Histopathological Examination of the Effects of Guided Tissue Regeneration (GTR) and Different Bone Graft Materials on the Repair of Experimentally Created Bone Defects¶

YÖNLENDİRİLMİŞ DOKU REJENERASYONU VE ÇEŞİTLİ GREFT MATERYALLERİNİN KEMİK DEFEKTLERİ ÜZERİNE OLAN ETKİLERİNİN HİSTOPATOLOJİK OLARAK İNCELENMESİ

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Summary

Purpose: The aim of this study is to evaluate and compare the effects of allografts (Tutoplast®-Spongiosa-Pfrimmer-Viggo, Biodynamics, Inc, Germany), xenografts (Bio-Oss®, Geitslich Sons Ltd Wolhusen, Switzerland), and guided tissue regeneration (GTR, Tutoplast®-Dura Biodynamics Int Deutschland, GmbH, Erlangen) on bone defects.

Materials and methods: We used 40 males Sprague-Dawley rats. The rats in the first group were sacrificed at the end of the 3rd week, whereas the rats in the second group were sacrificed at the end of the 6th week. These two main groups were divided into four subgroups according to the material used.

Results: Membrane is the most effective bone forming material when compared to allograft and xenograft materials.

Conclusion: The success of membranes in bone healing was found to be a statistically significant effective when compared to xenografts and allografts

Key Words: Allograft dehydrated with solvents, Xenografts, Guided tissue regeneration

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Özet

Amaç: Allogreft (Tutoplast®-Spongiosa-Pfrimmer-Viggo, Biodynamics, Inc, Germany), ksenogreft (Bio-Oss®, Geitslich Sons Ltd Wolhusen, Switzerland), ve yönlendirilmiş doku rejenerasyonunun (GTR, Tutoplast®-Dura Biodynamics Int Deutschland, GmbH, Erlangen) kemik defektleri üzerindeki etkilerinin incelenmesi ve bu materyallerin etkisinin karşılaştırılması

Materyal ve metot: 40 adet sıçan kullanılmıştır. İki ana grup oluşturulmuştur. İlk grup 3.haftanın sonunda ,ikinci grup da 6.haftanın sonunda sakrifiye edilmiştir. Kullanılan materyale göre de gruplar dört alt gruba ayrılmıştır.

Bulgular: Membranın diğer iki materyale göre kemik oluşumu yönünden daha başarılı olduğu tesbit edilmiştir.

Sonuç: Kemik iyileştirici etkisi yönünden membranlı grubun daha başarılı olduğu gözlenmiştir.

Anahtar Kelimeler: Solventlerle dehidrate edilmiş allogreft, Ksenogreft, Yönlendirilmiş doku rejenerasyonu

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Autogenic, allogenic, and xenogenic bone grafts, ceramic, polymethylmetacrylate, metal plaque and pins, GTR, and distraction osteogenesis are among the most frequently used methods for the repair of bone defects (1-7).

Rapid connective tissue formation is the main difficulty for optimal bone repair and new bone formation. Guided tissue regeneration, which origins from the idea that, preventing fibrous tissue from entering the defected area allows new bone formation, has been studied by periodontologists in the early eighties (1,8,9). This article aims to study and compare the effects of allograft to xenograft and membrane when used alone.

Material and Method

This study was carried out at the University of Istanbul, Experimental Medicine Research Institute using 40 male Sprague-Dawley rats weighing 400-450 g. Materials that we used in our study were allograft (Tutoplast®-Spongiosa-Pfrimmer-Viggo, Biodynamics, Inc, Germany), xenograft (Bio-Oss®, Geitslich Sons Ltd Wolhusen, Switzerland) and membrane (Tutoplast®-Dura Biodynamics Int Deutschland, GmbH, Erlangen). Anesthesia of the rats was achieved using i.m. Ketalarâ injection (100 mg/kg). Then bone cavities which were 10 mm long, 3 mm deep and 2 mm wide were formed by fissure burs with low rpm, under irrigation with sterile saline solution and standardised with the help of wooden models of the same dimensions on the tibias of the rats.

All of the rats were divided into two main groups. The rats in the first group were sacrificed at the end of the 3rd week, whereas the rats in the second group were sacrificed at the end of the 6th week. These two main groups, were divided into four subgroups according to the material used.

Group 1: Bone cavities were filled with allograft material.

Group 2: Bone cavities were filled with xenograft.

Group 3: Bone cavity was only covered with membrane.

Group 4: Bone cavities didn't receive any application (control group).

