Healing of mandibular through-and-through osseous defects by Guided Tissue Regeneration in ferrets

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INTRODUCTION

The ultimate goal of endodontic surgery is the predictable regeneration of periapical tissues, including a complete repair of osseous defects. One of main concerns in treating an endodontically involved tooth with a through-and-through osseous defect is that incomplete bone healing may be inevitable[1,2]. Despite the fact that the periapical area and most canal systems are free of infection following endodontic treatment, bone healing does not always occur due to an ingrowth of connective tissue into the osseous defect. This can result in periapical scarring which is often misdiagnosed as pathology and may lead to unnecessary surgical reentry.

In 1982 Nyman et al[3,4]. defined and presented a new biological principle relating to regenerative wound healing, described as guided tissue regeneration(GTR). The principle of GTR, as applied to bone healing, is based on the prevention of connective tissue from entering the bony defect during the healing phase. This allows the slower bone producing cells to migrate into and reproduce bone within the defect.

Previous studies[5,6] using the polytetrafluoroethylene (PTFE) membrane (Gore-Tex ; W.L. Gore an Associates, Flagstaff, AZ) on mandibular defects of rats and monkeys have shown that complete osseous repair occurs within as few as 3 weeks.

Lundgren et al.[7] used the principle of guided tissue regeneration to test if Polyglactin 910 (Vicryl ; Ethicon, East Brunswick, NJ), which is a resorbable membrane, can be used to achieve proper bone regeneration in through-and-through bone defects in the skulls of rabbits. Results showed regeneration of bone extending from one edge of the defect to the other; whereas control groups contained only soft tissue repair. Pecora, et al.[8] demonstrated that the use of GTR principles can enhance the quality and quantity of bone regeneration in the periapical lesion in humans and that the GTR procedure accelerates bone growth

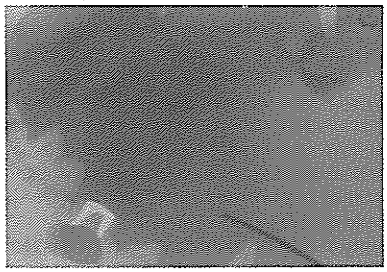

Fig. 1. Radiograph after periapical surgery. The through-and-through osseous defect was created.

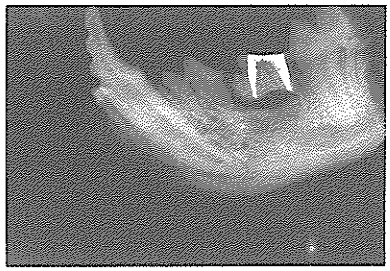

Fig. 2. Radiograph after 12weeks in control group.

Fig. 3. Radiograph after 6 weeks in Gore-Tex group.

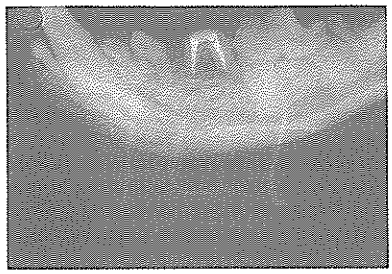

Fig. 4 : Radiograph after 12weeks in Gore-Tex group.

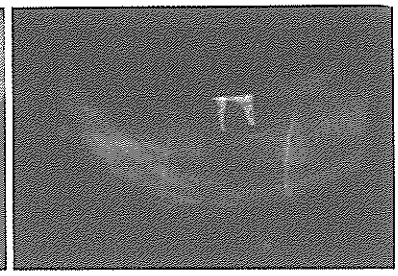

Fig. 5 : Radiograph after 12weeks in Vicryl group.

in circumscribed bone cavities after endodontic surgery.

Up date however, there have been few published studies using biodegradable membranes on maxillary or mandibular jawbone defects. Furthermore, there have been no studies which compare the effectiveness of the expanded PTFE membrane versus biodegradable membrane (Vicryl, Guidor).

The purpose of this study was therefore to evaluate whether improved bone regeneration can be achieved in dental bone with the different membrane barriers (Gore-Tex, Vicryl, and Guidor), following the creation of a through-and-through lesion.

