A Histological Study of the Periodontium in a Tooth Bearing Bone Graft

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A HISTOLOGICAL STUDY OF THE PERIODONTIUM
IN A TOOTH BEARING BONE GRAFT

by

ROBERT F. MISIEWICZ

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

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LIFE

Robert Felix Misiewicz was born in Boston, Massachusetts, on May 31, 1936.

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CHAPTER I

INTRODUCTION

Bone grafting and tooth transplantation are processes which have been known for many centuries. Literature reveals little, however, on the subject of tooth bearing bone grafts. The surgical approach to the problem has been investigated. It has been suggested that due to pulpal necrosis, future studies should be carried out using root filled teeth.

This paper will report on the histological findings of the periodontium of such a tooth bearing bone graft.

The ultimate aim of this study will aid in determining the feasibility of transplanting teeth using such a graft.
CHAPTER II

REVIEW OF THE LITERATURE

A. Transplantation of Teeth:

The literature concerning the transplantation of teeth is copious and varied. A review could be focused on many aspects of the subject. Interest in the subject has vasculated since its recorded beginning in the writings of Abulcasis (1050-1122), an Arabian surgeon. He described the binding of loose or accidently removed teeth with a gold wire which was allowed to remain in place for a lifetime.

Ambrose Pare (1517-1592) advocated replantation and was the first to mention transplantation.

Charles Allen in 1685, wrote the first book in English on dentistry. In his book he discussed homogenous transplantation which he described as "robbing Peter to pay Paul".

Pierre Fauchard (1690-1761) in writing on replantation and transplantation stated that teeth, "may be replaced in the jaw; or they may be taken out to be placed in another person's mouth".

John Hunter (1776) was a strong advocate of transplanting and replanting teeth and he described these operations more fully than
had been done before. He also described resorption of a planted tooth and likened it to that of an exfoliating deciduous tooth.

At the end of the eighteenth century, American dentistry had its beginning and we note much clinical activity and little laboratory experimentation in the field of tooth transplantation.

In the late 1800's there were great extremes in thinking regarding replantation of teeth. Thompson reported, in 1881, that he replanted teeth in patients with periodontitis or caries extending below the gingival margin. He would remove the tooth for treatment or restoration and then replant it.

Leon Frederl (1887) was believed to be the first one to experiment with transplants on dogs and to examine the tissues microscopically. His views were that:

1) absorption does not occur in teeth protected by the periosteum,
2) periosteal remains were absolutely necessary to obtain reunion,
3) any consolidation was due to renewed vitality on the part of the periosteum, and
4) whenever a portion of the periosteum was destroyed
there absorption began."

Julius Scheff of Vienna and Mendell-Joseph of Paris both shared Fredel's views.

Curtis (1888) reported that replanted teeth became ankylosed and could be used for bridge abutments.

In 1893, Younger expressed the belief that the presence of periodontal membrane was necessary to the success of a transplantation.

Barrett theorized in 1901 on whether or not replanted teeth became ankylosed. He thought that a new periodontal membrane was formed and that it in turn formed bone and not cementum.

From 1900 to 1935 the literature reveals an increasing number of animal studies. In 1917, Wilkinson reported on the histopathology of replanted teeth in the monkey. He found that the tooth stripped of periodontal membrane had less resorption than that on which the membrane was retained. Bodecker and Lefkowitz (1935) replanted teeth in dogs. They utilized root canal therapy prior to replanting the teeth which, they observed, became firm functioning units.

In the late 1930's attention was focused on the role of reattachment of the periodontal membrane to cementum. These works deal mainly with the periodontal pocket but they are of interest in studying the
reattachment of transplanted and replanted teeth.

Skillen and Lundquist, in 1937, created periodontal pockets in dogs and showed that epithelial downgrowth prevented the connective tissue reattachment in such pockets.

