, the value of biomechanical parameters of operated bones of each dog was less than those of intact bones of the same dog. Significant differences in the examined mechanical properties were found between percent of decreased values of the operated bones of experimental and control dogs. Decrease in biomechanical values of operated bones is shown in table 2. The

percent of reduction in biomechanical properties of the operated bones in experimental group was significantly less than those in control group, which revealed that radial osteotomy sites were stiffer in the former group.

Table2. Decrease in mechanical properties of operated bones in control and experimental groups.

	Maximum load (%)	Ultimate strength (%)	Young's modulus of elasticity (%)
Control	74.3	75.09	72.04
Experiment	50.68	51.05	47.62

Discussion

In this study, we have investigated the role of autogenous greater omentum as a free non-vascularized graft, on experimental bone healing with regard to its plausible ability to enhance the mechanical properties in bone healing.

Since increased metabolic demands during bone repair require an increased blood flow rate (BFR) and functional vascular density (VD),[14] reconstruction of the circulation is one of the earliest and most important events during bone repair.[15] Furthermore, establishment of a functionally intact vascular network appears not only to precede the event of bone formation, but also to have a substantial influence on the result.[16, 17] Moreover the 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.[18] Thus, microcirculatory properties may play an essential role during osteogenesis [19] and in this regard vascular reorganization and blood supply at the fracture site can have an important role in the healing of fractures and the return of mechanical integrity of the repaired bone.[20]

The great ability of omentum in revascularization of tissues has been well documented. This process of neovascularization allows the omentum to provide vascular support, promote function and healing in ischaemic or inflammed tissue.[21, 22] A number of polypeptide growth factors that possess potent angiogenic properties have recently been identified and in this regard Zhang and co-workers demonstrated that vascular endothelial growth factor (VEGF) is the major angiogenic factor produced by omentum and possibly underlies the mechanism of omentum-induced angiogenesis.[23] They also demonstrated that omental adipocytes, are the primary source of VEGF protein. [23] Street and co-workers showed that VEGF can stimulate bone repair not only by promoting angiogenesis but also by accelerating and enhancing bone turnover.[24] 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. [24]

In our study, the mean mechanical properties of the operated bones tended to be less than the respective mechanical properties of the intact bones in both control and experimental groups. On the other hand, statistical analysis showed that the operated bones in experimental group exhibited a significantly less decrease in mean maximum load, ultimate strength, and Young's modulus of elasticity than operated bones in the control group ($P< 0.05$). Our results are in agreement with those of White and co-workers. They investigated temporal changes in physical properties of healing fractures. Their results revealed that the maximum load to failure changed

markedly toward increased strength and stiffness during the fracture healing process.[25] In fact in the sequence ossification during bone repair a continuum of increasing stiffness and strength is observed in tissue types from granulation tissue to cartilage and finally to bone.[26] In the bones with same structural properties (e.g.: geometry of whole bone, bone length, and bone curvature), the biomechanical properties such as maximum load, ultimate strength and stiffness (Young's modulus of elasticity) are strongly associated with their material properties, such as bone mineral density (BMD).[27] In our study, higher values of maximum load in the operated bones of experimental group could reflect larger callus size in compare to the control group. Higher values of ultimate strength and Young's modulus of elasticity of operated bones in experimental dogs in compare to the control dogs, might suggest more advanced healing of osteotomy site in the former. It might be due to more advanced mineralization of the callus, higher bone mineral density (BMD), and even increase in collagen content. Since the only variable between the two groups was the presence of autogenous greater omentum in osteotomy site in experimental group, these differences can be attributed to it. It is plausible that the greater omentum used as a free autograft in this study, acted through its major angiogenic factors to induce angiogenesis, reconstruct circulation and enhance blood supply to the osteotomy site. Accordingly, the process of bone healing hastened and the recovery of biomechanical properties of operated bones in experimental group promoted.

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 peritonism, intestinal obstruction, and perineal hernias.

Based on the results of this study, autogenous greater omentum as a free graft might has clinical application in the treatment of fractures in patients at risk of developing delayed or nonunion fractures.

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چکیده:

اثر پیوند آزاد پرده چادرینه خودی بر اختصاصات بیومکانیک التیام استخوان در سگ

سیامک سیف زاده[1]، قادر رضازاده[2] ، بهرام دلیر نقده[1] ، بهزاد پوررضا[3]، سیامک کاظمی[3]

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هدف- ارزیابی پرده چادرینه بزرگ به عنوان یک پیوند آزاد خودی در تشویق التیام استخوان.
طرح مطالعه- مطالعه آینده نگر توصیفی.
حیوانات- در این مطالعه از ده قلاده سگ بالغ، ۴-۳ ساله با وزن ۳۱-۲۵/۲ کیلوگرم، در دو گروه همسان آزمون و شاهد (۵ سگ در هر گروه) استفاده شد.
روش کار- در تمام سگ ها، استئوتومی در میانه استخوان زند زبرین راست و امنتکتومی ناقص انجام شد. قطعه ای از چادرینه به ابعاد ۳۰×۳۰ میلی متر مربع به عنوان پیوند آزاد آماده شد. سپس در سگ های گروه آزمون پیوند مذکور برروی ناحیه استئوتومی کارگذاری شد. بعد از ۸ هفته، سگها آسان کشی شده و استخوان های زند زبرین جراحی شده و سالم در هر قلاده سگ جدا شده و تحت آزمون خمشی سه نقطه ای قرار داده شدند.
نتایج- ارزش عددی پارامترهای بیومکانیکی در استخوان های جراحی شده در هردو گروه، در مقایسه با استخوان های سالم، پایین تر بود. درصد کاهش مقادیر عددی پارامترهای بیومکانیکی در استخوان های جراحی شده در گروه آزمون بطرز معنی داری، کمتر از گروه شاهد بود.
نتیجه گیری و کاربرد بالینی - نتایج این مطالعه نشان داد که پیوند آزاد پرده چادرینه بزرگ خودی میتواند اختصاصات بیومکانیک التیام استخوان در سگ را بهبود بخشد. استفاده بالینی از پیوند آزاد پرده چادرینه بزرگ خودی در شکستگی های دیر جوش یا عدم جوش خوردگی استخوانی در سگ پیشنهاد می گردد.
کلید واژگان- پرده چادرینه، عروق زایی، التیام استخوان، بیومکانیک.