MATERIALS AND METHODS

Six adult male ferrets (Mustela putorius furo ; erdes Carnivora) were used. The age of ferrets ranged from 18 to 24 months to ensure that the apex of the mandibular premolars were mature and fully developed. Prior to the surgical procedures, the animals were anesthetized with an intraperitoneal injection of ketamine (30mg/kg body weight) and Atrophine and Xylozine. The mandibular premolars were isolated with cotton rolls, and the crowns sectioned approximately 2mm coronal to the free gingival margin to allow access to the root canal systems. The canals were

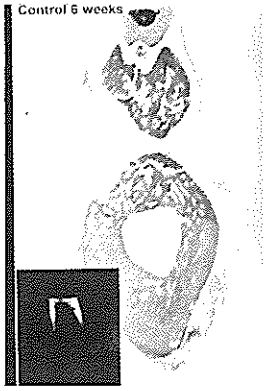

Fig. 6 : Control 6 weeks group : The rest of the defect was closed by a large bridge of connective tissue.

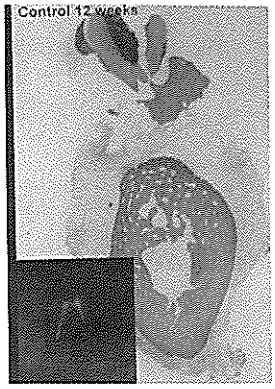

Fig. 7 : Control 12 weeks group shows partially formation fibered bone at limited area. but the ingrowth of sulcular epithelim into the defects.

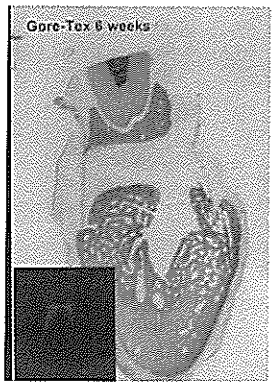

Fig. 8 : Gore-Tex 6 weeks group shows the moderate bone regeneration. but the remaining defect is filled with connective tissue.

cleaned and shaped to a size 25 with the use of standard files and a conventional step-back technique. Exact determination of working length was unnecessary, as the root and surrounding periapical tissue would be excised during the surgical procedure. The canal was dried with paper points, obturated with gutta-percha and sealer, and the coronal access was restored with IRM. A full thickness mucoperiosteal flap was elevated bilaterally from the midline to distal of the molar region to canine in the mandible. With #6 round burs and #557 straight fissure burs(SS White, Lancaster, PA.) in a high speed handpiece, a standardized round through-and-through osseous defect (3x5mm in diameter) was created bilaterally at the level of the root apices of the mandibular premolar. The exposed root was resected with a 169 fissure bur (SS White, Lancaster, PA.). Copious amounts of saline were used for irrigation.

A total of 12 defects were created and divided into four groups (Group 1-4). In Group 1, (control), the defects were covered with the mucoperiosteal flap without a barrier. In Group 2, the transosseous defects were covered both buccally and lingually with a Gore-Tex membrane extending 2 mm beyond the defect. The flap was carefully repositioned on the outer side of the test material and sutured with 5-0 Catgut sutures so that the material would be totally covered. In Group 3, the defects were covered with Vicryl on both sides. In Group 4, the defects were covered with Guidor (Guidor Co., Bensenville. IL) on both sides. Radiographs were taken after surgery. Each group was subdivided into two subgroups with healing periods of 6 weeks and 12 weeks. At 6 and 12 weeks postsurgically, each animal was sacrificed with an overdose of pentobarbital. At this time, radiographs were taken.

The defect areas including the surrounding tissues were sectioned for histological preparation. The tissue blocks were fixed in 10% buffered

Fig. 9. Gore-Tex 12 weeks group shows the defect is filled with the regenerated bone.

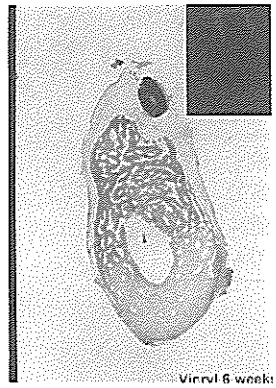

Fig. 10. Vicryl 6 weeks group shows the defect is almost filled with the regenerated bone.

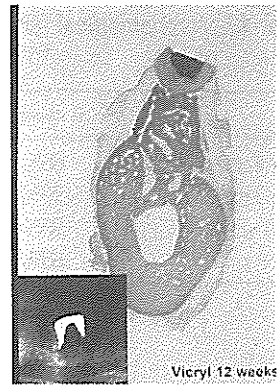

Fig. 11. Vicryl 12 weeks group shows the defect has undergone extensive bony healing.

formalin solution.

