June 1983

Autogenous Transplantation of Teeth into Surgically Prepared Sites in Rhesus Macaque Monkeys

Curtis Beck

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Autogenous Transplantation of Teeth into Surgically Prepared Sites in Rhesus Macaque Monkeys

Curtis Beck, D.D.S.

A Thesis
Submitted in Partial Fulfillment of the Requirements for the Degree of Masters of Science
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Master of Science Thesis

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1983
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I dedicate this thesis to my parents and family who have helped and supported me in all ways through the twenty-three years of education which has helped to produce this thesis.
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INTRODUCTION

A number of studies of periodontal wound healing, regeneration and development have been reported and it is clear that a distinction needs to be drawn between the two forms of autogenous transplantation; viz., transplantation to an extraction site (replantation) or transfer to a newly prepared socket (transplantation). In the former procedure, healing occurs by the union of the severed halves of the periodontal supporting apparatus (Andreasen, 1980b), while in the latter case, the bone-related half of the attachment apparatus is not present and must be re-established during healing.

Most of the previous studies have focused on tooth replantation (Hale, 1954, 1956; Agnew and Fong, 1955, 1956; Fleming, 1959, 1962; Hammer, 1955; Loe and Warchaug, 1961; Costich, 1962; Guralnick and Shulman, 1962; Cambell, 1963; Shulman, 1968; Natiella, Armitage and Greene, 1970; Conklin, 1976). Surprisingly, there have been only a few investigations of transplantation into surgical sockets in the mandible. These few pertinent reports (Apfel, 1954; Pafford, 1956) are clinical in design using functional and radiographic criteria to assess the success of the procedures. Histological description of the new attachment apparatus, or whether one indeed forms, are not available. Since these clinical reports imply that healing does occur and that new attachments do form, an understanding of the origin of the regenerated periodontal tissue needs to be clarified. Then, based on these findings the tissue interactions between transplanted teeth and the surgically prepared bony site can be evaluated.
The purpose of this study was to determine whether tooth re-attachment occurs following autogenous transplantation of fully formed teeth into surgically prepared sites. Radiographic and histological evidence of a functional periodontal ligament in transplanted teeth is provided.

LITERATURE REVIEW

As our understanding of periodontal wound healing, regeneration and development has expanded, it has become evident that a more pronounced distinction needs to be drawn between the two forms of autogenous tooth transplantation, transfer to an extraction site and transfer to a surgically prepared socket. In the former healing occurs by the union of the severed halves of the periodontal supporting apparatus mainly bone-ligament and ligament cementum (Andreasen, 1980b) while in the latter the bone ligament half of the attachment apparatus must develop de novo if a normal attachment is to be reestablished. The literature to date has focused primarily on tooth replantation (Hale, 1954, 1956; Agnew and Fong, 1955, 1956; Hammer et al. 1955; Fleming, 1959, 1962; Loe and Warchang, 1961; Costich, 1962; Guralnick and Shulman, 1962; Cambell, 1963; Shulman, 1968; Natiella et al., 1970; Conklin, 1976) and there has been little research in the area of transplantation to surgical sockets in the jaws. The reports in the literature (Apfel, 1954; Pafford, 1956) are of a clinical nature and depend on functional and radiographic evidence as their criteria of success giving no data on the histology of the new attachment apparatus or whether one indeed forms. Since the clinical reports imply that healing does occur and new attachments do form, an understanding of the origin of the newly developed periodontal tissue
needs to first be clarified before it is possible to determine how the new tissue affects healing.