Histopathologic investigation was carried out at the University of Istanbul Faculty of Veterinarian Dept. of Pathology. For histopathologic investigation, the specimens were fixed in formal saline (10%) and rinsed in saline solution containing 3% sodium citrate and formic acid. After this procedure, they were embedded in paraffin wax. Interrupted serial sections of 7µm thickness were cut and stained with haematoxylin and eosin. The results were evaluated with light microscopy (4).

Table 1. Modified Heiple Bone Regeneration Scoring System

0- No new bone formation
1- Limited new bone formation on the margins of the defect approximately 1/3 of the defect is filled
2- High amount of new bone formation. 2/3 of the defect is filled with new bone
3- High amount of new bone formation, leaving only a little gap at the center of the defect

Specimens were evaluated according to Modified Heiple Bone Regeneration Scoring System used for evaluation of bone healing (4) (Table 1).

Results

In the 1st group with allograft; at the end of 3rd weeks; in all sections bare fibrous tissue formation and osteoblastic activity around the graft material was observed.

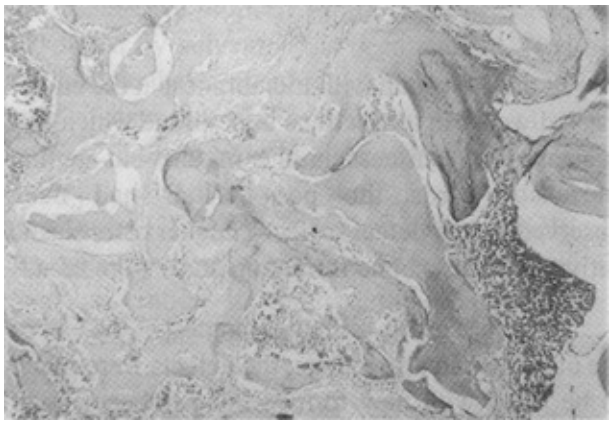

Figure 1. The specimen shows, more smooth and equal thickness trabeculae (10 X HE) (Tutoplast R group 6 th weeks)

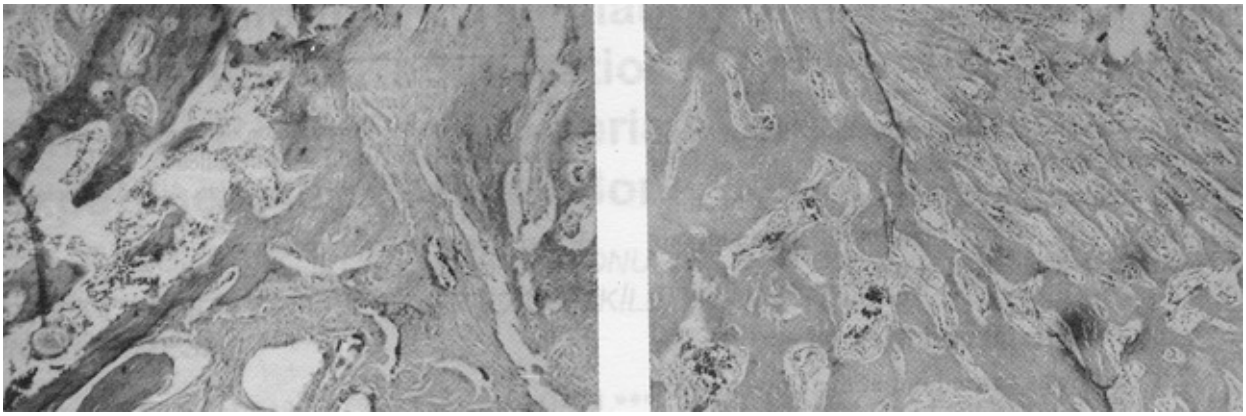

Figure 2. High density of bone trabeculae around the particles and in some areas different length of trabeculae were seen. (10 X HE) (Bio-Oss R group 6 th weeks)

Figure 3. Increased osteoblastic activity and bone marrow was seen. (10 X HE) (Tutoplast®-Dura- Membrane group 6 th weeks)

In the 2nd group with allograft; at the end of 6th weeks; all sections showed fibrous connective tissue formation in a capsule form, and in the various regions, more bone tissue formation compared to the 3 weeks group was observed. (Fig 1).

In the 3rd group with xenograft; at the end of 3rd weeks; in all sections, a very thin bone tissue formation was observed around the graft material. A connective tissue starting from around the bone spreading to the peripheral area was observed.

In the 4th group with xenograft; at the end of the 6th week; centers of fibrous tissue formation around the graft material was seen with some bone tissue covering some parts a graft like capsule (Fig 2).

In the 5th group with membranes; at the end of the 3rd week; in all sections bone tissue which had decreased of hardness in the middle but getting fibrous towards the peripherium had been observed. In the defect surfaces, higher osteoblastic activity and new bone formation areas were seen.