Beube (1947) used dogs in a study of periodontal pockets and their repair. He provided interesting information on the process of reattachment. He noted polymorphonuclear leukocytes and acute inflammation in twenty-four hours. In three days there was a predominance of monocytes. Fibroblastic tissue was present in eight days and new bone and cementum were present in twenty-seven days.

Between the years of 1945 and 1950 there was a revived interest in replantation of teeth. The majority of the reported cases utilized root canal therapy. In 1948, Kromer presented a rational technique for replantation. He wrote of the importance of root canal treatment and the retention of the periodontal membrane. Infection was not considered to be an important postoperative complication.

From 1950 to present there has been renewed interest in transplantation. Between 1950 and 1955 there appeared a series of publications on the autogenous transplantation of human teeth by Apfel, Miller, Fong, Hale and Clarke. Varying degrees of success
have been obtained. Between 1952 and 1957 Fleming and Glasstone successfully transplanted developing tooth buds of experimental animals to various sites such as the brain and the anterior chamber of the eye. The ability of teeth to survive when transplanted to areas of the body other than the mouth is encouraging to those attempting transplantation within the mouth. In 1955, Butcher and Vidair reported on replantation of teeth in the monkey. Their conclusion was that vitality was not a decisive factor in reattachment. Reattachment occurred by the formation of new layers of cementum, engulfing the free ends of the periodontal fibers. Areas of ankylosis and resorption were found along the root surfaces of the replanted teeth.

Hammer reviewed the subject of replantation and implantation in 1955. Previously he had performed autogenous transplantations in which teeth with periodontal membrane were implanted in the greater omentum and in bone cavities of dogs and monkeys. He concluded that the attached periodontal membrane survived and was an important factor in the reattachment of reimplanted and transplanted teeth. He also reviewed his work on replantation with and without periodontal membrane. He found that without periodontal membrane there was a rapid and complete bony ankylosis with resorption of the tooth and
bony replacement. He concluded that the life of a replanted tooth was directly proportional to the amount of periodontal membrane attached to it at the time it was replanted. He also mentioned implants and suggested the use of a screw-type pin for a root filling that would be locked in bone when the root was resorbed and replaced by bone.

In 1956 a symposium devoted to the subjects of transplantation, replantation and implantation was published in *Oral Surgery, Oral Medicine and Oral Pathology*. Agnew and Fong reported on tooth transplantation in the Rhesus monkey. The process of repair, pulpal changes, root resorption and ankylosis were found to be similar to those found in humans. Waite reported on transplantation of teeth in dogs following storage by freezing. The results were encouraging even though root resorption was noted. Emmersten reported in the symposium on the replantation of teeth that were removed because of periapical lesions, were root filled and then replanted following curettage of the granulation tissue. His results were similar to those of Kramer.

P. C. Alexander, Apfel, Baden, Hale, Holland, Olech, Tam, and Thoma participated in the symposium. All the papers reported some success and some failures. "Success" appeared generally to consist of
(1) repair of soft tissue and bone around the transplant or replant, (2) no discomfort to the patient, and (3) functional retention for at least two years. The most frequently listed problem was resorption of transplanted and replanted teeth.

Costich (1963) in a paper entitled "Basic Problems of Regeneration and Transplantation" pointed out that root resorption and provision for complete bony support about the roots of transplants present the major problems to be solved before success can be consistently achieved.

Liaros (1961) performed a preliminary investigation on dogs of the surgical approach in transplantation of a bone graft with a tooth in situ.

Dal Pont (1962) clinically described a new method of tooth transplantation in humans by means of a free dento-alveolar transplantation.

It is in light of Liaros' work that this study will concern itself.

B. Repair of Bone Grafts:

The first reported bone transplantation seems to have been made by Meek'ran (1682) who filled the defect in a soldier's head with skull bone taken from a dog, but he had to remove the transplant because of
religious interference. Hunter (1728-1793) recorded the ability of bone splinters to survive and the resorption and remodeling of bones. Ollier (1867) reported very extensive investigations on transplantation of periosteum, bone marrow and bones in different animals and it was his opinion that the major part of a bone graft survived. He also stated that what he considered new bone formation arose from the periosteum and endosteeum of the bone graft.