The radiographic evaluation was done using Sigma Scan/Image software (Jandel Scientific Software, San Rafael, CA). The size of lesions were quantified morphometrically.

The histologic evaluation was done by using sawing and grinding technique, developed by Donath and Breuner[9] for the examination of uncalcified bone and teeth with attached soft tissue.

RESULT

Radiographic finding

The results of the morphometrical analysis of the radiograph are presented in table 1. Control 6 weeks and 12 weeks group showed some bone growth , but large defects remained on these teeth. Gore-Tex 6 weeks group showed reasonable healing with only a small remaining defect. Gore-Tex 12 weeks group radiographically healed better. Vicryl 6 weeks and 12 weeks group showed that in the apical area the defect was filled with bone. Guidor 6 weeks group showed rather defect still present. And Guidor 12 weeks group showed bony like materials at the lesion but still large periodontal defect.

Histologic finding

1. control group

1) 6 weeks

Control 6 weeks group showed the insignificant bone regeneration at the basal edge of the defect. The rest of defect had hardly undergone bony healing but was closed by a large bridge of connective tissue. Bone structure was fibered bone and osteiod.

2) 12 weeks

Control 12 weeks group showed the limited bone formation in the crestal region of the defect. Bone structure was fibered bone, osteoid and immature lamellar bone.

Table 1. Morphometrical analysis of the radiograph.

	6 weeks	12 weeks
Control group	65%	80%
Gore-Tex group	90%	95%
Vicryl group	95%	95%
Guidor group	65%	90%

Fig. 12. Gore-Tex 6 weeks group shows immature spongy bone growth into the defect.

Connective tissue infiltration and ingrowth of sulcular epithelium into the defects were found. The alveolar bone resorption with inflammation soft tissue infiltration was found.

In the control group, histologic finding showed partially formation of fibered bone on the side of the defect at the limited area and intense bone

2. Gore-Tex group

1) 6 weeks

Gore-Tex 6 weeks group showed the moderate bone regeneration. Only a thin layer of new bone remodeling below the tunneling defect but the ingrowth of sulcular epithelium into the defects . had been created. The quality of regenerated bone was mostly immature woven bone, rarely lamellar bone. The remaining defect was filled with mostly connective tissue and ingrowth sulcular epithelium. The mandibular canal was closed by regenerated bone.

2) 12 weeks

In Gore-Tex 12 weeks group, the defect was filled with the regenerated immature bone, which is partially remodeled into lamellar bone, up to the end of root but a certain area of the defect was filled with the connective tissue. Paramembraneous inflammation with signs of alveolar bone resorption was found. The membrane had been infiltrated by granulocytes. Its outer surface wassurrounded by macrophage and was separated by a connective tissue pseudomembrane. The direct bone apposition to the membrane was found at the limited area.

It was clearly discernible that the membranes, which protrude into the oral cavity and are thus contaminated by bacteria, adversely affect bone resorption. However the limited, direct membrane -bone contacts reveal the osteoconductive potential of the Gore-Tex membrane.

3. Vicryl

1) 6 weeks

In Vicryl 6 weeks group the defect had undergone complete bony healing. Woven bone -partially fibered bone, partially lamellar bone- with osteoid apposition was found at the surfaces of the trabeculae. Intense bone remodeling was found at the margins of the former defect. Generally hardly any inflammatory reaction was seen. Some macrophages and granulocytes are located near the buccal and lingual membrane residues.

Fig. 13. Direct bone apposition to the Gore-Tex membrane is shown at the Gore-Tex 12 weeks group.

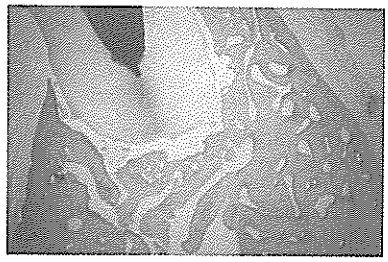

Fig. 14. Vicryl 12 weeks group shows the defect is completely filled with the regenerated bone.