Development of Periodontal Tissue

The origin of the cells in the tooth supporting structures have been extensively discussed in the literature. Hoffman (1960) transplanted neonatal hamster molars stripped of their dental sac to subcutaneous tissue sites in adult hamsters. Teeth continued to develop in this site and a periodontal ligament and bone was formed. Hoffman (1960) was unable to identify the origin of the cells which were responsible for the ligament and bone formation. He suggested that the precursor cells might have originated from: (1) proliferation of cells of the dental papilla which had migrated; (2) mesenchymal cells which had adhered to the outer enamel epithelium and differentiated after transplantation; or, (3) host tissue which had been stimulated to undergo differentiation into osteoblasts under the influence of Hertwig's sheath. Ten Cate et al. (1971), with an autoradiographic study of transplanted neonatal mice molars to mouse cheek subcutaneous tissue, showed that the few adherent ectomesenchymal cells of the inner layer of the dental follicle contributed to the formation of the periodontal ligament and cementum. Their role was doubtful as to their part in the new bone formation recorded. Though labelled cells were found associated with the new bone, labelling was minimal and might have been explained by background radiation. Host lymphocytes were found associated with the external aspect of the new bone, suggesting that the host was undertaking a rejection reaction to expel donor-derived bone. Ten Cate (1975b), using tritiated proline as a marker, showed alveolar bone development occurred at the same time the periodontal ligament was forming.
transplant study Freeman et al. (1975) showed that a tooth germ transplanted into the parietal bone could develop a gomphosis. Al-Talabani and Smith (1978) performed a study on transplantation of five day old tooth germs to synergic hamster cheek pouch again were unable to designate the origin of the periodontal ligament and bone which formed around the transplanted tooth. However, since bone only started to develop with extension of Hertwig's epithelial root sheath and periodontal ligament was formed only in association with the root, they felt that Hertwig's epithelial root sheath had the power to induce bone and periodontal ligament development from different types of fibrous connective tissue that might surround the transplanted tooth germ (Andreasen, 1980b). Barrett and Reade (1981) in a study correlating the degree of development of a tooth and the subsequent formation of bone and periodontal ligament showed that fully formed teeth, when transplanted to the renal subcapsular site in mice, did not retain the capacity to form alveolar bone and periodontal ligament while immature teeth did. The subcapsular site is highly resistant to bone induction. Thus, the formation of bone in this area gives circumstantial support to the concept that the donor periodontal tissue was the source of bone forming cells. This concept, however, is not conclusive because recruitment of host cells with bone forming potential could not be ruled out. Andreasen (1980b) demonstrated bone formation around fully formed teeth transplanted to submucosal sites in monkeys again supporting the idea that the periodontal remnants on the tooth either contain certain osteoprogenitor cells or can induce surrounding tissue to form bone.

Atkinson (1972) disagrees with the concept that the root sheath plays
an active role in root-formation. Atkinson (1972) contends that the root sheath plays a passive role and the deeper stromal cells of the dental papilla are endowed with the potential to form odontoblasts at the same time and by the same interaction with the inner dental epithelium which created the original odontoblasts of the crown. These cells are inhibited from histodifferentiation into odontoblasts by a substance released by fully differentiated odontoblasts. Along with this concept of an inhibitory substance, Atkinson (1972) further proposed that the root sheath acts only as an extension of the enamel-dentin junction in which inhibitory mechanisms are absent and, thus, passively becomes colonized by previously induced presumptive pre-odontoblasts.

Yoshikawa and Kollar (1981) have deduced from a series of experiments that not only does the dental follicle give rise to the tooth supporting apparatus such as alveolar bone, periodontal ligament, and cementum, but that the dental papilla cells retain their inductive capabilities to regenerate or repair the dental follicle and possibly its derivatives (Kollar, 1972a, 1972b; Kollar and Baird, 1970a, 1970b). From the limited range of ages tested these authors conclude that repair is possible at least until a definitive root length is achieved.