Ollier's theories were opposed by Barth (1893) and Marchand (1899) who stated that the major part of the graft dies when transplanted, and that the periosteum and the end-osteum of the graft do not influence the outcome of the transplantation. Later, Marchand (1901) and Barth (1908) changed their opinions and admitted osteogenic properties of the periosteum. They also took back their opinions of the prevalence of a special kind of bone resorption attached to the healing of bone transplants.

Axhausen (1908) published many thoroughly planned experiments of bone grafts on animals and humans. His findings included the theory that most of the cells in a bone graft die, with the exception of some cells in the periosteum and endosteeum and also in the superficial Haversian canals. According to his opinion, the
substitution of a transplant when grafted to skeletal surroundings
takes place principally by the process of bone resorption and
apposition by vascular tissue from the host and only to a lesser extent
by surrounding cells in the graft. The present opinion of the fate of
bone grafts differs little from Axhausen's views.

The many studies of later authors have been to a great extent
directed towards settling the question of bone graft survival and the
origin of cells which give rise to new bone.

Macewen (1912) and Groves (1920) found the graft viable, and their
opposition to Axhausen's findings seem, in part at least to have been
caued by Macewen's failure to recognize the cambium layer as part
of the periosteum.

Orell (1934-1937) showed that cooked bone and os purem, when
buried subcutaneously in humans, produced new bone formation, but
at a much slower rate than fresh autogenous grafts.

H. Engstrom and Orell (1943) by transplantation of dead bone,
showed that new bone formation was produced at the same rate as in
fresh bone transplants. They also stated that the osteogenic properties
of a graft were not necessarily bound to viable bone cells, but must
have been the result of specific chemical substances in the bone graft
which transform cells in the connective tissue into osteogenic bone cells. Results by Mosiman (1952) indicate that no specific chemical osteogenic organizers have yet been isolated.

Ray and Ward (1951) stated that hydroxyapatite crystals acted on an "ossifiable medium", but the healing was never as rapid as when fresh autogenous grafts were used.

The first autogenous graft on humans was reported by v. Walther (1821). The superiority of fresh autogenous transplants over other kinds of transplants is generally admitted (Campbell, Bower, McFadden, Payne and Doherty, 1953).

According to the modern literature on bone grafting the general opinion of the authors is that the grafting of an autogenous cortical bone transplant into a long bone is followed by death of most of the cells in the graft while the periosteum of the graft survives in part.

When the bed for the transplant is cut in any bone, the osteocytes die near the line of the cut. The graft is fixed in its place through the efforts of vascular connective tissue which is derived mainly from the periosteum and the marrow of the host bone. Primarily from this tissue, and also from surviving cells in the periosteum and endosteum of the graft, the Haversian canals in the
transplant are invaded and by processes of resorption and deposition, the graft is slowly replaced (Chase and Hevendon, 1955) and (Ham and Harris, 1956).

Cancellous bone, according to Ham and Gorden, 1952, might survive transplantation. There seems to be an agreement that the cells in a homogeneous graft all die when transplanted (Peer, 1955, Ham and Harris, 1956).

Sicher (1962) stated, "It is clear that even autogenous bone grafts will, under the most favorable conditions, have but a restricted life span. Not only must they share the fate of normal bone but, in addition, they must be subject to 'functional reconstruction'." It has been known for a long time that the microscopic and macroscopic structure of bone tissue is adapted to the mechanical function in a suprisingly detailed way. Whenever a bone is subjected to altered function its shape and structure change. Wolff's law, so often misquoted or misinterpreted, is based on the beautifully functional adaptation of the skeleton. Any graft will therefore undergo internal reconstruction and can never survive for any length of time because it now has an altered function.