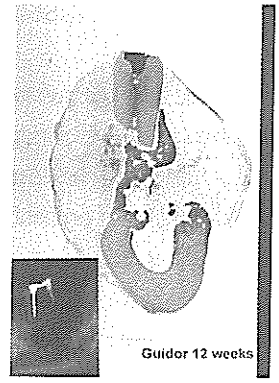

Fig 15. Guidor 12 weeks group shows the sefect has severe inflammatory reaction with massive bone loss.

2) 12 weeks

Vicryl 12 weeks group showed the defect had undergone extensive bony healing. The regen erated bone was mainly lamellar bone, partially fibered woven bone. Fewer microgranular membrane residues could be found buccally and lingually. Signs of increased bone remodeling in the zones adjacent to the defect was found.

After 6 weeks postoperatively the defect had undergone nearly complete bony regeneration. After 12 weeks postoperatively, the defect was filled with mainly lamellar bone. The extremely good biocompatibility with an only limited inflammatory potential.

4. Guidor

1) 6 weeks

The Guidor 6 weeks group showed the tooth was only anchored by the connective tissue. Massive inflammatory reactions reached into the depth within the buccal and lingual connective tissue in the region of the membrane insertion site. The inflammatory reaction had been caused by numerous large membrane residues. The localized formation of fibered bone was found on the basal side of the defect. Several areas of bone resorption on the lingual and buccal sides were found.

2) 12 weeks

The connective tissue anchorage of the tooth was found in Guidor 12 weeks group. The alveolar bone had been completely resorbed except for a small bone residue, which was the newly formed structure. This newly formed bone was mainly fibered bone, partially immature lamellar bone with an irregular cancellous structure.

The severe inflammatory reaction with massive lost of bone substance and the negative effect on bone regeneration were found. The limited bone formation, which was mainly fibered bone, was founded.

DISCUSSION

Periapical lesion healing with scar tissue after surgical treatment of periapical granulomas or cysts was described by Andreasen and Rud[1,2]. Fibrous scar tissue is probably formed by the soft tissue outpacing the slower bone regeneration from the osseous part of the cavity. The different turnover of these two types of tissue may influence the rate of growth of bone into the surgical wound.

The size of the experimental circumscribed lesion was found to be great importance in bone healing(Boyne et al[10]., Hjorting-Hansen and Andreasen.[11], Schmitz and Hollinger.[12]), because the distance between the soft and the hard tissues will determine which kind of tissue is formed. If fibrous tissue has been established first, it will probably act as a barrier to prevent further bone formation. Kaban and Glowacki[13] and Kaban et al.[14] created 4mm diameter through-and-through defects in the mandibular ramus of rats. These defects failed to heal at 16 and 24 weeks. Hjorting-Hansen and Andereasen.[11] created 5mm, 6mm, and 8mm defects in the mandible at adult Mongrel dogs. At 16 weeks, 8mm defects exhibited healing with fibrous tissue. But Schmitz and Hollinger[12] suggested at least 20mm defects in dog and monkey mandible as critical size defects which would not heal during the life time of the animal. Under experimental conditions in ferrets, 3x5mm through-and-through osseous defects were created. In this study, control group showed partial formation of immature fibered bone and connective tissue and sulcular epithelium ingrowth into defects.

GTR was first introduced as a treatment modality in the regeneration of the periodontium at the site of previous periodontal breakdown[15-19]. The treatment procedure involves the placement of a membrane barrier which prevents the gingival tissues from contacting the root surface during the healing process. This membrane protects the root surface from the growth of gingival epithelial cells. This gives the slower migrating periodontal ligament cells an opportunity to produce a new connective tissue attachment which can then proliferate coronally.

The principle of GTR can also be applied to endodontic surgery and cranial surgery[20,21] in the treatment of through-and-through osseous defects. Dahlin et al.(6) have shown complete healing in maxillary and mandibular defects using a membrane technique in monkeys. The defects were covered buccally as well as lingually with expanded PTFE membranes(Gore-Tex) and complete bone healing was seen after a 3 month period. Dahlin et al.[5] showed complete bone healing of bony defects using PTFE membrane by GTR in rats after a healing period of 6 weeks. In both of these studies, the results showed that none of the control defects(containing no membrane) healed spontaneously, but were filled with connective tissue to varying degrees. It is probable that the ingrowth of connective tissue prevents the formation of new bone tissue, resulting in a permanent bony defect.

