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Evaluation of Compressive Mechanical Properties of the Radial Bone Defect Treated with Selected Bone Graft Substitute Materials in Rabbit

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Abstract

Objective- To determine the effect of selected bone graft on the compression properties of radial bone in rabbit.
Design- Experimental in vivo study.
Animals- A total of 45 adult male New Zealand white rabbits.
Procedures- The rabbits were anesthetized and a one-cm-full thickness piece of radial bone was removed using oscillating saw in the all rabbit. The rabbits were divided into 5 groups on the basis of the material used to fill the bone defect: group 1: the paste of bone cement calcium phosphate; group II: the paste of calcium phosphate mixture with type I collagen; group III:

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tricalcium phosphate mixed with hydroxyapatite (TCP & HP) with 5% porosity; group IV: the same scaffold as group III with 10% porosity; and group V: the same scaffold as group III and IV with 20% porosity, with 9 rabbits in each group. Subsequently subdivided into 3 subgroups of 3 rabbits each.
Results- There was a significant increase in compression properties of radial bone in the group II and V in 2nd and 3rd months as compared with groups I, III and IV. The mean endurable crack-strength in group II and V were slightly higher than that of normal radius ($P<0.05$).
Conclusion and Clinical Relevance- Application of calcium phosphate paste with type I collagen and scaffold of tricalcium phosphate with hydroxyapatite having 20% porosity indicated to have positive effect in integral formation of qualitative callus at the site of fracture and early re-organization of callus to regain mechanical strength too.
Key Words- Calcium Phosphate, Tricalcium Phosphate, Hydroxyapatite, Radial Bone, Compressive Properties, Porosity, Type I collagen, Rabbit.

Introduction

There is a continuing demand for bone substitute materials in orthopedic and large skeletal defects that result from congenital, lytic, infectious, or neoplastic processes; represent a major challenge for reconstruction procedure.[1] Various animal models have been developed for testing bone substitute materials for potential clinical applications. Non–healing osseous defects have been used to assess osteoconductive or osteoinductive[2,3] properties of implants. As the bone graft substitute materials are employed primarily to serve as scaffolds to facilitate the bone regeneration process[4,5] still the search for an ideal materials for bone grafting remains a formidable challenge. The idea and utilization of graft materials in bone surgery go back to the 17th century. However, discrepancies associated with grafting defective bone tissues demand focus on the elucidation of the biopotency of graft materials in relationship to the biology of the bone repairing process during the last two decades.[6] An ideal graft substitute should be bioresorbable and have osteoconductivity capacity.[7] It also should be nontoxic and no immunogenic to the organism, easy to sterilize[8,9] and not compromise mechanical stability.[9,10] The objective of this experiment was to examine the possibility and comparing the local effects of different bone substitutes and their mechanical properties in the radial bone defect of rabbits.

Materials and Methods

This study was performed in accordance with the Islamic Azad Sciences and Research Branch Law on animal experimentation and this research project was approved by the Faculty of Specialized Veterinary Sciences Research Councils. 45 adult male New Zealand rabbits weighed 3.0 ±3.50 Kg were used for orthopedic surgery under general anesthesia, using 1mg/kg bw Acepromazine Maleate (2%) intramuscularly as premedication (Karon Co. Iran), and 35 mg/kgbw ketamine hydrochloride (10% Alfasan Woerden-Holland), combined with 5 mg/kg xylazine hydrochloride 2% (Alfasan.Woerden-Holland) intramuscularly for induction and maintenance of the anesthesia.
After securing each rabbit in lateral position, and under aseptic condition the craniolateral surface of right radius was exposed and a one-cm piece was removed from the mid shaft of the bone, using oscillating saw. The rabbits were divided into 5 groups. The animals were divided into five groups of nine rabbits on the basis of the material used to fill the bone defect: group 1: the paste of bone cement calcium phosphate; group II: the paste of calcium phosphate mixture with type I