What then is the biologic basis of grafting bone? Brought down to
the shortest and most basic statement: grafted bone entertains the proliferation of loose connective tissue and its most important cells, the undifferentiated mesenchymal or reserve cells. This proliferation leads to the gradual resorption of the grafted bone but also, just as gradually, to its replacement by new bone. Later on, if the graft is successful, the newly formed bone and the "host" bone are reconstructed until full harmony between function and structure has been achieved.

One could even add that in its resorption lies the significance of the implant.

C. Gutta Percha:

1) General Description:

Gutta percha is the refined, coagulated milky exudate of certain trees indigenous to the Malayan Archipelago. It resembles rubber both in chemical composition and in some physical characteristics. It was introduced into dentistry as "Hill's stopping" in 1847 by Koch.

2) Reaction of Tissue to Gutta Percha:

Miller (1930) demonstrated inflammatory tissue reaction to gutta percha in dogs and rabbits.

Coolidge (1932) showed similar results using pups as the
experimental animals. However, in the same year Kronfeld (1932) reported cementum deposits completely covering the gutta percha canal filling at the apical foramen of a molar tooth.

Boylger (1933) states that, "Blaney, Coolidge, Davis, Groove, Hatton, Kronfeld and Moen have shown the healing-in of gutta percha in pulp canal fillings." Boylger himself found that in the absence of infection, gutta percha is well tolerated by the connective tissue of both man and rat.

Dixon and Rickert (1938) using dogs, found similar histological reactions in humans and dogs to gutta percha root filled teeth.

Gutta percha satisfies more of the qualifications of a good root canal filling material than any other known material.
A. Surgical Procedure:

Autogenous replanted bone grafts were performed on young adult, mongrel dogs with a full complement of permanent teeth. The dogs were selected without regard to sex or breed. All instruments utilized in both surgical and endodontic procedures were sterilized prior to their use.

The mandibular third premolars were selected for the surgical and endodontic procedures because of their position and accessibility.

The basic surgical technique involved two stages:

STAGE I: The animals were anesthetized by intraperitoneal injection of 1 cc. of a 5% solution of sodium pentobarbital (nembutal, Abbot) per 3.5 lbs. body weight. All animals were intubated with (McGill) endotracheal tubes to facilitate respiration. Each dog was draped with sterile towels and a mouth prop was put into place.

a) Root Fill:

The immediate root fill of the third premolars was performed under aseptic conditions. The crown of the tooth was
reduced from occlusion with a rotating diamond stone and flushed with sterile saline. The vital pulp was extripated with an appropriate broach. The canals were reamed and filed using microcide A as a lubricant. Diagnostic radiographs were taken with reamers in place to determine complete bio-mechanical preparation. The third mandibular premolar was root filled with gutta percha cones using Procasol cement as the sealing agent. The remaining coronal portion of the tooth was filled with zinc oxyphosphate cement.

b) Extractions:

Extraction of the second and fourth mandibular premolars was performed so as to insure a sufficient table of adjacent alveolar bone for the tooth bearing bone graft. Mechanical splitting of said premolars with a diamond disc and careful luxation of the fractured roots with a bicuspid extraction forceps (#115) facilitated the extractions.

STAGE II: Approximately six weeks after the premolars were extracted sufficient healing and filling in of bone was noted in the extraction sites via radiographic determination. Anesthesia of the dog was induced in a similar manner as for the initial step and the animals were prepared for surgery (Figure 1). With a number
fifteen blade mounted on abard parker scalpel, an incision was made
along the crest of the gingiva from the mandibular first molar to
the distal of the mandibular canine on both the lingual and buccal
aspects of the root filled third premolar. The mucoperiosteal flaps
were reflected lingually to the inferior border of the mandible
and buccally to the mucobuccal fold (Figure 2).