The results of Gore-Tex group allowed only limited comparison with those of the other three groups, because membrane exposed to the oral cavity and were contaminated resulting in bone resorption. Despite of these limitation, the good osteoconductive potential of the Gore-Tex membrane , which has been described by several studies, was discernible in a few sites with direct bone-membrane contact.

The Vicryl group showed nearly complete bone regeneration 6 weeks after surgery. The defect was filled primarily with lamella bone in the 12 weeks Vicryl group. The Vicryl membranes showed the highest osteoconductive potential.

The Guidor group showed the defects filled with connective tisssue instead of bone and severe inflammtory reaction with massive bone loss. This group showed less bone regeneration than the control group. And at certain area showed fibered immature bone formation. Surprising the poor result of Guidor may be due to thickness of Guidor. The reason of severe periodontal defect may be not from physical property of Guidor but twice thickness of other membrane. In ferrets gingival tissue is too thin to adapt and enclose with Guidor. Perhaps this reason is one of the reason that Guidol give us not a good result.

Results of the present study suggest that the membrane barrier technique generally improved the bone regeneration. The Vicryl membrane resulted in the highest osteoconductive potential under the present experimental conditions.

As more endodontist become proficient at periapical surgery, GTR not only has a place for the repair of endodontic defects but also periodontal defects which are exposed upon flap reflaction. The result of this project will provide a comparative study among the different membrane barriers in regards to osseous healing potential.

CONCLUSION

1. The control group did not show any substantial bone regeneration.
2. Despite of the exposure of the membrane into the oral cavity the result of the Gore-Tex group showed the good osteoconductive potential of the membrane.
3. The Vicryl membrane showed the highest osteoconductive potential under the present experimental conditions. The defect had been filled almost completely with lamellar bone, and the inflammatory reaction by membrane resorption was extremely weak.
4. The Guidol group showed limited bone regeneration. The Guidol group showed severe inflammation caused by membrane resorption.
5. The results of the present study suggested that the membrane barrier technique generally improved the bone regeneration.

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- 국문초록 -

흰 족제비에서 조직유도재생술을 이용한 하악골의 관통된 결손 부위의 치유에 관한 연구

강릉대학교 치과대학 치과보존학 교실

외래강사 백 승 호

본 연구의 목적은 관통된 뼈의 결손부위를 각기 다른 membrane(Gore-tex, Vicryl, Guidor)을 사용하여 보다 나은 골 재생을 얻을 수 있는 가를 평가하는 데 있다. 여섯 마리 흰 족제비의 12개 하악 소구치를 근관치료한 후, 치근단 절제술을 실시하여 3mm x 5mm 크기의 관통된 뼈의 결손을 소구치의 근첨부에 형성하였다. 전부 12개의 결손부위가 형성되었고, 이를 4개의 군으로 나누었다. 대조군으로 결손부위를 membrane barrier없이 점막골막피판으로 덮었다. 다른 각 군은 결손부위를 각 Gore-Tex, Vicryl, Guidor membrane을 사용하여 설측과 협측 모두 덮었다. 각 군을 치유기간 6주와 12주로 두 아군으로 분리하였다. 방사선학적 소견으로 6주군에서 대조군은 65%, Gore-Tex군은 90%, Vicryl군은 95%, Guidor군은 65%의 결손부위의 치유를 보였다. 12주 군에서 대조군은 80%, Gore-Tex군은 95%, Vicryl군은 95%, Guidor군은 90%의 치유를 보였다. 조직학적 소견으로 대조군에서는 완전한 골 재생이 일어나지 않았으며, 결손부위로 결합조직이 자라들어온 것이 관찰되었다. Gore-Tex 6 주군에서는 대부분 fibrillar bone이 관찰되었고, 12 주군에서는 부분적으로 lamellar bone이 형성되었다. Vicryl군에서는 거의 완전한 골의 재생이 관찰되었다. 6주군에서는 재생된 뼈는 fibrillar bone이고 부분적으로 lamellar bone을 관찰되었고, 12주 군에서는 주로 lamellar bone으로 구성되었다. Guidor군에서는 제한적인 골 재생과 함께 심한 염증이 관찰되었다. 본 연구의 결과에서 조직재생유도술은 일반적으로 골 재생을 증진시킴을 볼 수 있었고, Vicryl membrane이 가장 뛰어난 골 재생유도 가능성을 보였다.