Yoshikawa and Kollar (1981) were able to reach this conclusion because they used an explantation site that offered a unique advantage. Whereas intramuscular or subcutaneous explantation sites are able to contribute precursor bone cells around grafted decalcified bone or dental tissue, the intraocular anterior chamber does not contribute bone forming precursor cells. When the tooth germ was subdivided into its component parts such as the enamel organ, the dental papilla, and the dental
papilla, and the dental follicle and explanted to the anterior chamber, the contributions of these components to the periodontium could be ascertained. The presence of bone in such experimental combinations has to arise from the grafted tissue and did not come from the host tissues. The fact that the combination of enamel organ and dental papilla (denuded of the dental follicle tissue) gave rise to the tooth and its supporting structures supports the conclusion that the dental papilla retains the capacity to form the dental follicle \textit{de novo}. Combinations of the enamel organ and the dental follicle from different ages, however, did not all produce complete teeth. Younger follicle tissue could produce complete teeth while older dental follicle tissue could not support odontogenesis. However, older dental follicle tissue was able to make bone in the explanted tissue. This gave rise to the conclusion that though both the dental follicle and the dental papilla in the early stages of development of the tooth have the same capacity to form a tooth \textit{de novo}; at later stages the dental follicle loses its general function and becomes specialized for the production of the supporting apparatus whereas the dental papilla remains developmentally labile. The fact that both the dental follicle and the dental papilla share the same functional capacities during early stages of tooth development is consistent with the contention that these two cell populations are derived from the same neural crest cell line.

Roberts' studies on parathyroid stimulated periodontal ligaments support the concept that the follicular cells present in the adult periodontium in the form of the cell rests of Malassez maintain their functional capacity for development throughout life (Roberts 1975; Roberts et al., 1982). Roberts described the periodontal ligament as a
mixed population of morphologically indistinguishable, relatively undifferentiated cells committed along separate pathways of cytodifferentiation to osteoblasts, osteoclasts, and cementoblasts with osteoblasts and osteoclasts as the primary differentiative pathway as evidenced by the slow rate of turnover of cementoblasts (Roberts and Jee, 1974; Roberts, 1975; Roberts et al., 1982).

**Periodontal Wound Healing**

Our understanding of periodontal wound healing has come from replantation studies or studies of limited injury to the periodontal supporting apparatus. Though these experiments represent true healing and not development *de novo* of an attachment apparatus, much of the information obtained in these studies shed light on the present understanding of what might be taking place in reorganization during the process of establishing a new attachment apparatus in an adult transplanted tooth.

**Epithelial and Connective Tissue Reattachment**

Three types of healing have been described in the literature (Daryabegi, 1980): (1) **Scar repair**, a reattachment procedure occurring when the soft tissues of the epithelial cuff heals to the ends of residual Sharpey's fibers or other tissue remnants on the tooth; (2) **Collagen adhesion**, a form of new attachment where no new cementum is formed but close apposition of soft tissue and tissues on the tooth allows collagen fibers to adhere; and (3) **Cemental repair**, a form of new attachment where cementum or cementum and dentin are first resorbed and new connective tissue fibers become embedded as new cementum is laid down, thus creating an attachment. A fibrous reattachment is seen to form consistently at
the level at which the periodontal ligament fibers are preserved (Nyman et al., 1979). In re-establishing attachment, hemidesmosomes appear before the lamina densa forms. Hemidesmosomes are apparent at 18 hours while the lamina densa is first seen at 24 hours and has established continuity at 48 hours. By 72 to 96 hours an intact attachment structure consisting of tonofilaments inserting into the attachment plaques of the hemidesmosomes and anchoring fibrils inserting into the connective tissue side of the lamina densa has formed.

Crevicular and junctional epithelium along with internal and external basal lamina begin to regenerate immediately after replantation and reach their peak at three days (Nasjleti et al., 1975; Melcher, 1976). Junctional epithelium will attach to enamel, cementum, dentin and under certain circumstances to calculus. The new junctional epithelium is believed to differentiate from the germinal layers of keratinized masticatory epithelium (Melcher, 1976) and establishes functional integrity in 7 to 9 days (Caffesse et al., 1968; Nasjleti et al., 1975). Listgarten (1972) proposed that the lack of keratinization in the new junctional epithelium despite its keratinized tissue origin is due to environmental factors. Differentiation of gingival fibers accompanied by regeneration of the gingival lamina propria readily follow wounding.

Andreasen (1980b) reported the formation of a normal cervical periodontal ligament despite extended drying or surgical removal of periodontal remnants on replanted teeth. In cases where crestal bone was removed, connective tissue which was apposed to the tooth was capable of inducing resorption while viable cells remaining on the tooth appeared capable of inducing new crestal bone formation. This latter finding was confirmed by Nyman and Karring (1979). These findings have led Andreasen
to propose that a differential in healing potential exists for different cells populating the sockets area.