Using gentian violet, a trapezoidal outline of the proposed graft
was drawn including bur hole positions for stabilizing interosseous
wiring. A trapezoidal outline was used to obtain greater stabilization,
especially against occlusal displacement.

Oblique cuts were made with high speed water cooled diamond
discs, following the premarked graft outline and extending apically
to the mandibular canal. The horizontal cut was made parallel and
immediately superior to the mandibular canal (Figure 3).

A 701 tapered fissured bur was utilized to prepare holes for
interosseous wiring in both the graft and host bone. Appropriate
sterile saline solution was used as a coolant during all bone
cutting procedures.

The bone graft was then slipped laterally from its bed and
placed in a super saturated solution of penicillin (Figure 4).
The tooth bearing bone graft was reimplemented into its original site and secured with 23 ga. stainless steel interosseus wires (Figure 5).

The time lapse between removal and replanting of the compound graft ranged between fifteen and thirty-five minutes.

Careful consideration was given to the immobilization of the graft at this time.

The recipient site with the compound graft in position was treated as follows: The mucoperiosteal flaps were replaced and approximated with care and interrupted sutures were placed along the entire length of the incision (Figure 6).

The opposing cusps of the maxillary teeth were removed as a protective measure to enhance greater stability for the mandibular graft.

B. Post-Operative Care:

Following the operative procedure, all of the animals were injected intramuscularly with 600,000 units of long acting penicillin (Bicillin, Wyeth). To decrease trauma during mastication the animals were placed on a soft diet which consisted of canned horse meat and pre-softened Purina dog meal. Water was available at all times.
Immediate post-surgical radiographs were taken of the graft area on all animals. Radiographs were also taken of all the specimens at the time of sacrifice.

C. Preparation of the Specimens:

By means of a lethal dose of sodium pentobarbital the animals were sacrificed at intervals of three weeks, four weeks, six weeks, eight weeks and three months. To remove the specimen, the lower lip, cheek, and floor of the mouth were resected and the mandible cut with a gigly saw. The specimens were placed in 10% formalin solution. The formalin solution was changed every twenty-four hours until fixation was completed. The specimens were then decalcified in formic acid-sodium citrate solution, embedded in paraffin or celloidin and sectioned in the mesiodistal direction. The sections were stained with hematoxylin and eosin and trichrome connective tissue stain.
CHAPTER IV
FINDINGS

A. Macroscopic Findings:

In all specimens, submaxillary and buccal swelling was noted during the first forty-eight hours. The mucoperiosteum about the grafts appeared to be moderately inflamed.

The three week specimen showed pocket formation associated with sloughing of the attached gingiva and slight tooth movement.

The eight week specimen demonstrated clinically similar findings to those of the three week specimen along with a severe weight loss of the animal from the time that the tooth bearing bone graft was performed.

All of the grafts were non mobile at the time of sacrifice. This indicates that some degree of attachment had taken place in all instances.

Intraoral radiographs of the compound grafts and associated paraimplantal tissues were taken at the time of sacrifice on all specimens. No callus formation was found on the inferior border of the mandible in the graft area as was noted by Liaros (1961). The
three week and eight week specimens show a marked radiolucent area between the host and donor bone indicating an apparent lack of bony union.

B. Microscopic Findings:

The general histologic picture of the three week specimen is one of rejection of graft and tooth. No epithelial attachment exists at the cemento-enamel junction. The periodontal ligament is absent but for a few loosely attached connective tissue fibers about the right apex. Both roots show multiple layers of cellular cementum on the surface of acellular cementum, which in turn are covered by acellular cementum (incremental lines). The lacunae of the cellular cementum are empty. Root resorption is evident at the apices.

The donor bone appears necrotic with associated thin, acellular trabeculae and lymphocytic infiltration.