collagen; group III: tricalcium phosphate mixed with hydroxyapatite (TCP & HP) with 5% porosity; group IV: the same scaffold as group III with 10% porosity; and group V: the same scaffold as group III and IV with 20% porosity. Each group was then subdivided into 3 subgroups of (Ia,IIa ,IIIa,IVa,Va) of one month, and (Ib , IIb, IIIb, IVb, Vb) of two months, and (Ic, IIc , IIIc , IVc,Vc) of three months duration with three rabbits in each subgroup. The external cast was used to immobilize the limb for the first two weeks. All mechanical testing were performed by a Zwick/Roell 2005 with a crosshead speed of 0.01 mm/sec.
Each rabbit was kept in a isolated cage with free access to commercial platelets (Dam Pars Comp. Tehran) and water. Intramuscular injection of 0.05 mg/ kg dexamethasone (Vetacoic®, Aburaihan Co. Iran) and 40000 IU/kg Penicillin G, Benzedrine, Procaine and Potassium 2:1:1 (Nasr Fariman Co. Iran) was performed for five days. Tramadol Hydrochloride (ZMC HAMBURG GMBH, Germany) was administered as an analgesic for 3 post-operative days (5 mg/Kg IM bid).
All mechanical testing were performed using a Zwick/Roell 2005 with a crosshead speed of 0.01 mm/sec. A load-distance curve was recorded to determine mechanical properties. Load bearing was obtained with maximum load recorded of the linear portion of the load-distance curve. The mechanical properties of normal radial bone were measured to provide reference values. Three specimens were tested for each condition, and data were presented as mean ± standard deviations (SD). Statistical analysis was carried out on the load bearing data using one way analysis of variance with the software program SPSS for Windows, version 9 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant. Tukey HSD multiple comparison testing was used to determine the significance of the deviations in the mechanical property of each sample for different times.

Results

No operative or postoperative complications were encountered. All the rabbits tolerated operation well and survived until the final experimental time. No wound complication was observed. After sacrifice, the macroscopic evaluation revealed maintenance of the correct position of bone fragments and the substitutes. The load bearing properties of intact and five treatment groups are compared in table 1. Statistical analysis of the mechanical testing using a one way factorial ANOVA design showed significant differences in load bearing between the groups at 1, 2 and 3 months after surgery (Table 1). Significant differences for load bearing were found between intact bone with that of 1, and 2 months durations ($P<0.05$), while individual significant differences between groups were found for all groups ($P<0.05$) after 1 month, but not between groups II and V ($P>0.05$) two months postoperatively. There were no significant differences between groups II and IV and V after 3 months ($P>0.05$). The results indicated that there was significant increase in compression properties of radial bone in groups II, IV and V in 2nd and 3rd months as compared to intact bone and group I (Fig. 1). The values for biomechanical testing was 386.4±16.8 N for normal bone and 278.3±17.6 N, 362.2±20.2 N, 312±16.8 N, 346±24.04 N, and 366±8.6 N for group I to V, respectively, recorded in 3 months duration. The mean endurable crack-strength in group II and V were slightly higher than that of normal radius ($P<0.05$).

Table1. Load bearing values of groups I, II III, IV,V and intact bone (Mean ± SD)

Time of implantation	Load bearing (N)					
	Intact bone	Group I	Group II	Group III	Group IV	Group V
1 month	386.4±16.8	158.2±16.4	192.2±17.8	162±20.8	170±12.08	171±6.6
2 months	386.4±16.8	198.4±18.6	254.3±21.6	204.1±24	228±18.06	268±10.2
3 months	386.4±16.8	278.3±17.6	362.2±20.2	289±19.7	346±24.04	366±8.6

Discussion

The initial attachment and subsequent proliferation of osteogenic cells, as well as their ability to form bone are the most important factors affecting the success of a bone implant.[2,3] The current study aimed to evaluate the positive effect of a new biocompatibility of calcium phosphate mixed with collagen type I and scaffold tricalcium phosphate mixed with hydroxyapatite having different porosity on bone response in term of tensile strength.[7,11] In this study both combinations of collagen type I with bone cement in the form of the paste and also the scaffolds of tricalcium phosphate remained intact at the defect site which augmented the stiffness of the bone. This was really true with group II using collagen type I mixed with calcium phosphate and scaffold tricalcium phosphate with 20% porosity at the end of 3 months. It seems that mixing both collagen type I and bone cement and tricalcium phosphate with hydroxyapatite, due to its high porosity, become stronger on other loading modes such as tension, shear, and bending as the remodeling process continues. It also seems that there have been direct resorption of the collagen type I and bone cement and more penetration of host cells into the scaffold having 20% porosity, followed by deposition of new bone. This combination showed to have osteoconductive property to form new bone in the defected area.[12,13] The results of this study demonstrate the large disparity that can occur between the stiffness of bone defects treated with mixture of bone cement and collagen, cement and scaffold with different porosity and the normal compressive stiffness at the site. These radial bones have been attributed to the stress shielding resulting from the large disparity between the stiffness of the materials used in the host bone.[14,15,16] There are other situations in which an increase in the density and modulus of bone itself has been implicated in the accelerated breakdown of surrounding tissue.[17] The defect produced in this animal was not intended to serve as a critical size defect. The model was rather developed to evaluate the role of time in changing the mechanical properties of treated site. According to the results of this study, it could be expected that during the time, the rate of increase in the compressive strength of the bone defect, received the mixture of cement with collagen type I and scaffold with more porosity would be higher in comparison with other groups. This is explained by the increased density offered by the presence of the bone cement mixed with collagen type I and scaffold having hydroxyapatite with high porosity and attested to the mechanical continuity afforded by the bonding of bridging bone to the surface of the cement and scaffold materials.[18,19,20] The sites filled with these particles have a strength equivalent to the bone normally present at that anatomical site.[21,22,23] The much higher strength of the sites implanted with cement and collagen and scaffold with high porosity was documented by significant increase in stiffness.[24,25,26]