In the 6th group with membranes; at the end of the 6th week; in all sections, the defects were found to be filled with new formed bone trabeculaes of different lengths and thickness with dense fibrous tissue formation in some parts. After the staining, it was seen that some of these trabeculaes have transformed into lamellar bone, whereas some of them were not completely mature (Fig 3).

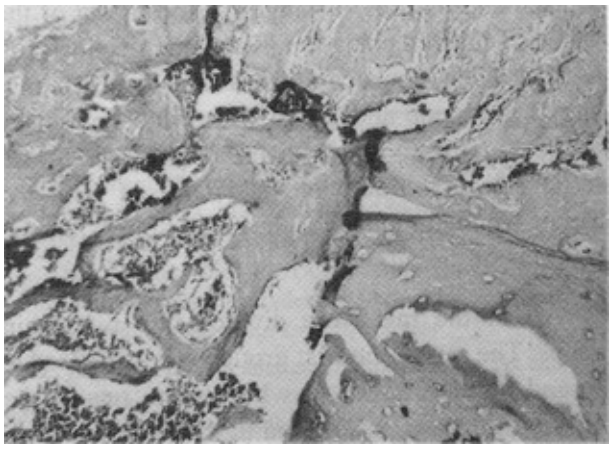

Figure 4. Areas of immature bone tissue (10 X HE) (Control group 6 th weeks)

In the 7th group which is also the control group; at the end of the 3rd week; in all sections, a tissue containing fibrous material was seen on the defect surfaces (Fig 4).

In the 8th group; at the end of the 6th week; in most of the sections, defects were found to be filled with fibrous tissue.

Table 2. The values of groups according to Modified Heiple Bone Regeneration Scoring System

	Allograft		Xenograft		Membrane		Control	
	3rd week	6th week	3rd week	6th week	3rd week	6th week	3rd week	6th week
0	-	-	-	-	-	-	3	1
1	6	3	5	3	1	-	5	6
2	4	6	5	7	6	2	2	3
3	-	1	-	-	3	8	-	-

In this study, statistical tests were carried out using GRAPHPAD V.2.02 package program. In the comparison of four groups ANOVA was used, the comparison of sub groups was carried out using Tukey-Kramer Multiple Comparison Test. In the comparison of two groups, Paired Student's t test was used (Table 2).

When the groups are compared for the increase in bone formation on the 3rd and 6th weeks:

In the only membrane group, with t: 2.49, p: 0.02, a statistically significant result was observed. On the 3rd week: A significant difference between the membrane and allograft groups was seen with $p<0.05$, between the membrane and control groups, again a significant difference was found with $p<0.001$.

On the 6th week: Significant differences were observed between the membrane and allograft groups with $p<0.01$, between membrane and xenograft groups with $p<0.001$ and between membrane and control groups with $p<0.001$ on the behalf of the membrane group.

Discussion

It is generally conceded that bone autografts are far superior to any type of bone grafts; therefore, the overwhelming majority of surgeons prefer autologous bone. However with the more widespread application of bone grafting, the replacement of large bony defects caused by trauma or wide resection of tumors, large amounts of bone are required and are not always as available as autografts. Moreover procuring autografts requires an additional surgical procedure on the same patient increasing the risk of infection, increasing blood loss, lenghtening the operating time and leading to possible increased morbidity. Consequently extensive research and various methods of preparing preserved allografts and xenografts have been explored (4,7,10,11).

Akal et al (3) have applied allograft (dehydrated Tutoplast®-Spongiosa chips) to the bone defects after cyst enucleation and apical root resection operations. On the 1st week, 1st, 3rd, 6th months and 1st year post operatively, clinical and radiographical examinations were carried out. As a result of these examinations no trabecular anomaly, migration or foreign tissue reaction was detected. Complete healing was observed. We also have observed more trabecular formation in the 6th week groups than the 3 rd week groups, without any foreign body reaction.

Successful results in experimental and clinical studies and in usage of barrier membranes for the improvement of healing of bone defects have been reported (1,4,5,6,12). In our study, the osteoconductive property of allografts (Tutoplast® dehydrated by solvents) has been studied. Nonresorbable synthetic membranes are reported to be cause of exposure and infection because of their hardness. Requirement of a secondary operation is another disadvantage of nonresorbable membranes(13). In our study, such a complication was not observed. However requirement of a second operation to remove the nonresorbable PTFE membrane is considered as a disadvantage (14,15).

Sayan et al (13), examined and compared the effects of nonresorbable polytetrafluoroethylene (PTFE) membranes with Tutoplast® Dura on

healing of bone defects. They used 8 dogs and evaluated the results both clinically and histopathologically at the end of post operative 3rd and 6 th weeks . They didn't find any significant differences between two membranes on healing of bone defects and reported that due to the resorbable material Tutoplast® Dura doesn't need second surgical procedure. We also think that resorbable materials have advantages in comparison to the others.