A layer of fibrous connective tissue extends horizontally between the donor and host bone. The host bone exhibits resorption via osteoclastic activity in some places and in other places incipient bone formation (thin trabeculae covered by osteoblasts and osteoid tissue).
The four week specimen shows a firm attachment of the epithelium at the cemento-enamel junction. The periodontal ligament appears to be moderately infiltrated with mononuclear cells (lymphocytes), polymorphonuclear neutrophils and newly formed fibroblasts. The periodontal ligament fibers are aligned parallel to the roots of the tooth.

The intraradicular area of the periodontal ligament exhibits marked ankylosis along the inner aspect of both root surfaces. Areas of resorption of both cementum and dentin are noted with associated osteoclastic activity. The cementum in the apical regions is highly cellular and lamellated.

Both apices are covered over with a fibrous connective tissue scar. In the intraradicular septal apex area there is an infiltration of stratified squamous epithelium (Figure 13). The histology of the bony tissue indicates resorption of the donor graft with proliferation of osteoblastic and osteoclastic cells.

At the junction of the donor and recipient bone there is an inflammatory process with edema and proliferation of new bone and fibrous tissue formation. The overall picture is one of successful bone graft with resorption of the tooth in situ.
The six week specimen shows a break at the cemento-enamel junction of the epithelial attachment. Infiltration and inflammation of the coronal third of the root surface is noted with resorption of dentin (Figure 10).

Loose collagenous fibrils of the periodontal ligament (membrane) run parallel to the root surfaces (Figure 8c).

Ankylosis is apparent. The cementum displays superficial resorption with incipient reparative deposition of cellular cementum.

The alveolar osseous wall exhibits mostly lamellated bone with some bundle bone.

The apical areas of the tooth show a marked mononuclear cell infiltration with a connective tissue capsule below.

Osteoclasts, osteoblasts, and proliferating capillaries are seen at the junction of host and donor bone (Figure 12). The interradicular septal apex shows loose connective tissue with numerous fibroblasts (Figure 11). Once again, the picture is one of the graft taking and moderate resorption of the involved root filled tooth.

The eight week specimen exemplifies fibrosis overtaking osteoid formation in relation to the donor and host bone (Figure 15). There
is no epithelial attachment at the cemento-enamel junction. The periodontal ligament is totally absent. The cementum exhibits acellular lamellation.

The donor bone has empty lacunae and infiltration of mononuclear cells is seen throughout the marrow spaces.

The recipient site bone is covered with a dense fibrous connective tissue layer.

Resorption and apposition of the trabeculae of the recipient bone is evident. This specimen indicates an early failure as was shown previously in the three week specimen.

At three months the microscopic picture is one of a graft having found an adequate environment with subsequent proliferation and apposition of bone.

There is attachment of the epithelium at the cemento-enamel junction. The periodontal ligament space has been reduced and areas of ankylosis consisting of bundle bone attached to cementum are noted (Figure 16). Those periodontal fibers that remain are aligned parallel to the root surfaces (Figure 18).

The graft or donor bone shows the typical picture of decreased
function. The spongy bone around the alveolus exhibits marked rarefaction; the bone trabeculae are less numerous and very thin.

The apical areas of both roots demonstrate a fibrous encapsulation with a proliferation of lymphocytes and plasma cells (Figure 17).

The interradicular septal apex shows ankylosis with increased vascularity in the wide marrow spaces.

C. Control:

A root filled mandibular third premolar was utilized as a control.

This animal was utilized in an attempt to ascertain the histological picture of the periodontium and apical reaction of a root filled tooth. The root fill was accomplished in the same manner as the previous specimens. The histological picture of a healing graft is well known.

The control specimen (four weeks) showed attachment of the epithelium at the cemento-enamel junction. The periodontal ligament was quite normal demonstrating directional changes due to non-function.

Hypercementosis of both apices was noted as is often the case in non-functional teeth.
A chronic inflammatory reaction about the apical areas was exhibited along with areas of resorbed cementum (Figure 14).