The present compressive mechanical properties demonstrated that, despite the duration of the experiment was too short, there was drastic changes to regain compressive strength in second and fifth groups which were near the value of intact bone.

The results of present study suggested that the combination of xylazine and ketamine (0.17 mg kg^{-1} and 1 mg kg^{-1}, respectively) induced analgesia with rapid onset and a longer duration compared to xylazine or lidocaine alone. In spite of the fact that co-administration of xylazine and ketamine did not cause adverse effects on physiological values (HR, RR, and RT); in a clinical point of view, this combination at these doses is not suitable for standing surgeries of hindquarters in donkey because there was unacceptable likelihood of recumbency. Further studies are needed to achieve an optimal combination of these drugs providing satisfactory analgesia without any complications.

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چکیده

ارزیابی خصوصیات فشردگی استخوان با استفاده از مواد جایگزینی گرافت استخوانی در نقیصه استخوان رادیال در خرگوش

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هدف بررسی - خصوصیات فشرده گی مواد جایگزین شده گرافت استخوانی در خرگوش.

طرح مطالعه- تجربی در حیوان آزمایشگاهی.

حیوانات- ۴۵سر خرگوش نر سفید بالغ.

روش کار- تحت بیهوشی عمومی درشرایط کامل آسپسی بعد از کنار زدن پوست و بافت همبند سطح جانبی داخلی دست راست و مشخص کردن استخوان رادیوس با استفاده از اره برقی قطعه ای تمام قطر به طول ۱ سانتیمتر از وسط استخوان راست برداشته شد و سپس این خرگوش ها بهپنج گروه۹ تایی گروه (I) سیمان کلسیم فسفات و گروه (II) سیمان و کلاژن تیپ و در گروه (III) گرانول تری کلسیم فسفات و هیدروکسی آپاتیت با تخلخل ۵٪ در گروه (IV) از گرانول با تخلخل ۱۰٪ و در گروه (V) از گرانول با تخلخل ۲۰٪ جهت پر کردن نقیصه استفاده گردید. در پایان هر دوره آزمایش با اخذ طول کامل استخوان رادیال راست و چپ با استفاده از دستگاه Zwick/Roell 2005 با جابجائی گیره های دستگاه با سرعت ۰.۰۱ میلیمتر بر ثانیه میزان فشرده گی استخوان سالم و آزمایش بر واحد نیوتن ثبت گردید.

نتایج- نشانیهای بالینی در هر سه گروه در طول دوران مشاهدات بالینی از یک فعالیت فیزیکی برخوردار بودند میزان افزایش مقایسه ای فشرده گی مواد جایگزین شده در سه گروه نشان داد که قدر ت و توان مواد در برابر نیرو وارده در استخوان رادیال گروه II , V در زمان دو ماه و سه ماه در مقایسه با ارزش ان با نرمال و گروه های دیگر ببسیار معنی دار بود. بطوریکه برای استخوان نرمال ۱۶.۸ ±۳۸۶.۴ نیوتن واستخوان رادیال در گروهI مقدار ۲۷۸±۱۷.۶نیوتن و در گروه II این میزان ۲۰.۲±۳۶۲.۲ نیوتن در گروه III این میزان ۱۶.۸±۳۱۲ و در گروه IV میزان فشرده گی ۲۴.۰۴±۳۴۶ نیوتن ثبت گردید.این میزان در گروه V حدود ۸.۶± ۳۶۶ نیوتن بود که در پایان دوره سه ماهه ثبت گردید.

نتیجه گیری و کاربرد بالینی- بر اساس نتایج حاصل از این مطالعه و می توان اظهار داشت که استفاده از ترکیب سیمان استخوانی و گرانول تری کلسیم فسفات با هیدروکسی آپاتیت با ۲۰ ٪ تخلخل می تواند در تحریک استخوان سازی و I و کلاژن تیب بازیافت استحکام اولیه محل شکستگی مهم و موثر باشد.

کلید واژگان- قدرت فشرده گی استخوان، کلاژن تیپ I، استخوان رادیال، تری کلسیم فسفات هیدروکسی آپاتیت، خرگوش.