Özgen at al (7), evaluated the effects of Tutoplast® Dura , polyurethane membrane and fascia lata on dog mandible's. Following the operation on the 14 th, 21 st, 28 th days , bone secimens were taken. They reported that, Tutoplast® Dura group gave more satisfactory results and none of the groups showed any inflammatory response. By the same way, in our study , we didn't find any inflammatory response on Tutoplast® Dura group .

Yazar at al (4),examined the effects of GTR in continuity bone defects on osteogenesis-without using bone graft materials. They divided rats was, into two main groups and then two main groups were divided into three subgroups. One of them is the experimental group and the other two of them were control groups. In the 1 st group, they removed periosteum together with bone and did not apply membrane, in the 2 nd group only bone was removed, periosteum was preserved and no apply membrane was applied, in the 3 rd group, bone and periosteum were removed and e- PTFE membrane was applied. They found an important statistical difference between the periosteum groups which had been preserved and had not been preserved . As a result, they reported that GTR technique is not uniquely sufficient for continuity bone defects.

Collagen membrane was applied to 5 mm wide bone defects (critical defect) formed on the zygomatic bones of a rabbits by Mundell et al (6). In the examination made after 4 weeks of healing period, the defects were found to have been healed with bone regeneration. In the control group, defects were found to be filled with fibrous connective tissue. As a result they have stated that collagene membranes could be used in the treatment of continuous bone defects. However Mundell et al (6) have reported a slight inflammatory reaction against the collagene membranes. Observation of no such side effects in our study, supports the idea that Tutoplast®-Dura is a biocompatible material.

Sandberg et al (16), have sealed the 5 mm wide defects (critical defect width) that they have formed in the rat mandibles using e-PTFE and resorbable polylactic/polyglycolic acid copolimer membranes, and found that the defects were mostly filled with new bone formation. They have stated that inflammatory reactions were due to the high resorbability of the membranes. They also have concluded that resorbable membranes did not have enough resistance to the pressure of the surrounding soft tissues. In our experimental study we have observed the duration of the membrane continuity, and the same areas of cartilage formation were also seen as in PLA/PGA membranes.

Berglundh et al(17), formed defects on one side of the dog mandibles and applied xenograft (Bio-Oss®, Geitslich Sons Ltd Wolhusen, Switzerland), then performed normal extraction on the otherside. As a result, they have stated that xenograft was completely replaced by bone and is an osteoconductive material. Our observation of dense bone trabeculae on the 6th week in the group to which xenograft was applied, confirms that xenografts are osteoconductive materials.

Hockers at al(18), examined the effects of a bioresorbable membrane (Bio-Gide ®) supported by xenografts (Bio-Oss®) or autografts in regenerating bone into periimplant defects. The animals were divided to for groups. 1 st group, received Bio-Gide ® membrane alone , 2 nd group received Bio-Gide ® membrane supported by Bio-Oss® , the 3rd group received the Bio-Gide ® membrane supported by autografts the fourth group, served as control. They concluded that the bioresorbable membrane enhances bone regeneration in particular in conjunction with the use of a supporting graft material. In addition, deproteinized bovine bone mineral and autogenic bone grafts appeared to be equally well integrated into regenerating bone.

In a study by Merkx et al (19), experimental defects were created in the goat bones and autogenic cancellous, cortical bone and xenograft (Bio-Oss®) were applied to compare their effects. They have reported that, in the end of 24 weeks xenograft was replaced by new bone formation, and this procedure was performed by multinucleated osteoclast like cells. In our study, the xenograft group showed new bone formation in the end of 6 weeks, without any foreign body reaction.

Merkx et al (20), evaluated the capacity of composite grafts consisting of either particulated cancellous or particulated cortical bone and anorganic bovine bone mineral (Bio-Oss®) to ınduce regeneration in standardized critical size bone defects overlying the frontal sinus. They used skeletally mature female goats. As a result, they reported composite grafts consisting of autogenous cancellous bone/ Bio-Oss® yield good results, combining the advantages of each material alone and reducing the disadvantages of each when used seperately.

Young et al (21), stated that, long term studies are needed to determine whether anorganic xenogenic bone may be regarded as a resorbable material and whether any side effects occur as a result of this material's tendency to linger in the recipient bed.

Conclusion

As a result, the success of membranes in bone healing was found to be statistically significant when compared to xenografts and allografts. Even though bone formation in the graft groups was more than the control group, no statistically significancy was found. It was also detected that there had been no significant difference between the allografts and xenografts, and all of the three materials were biocompatible.

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