The mononuclear cell infiltration (macrophages, lymphocytes, and plasma cells) was attributed to the numerous accessory canals which were not filled-in by the gutta percha. Fatty marrow was noted in the alveolar process.
CHAPTER V
DISCUSSION

Liaros (1961) studied the surgical approach to the problem of a tooth bearing bone graft. He suggested that future studies be carried out utilizing a larger block of alveolar bone, adequate interosseous wiring for stabilization of the graft and root canal treatment to combat pulpal necroses of the tooth involved.

Utilizing these suggestions this investigation studied the histology of the periodontium of such a graft.

The periodontium, or the functional unit of the tissues supporting the teeth, includes the gingiva, dento-gingival junction, periodontal ligament, cementum of the root surface and the alveolar process.

The three and eight week specimens demonstrated a lack of gingival attachment at the dento-gingival junction. The six week specimen showed a break in the continuity of the epithelial attachment and resorption of cementum took place on the involved root surface below this area (Figure 10). All other specimens exhibited normal epithelial attachment at the cemento-enamel junction. The periodontal
ligament was observed to behave as in any non-functional tooth. The fibers became loosely attached and ran parallel to the root surfaces. In all cases, excluding the three and eight week specimens, ankylosis was found scattered between normal periodontal fibers.

The cementum of the root surfaces indicated that the teeth involved were non-functional in that hypercementosis occurred at the root apices. Inflammatory reactions from the interosseous wiring caused numerous areas of resorption with subsequent proliferation and ankylosis.

The alveolar process exhibited sparse bundle bone formation and much lamellated bone in those grafts which were successful (four weeks, six weeks and three months). Fatty marrow and vascular proliferation was shown in the interradicular spongy bone.

The junction of the graft and host bone indicated that in the unsuccessful grafts (three and eight weeks), fibrosis overtook osteoid tissue formation and the graft was being rejected.

One might say that the environment must be good for the graft to survive. Before these results can be interpreted it must be taken into consideration that the material presented here is very limited. Also, when animals are used for intraoral investigation, lack of
cooperation and poor oral hygiene are but two factors which constitute a constant source of irritation to the normal healing process.

In general it may be stated that the autogenous transplantation or replantation of a bone graft with a root filled tooth in situ exhibits a picture of either death and subsequent sloughing or a "take", with proliferation and replacement of tissues.

Compounding such basic problems as stabilization and adequate apposition of the graft itself was the inevitable inflammatory process which occurred in all specimens at or about the apices of the root filled teeth. The problem was enhanced by the inflammation in the multiple accessory root canals which are prevalent in dog's teeth. Perhaps dogs are a poor choice as an experimental animal for such a study as was undertaken.
CHAPTER VI
SUMMARY AND CONCLUSIONS

Summary:

In the present work a histological study was performed on the periodontium of an autogenous tooth bearing bone graft with a root filled tooth in situ. The principle aim of this investigation was to discern the biological behavior of the graft and tooth during healing of the autoreplantation.

Six mongrel dogs were utilized for this experiment. The dogs were anesthetized with intraperitoneal general anesthesia and the mandibular tooth involved in the graft was root filled and removed from occlusion. Using bone burs and diamond discs a block of alveolar bone containing the root filled tooth was removed and replaced in a vascular bed. Interosseous wiring and trapezoidal graft outline gave the graft adequate stability. The dogs were sacrificed at periods of three weeks, four weeks, six weeks, eight weeks, and three months. A suitable control was run and sacrificed at four weeks.

The specimens were decalcified, sectioned and stained. The histologic results show that the environment must be good for the
graft to survive. In general, the transplantation or replantation of a bone graft with a root filled tooth in situ exhibits a picture of either death and subsequent sloughing due to acute inflammation or a "take", with proliferation, resorption and replacement of tissues.

Conclusions:

From this investigation it was found that:

Transplantation of autogenous tooth bearing bone grafts with a root filled tooth in situ are possible.