Alveolar Reattachment

Though there was some early disagreement over the necessity of maintaining an intact vital periodontal ligament covering the entire root surface recent researchers (Loe and Warehaug, 1961; Andreasen, 1966, 1972, 1977, 1980bcd, 1981b; Shulman, 1968; Shulman et al., 1968; Hammer et al., 1970; White, 1975) have all recognized the necessity of retaining the vitality of the periodontal ligament to encourage remodeling of a new attachment unit with a minimum of root resorption and ankylosis. Castelli et al. (1971) reported that the vasculature was re-established by growing out from the bone and developing a vascular network in the clot between the bone and the tooth. In the reimplantation studies it was postulated that repair can occur from two sources, the surviving cells of the periodontal ligament on the tooth and bone and from the surrounding soft connective tissue and bone marrow spaces. Melcher (1970) stated that small isolated areas of damage to the periodontal ligament are repaired by repopulation of the area via proliferation and migration of cells from the uninjured surrounding area as well as surviving cells in the area of injury. He also reported that the area of injury experiences an acellular phase immediately after injury followed by increased cell division during the course of repair. These findings are in agreement with Roberts' (1975) data on cell population dynamics in periodontal ligament stimulated with parathyroid extract. Those data implied that the increased cellularity associated with the parathyroid-stimulated osteoclasts occurred by two separate mechanisms; one rapid influx of migrating cells presumable via vascular channels, and, two, slower
cellular input via local proliferation of cells. These cells arise from the mixed population of relatively undifferentiated cells as well as G\textsubscript{1}- or G\textsubscript{2}-blocked (Gelfant and Smith, 1972) along separate pathways of cytodifferentiation to osteoblasts, osteoclasts and cementoblasts which comprise the periodontal ligament (Roberts, 1975; Roberts et al., 1982). These findings are particularly significant in light of the conclusions of Line et al. (1974) that periodontal ligament which repopulated from adjacent bone marrow ankylose while areas of the ligament repopulated from supracrestal connective tissue does not. Since the vasculature of the periodontal ligament would be totally disrupted in replantation and absent in transplanted donor material since it would be left behind in the bony half of the periodontal ligament, the lack of a vasculature would slow the influx of host reparative cells placing greater dependence on the dividing cells in the donor material (Roberts and Jee, 1974). The healing from the donor side out would according to Line result in less ankylosis. Andreasen (1981a) corroborated Melcher's (1970) work and carried it one step further reporting that the physical size of the area of injury to the periodontal ligament affected the type of healing with smaller lesions resulting in transient ankylosis and larger areas resulting in permanent ankylosis.

Atkinson (1972) reported that cells which repopulate untreated tooth isografts were capable of differentiating into odontoblasts which deposited reparative dentin. In 1976, Atkinson transplanted isografts which contained non-viable pulps (heat killed) and he reported that histodifferentiation did not occur. From these data he concluded that mineralized tissue in the donor tooth had no effect on the induction of odontoblasts from host tissues. He concluded, in addition, that the differen-
tiating cells in his earlier experiments (1972) must have been of donor origin since isografts with heat-killed pulps showed no such differentiation from host tissues.

Andreasen (1981b) in a study evaluating the interrelation between alveolar bone and periodontal ligament repair identified the cells in the vicinity of the root surface as the source of osteoinductive potential which determined the amount of repair of labial bone plate which would occur after its excision at the time of replantation of surgically extracted teeth. Nyman and Karring (1979) postulated similar reasons for their results in attempting to regenerate surgically removed buccal bone.