With adequate environment, successful bone grafts were attained.

Ankylosis of the root filled tooth is inevitable in such a tooth bearing bone graft.

Unsuccessful grafts were due to acute inflammation causing early failure or fibrosis overtaking osteoid tissue formation.

Newer techniques in the field of tissue culture, histo-chemistry, electron microscopy, microradiography, bone and tooth physiology and periodontology will contribute to the solution of the basic problems of resorption and repair and, thus, to increasing success in tooth transplantation.
BIBLIOGRAPHY


Hunter, W.: The role of sepsis and antisepsis in medicine, Lancet, pp. 78-86, January 14, 1911.

Koch, History of Dental Surgery, pg. 184, 1909.


FIGURE 1
PREPARED GRAFT SITE (X - REDUCED CROWN WITH ATTACHED GINGIVA)

FIGURE 2
MUCOPERIOSTEAL FLAP AND OUTLINE OF PROPOSED TOOTH BEARING BONE GRAFT

APPENDIX

A. PHOTOGRAPHS OF METHODS:

FIGURE 1

FIGURE 2
FIGURE 3
TOOTH BEARING BONE GRAFT

FIGURE 4
COLOR PHOTOGRAPH OF TOOTH BEARING BONE GRAFT
FIGURE 5
STABILIZATION OF REPLANTED TOOTH BEARING BONE GRAFT

FIGURE 6
PHOTOGRAPH SHOWING CLOSURE OF WOUND
FIGURE 7  GRAFT BONE WITH ASSOCIATED PERIODONTAL LIGAMENT (25X) H AND E.

FIGURE 8  MESIAL ROOT, DENTIN-a, CEMENTUM-b, PERIODONTAL LIGAMENT-c, INTERRADICULAR BONE-d. (25X) H AND E.

SIX WEEK SPECIMEN
Mesiodistal Section Through Mandibular Third Premolar

FIGURE 9  FIBROUS CONNECTIVE TISSUE RING CAUSED BY TISSUE REACTION TO INTEROSSEOUS WIRING. -x. (25X) H AND E

FIGURE 10  ARROW POINTS TO RESORPTION OF CEMENTUM BELOW THE CEMENTO-ENAMEL JUNCTION. (25X) H AND E.
FIGURE 11
INTERRADICULAR SEPTAL APEX, DENTIN-a, ACCELLULAR CEMENTUM-b, LOOSE CONNECTIVE TISSUE IN P.D.L. SPACE-c, INTERRADICULAR BONE-d. (25X) H AND E.

FIGURE 12
JUNCTION OF GRAFT AND HOST BONE DONOR BONE-a, NEW BONE AT JUNCTION-b, RECIPIENT BONE-c. (25X) H AND E.
FIGURE 13  INFILTRATION OF STRATIFIED SQUAMOUS EPITHELIUM IN THE AREA OF THE INTER-RADICULAR SEPTAL APEX.

FIGURE 14  CHRONIC INFLAMMATION ABOUT THE ACCESSORY ROOT CANALS.
FIGURE 15  JUNCTION BETWEEN DONOR AND HOST BONE. DONOR BONE-a, FIBROSIS-b, HOST BONE-c. (25X) H AND E.

FIGURE 16  ANKYLOSIS-a (25X) TRICHRome
FIGURE 17  APICAL AREA SHOWING FIBROUS ENCAPSULATION WITH A PROLIFERATION OF MONONUCLEAR CELLS, (25X) TRICHROME

THREE MONTH SPECIMEN

FIGURE 18   PERIODONTAL LIGAMENT ALIGNED PARALLEL TO ROOT SURFACE, DENTIN-a, CEMENTUM-b, PERIODONTAL FIBERS-c (100X) TRICHROME.
APPROVAL SHEET

The thesis submitted by Robert F. Misiewicz has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

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