Ten Cate (1975a) extended the idea of local tissue taking part in periodontal repair. He claimed that, if the periodontal ligament was to be replaced, a source of previously determined cells of dental origin was required, since it was not possible to induce new ligament tissue from nonspecific connective tissue cells. Experimental evidence has been presented that the periodontal ligament possesses the capacity to inhibit both osteogenesis and the progressive resorption of the connective tissues of the root. The latter may result in bony attachment to cementum, progressive resorption of the root and replacement by bone (Loe and Warehaug, 1961; Andreasen and Hjorting-Hansen, 1966; Sherman, 1968; Hodges, 1969; Melcher, 1969, 1970, 1976; Barbakow et al., 1977). From this research and the research on the development of periodontal tissue (above) it is apparent that the pleuripotentential cells in the periodontal ligament of ectomesenchymal origin, perhaps in the cell rests of Malassez, play a potential role in reforming a functional periodontal ligament. Toto and Magan (1966) provide additional evidence for pleuripotentential cells in the
periodontal ligament when they showed autoradiographically that multinucleated osteoclasts arose from the coalescence of cells in the ligament and that osteocytes that replaced these osteoclasts 12 hours after parathyroid stimulation were derived from cells present in the loose connective tissue.

Burnette (1976) reported isolating and culturing epithelium-like and fibroblast-like cells from porcine periodontal ligament explants. Other researchers have reported the survival of epithelial rests in transplantation studies (Sherman, 1968; Groper, 1970). Melcher (1970) also suggested that there is a homeostatic interaction between the ligament cells and their progeny. The bone of the alveolus, acted upon by external stimuli, maintains a uniform periodontal space. Ankylosis may be the result of an interference with this interaction rather than a diminished capacity for regeneration of the periodontal ligament relative to the capacity for regeneration in bone.

The purpose of the present study is to investigate (1) if healing and establishment of a functional periodontal ligament does occur with the proposed protocol for autogenous transplantation of fully formed teeth to surgically prepared sites and (2) if healing does occur, what form it takes.

We propose to examine the experimental advantages of transplanting fully formed teeth into surgically prepared sockets. Because recent investigations have implicated the dental follicle and the outer dental epithelium as precursors and organizers of the periodontal attachment apparatus, the nature of the periodontal attachment and its origin will be examined in the proposed study. Such a study has clinical significance because of the increasing number of adults with missing first molars who
seek orthodontic treatment.

MATERIALS AND METHODS

Animals

Three adult Rhesus macaque monkeys were used in this study. In each monkey three teeth were extracted and three contralateral teeth were transplanted. Central incisors, second premolars and second molars were extracted to maximize the number of transplanted teeth while still maintaining two adjacent teeth and to see whether tooth type and location might influence healing. The extraction and transplantation schedule of each monkey is summarized in Table 1.

Surgical Preparation of the Recipient Site

Animals were anaesthetized with 25 mg/kg Ketamine HCl (100 mg/ml) and 0.5 mg/kg Acepromazine (10 mg/kg body weight). At the time of tooth extraction special care was taken to maintain crestal bone as well as buccal and lingual plates of the alveolus. Three extractions were done at the same time and the monkeys were placed on a soft diet for 7 days. A minimum of 71 days of healing followed the extractions to ensure remodeling of the area with a loss of the lamina dura, socket filling and the re-establishment of crestal bone. A direct-bond space maintainer, adjusted to serve later as a transplant retainer, was bonded directly to the buccal surfaces of the teeth adjacent to the recipient site using Concise (3M, St. Paul, MN).

Transplantation Procedures

The transplantation procedure was devised from the many published surgical procedures for tooth transplantation (Hale, 1954; Guralnick and Shulman, 1962; Pafford, 1956; Costich, 1962; Apfel, 1954; Tam, 1956;
### TABLE I

EXPERIMENTALLY INVOLVED TEETH

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Kaqueler and Massler, 1969). After donor-tooth root morphology was determined radiographically, an incision was made midway buccolingually along the crest of the alveolar ridge of the former extraction site which was to become the recipient graft site. Tissues at sites were elevated to the buccal and lingual only to the extent necessary to allow exposure of the crestal ridge. Care was taken not to dislodge the existing space maintainer. Conservatively sized preliminary sockets were created using a 25 mm, #4 round, endodontic burr in a high speed handpiece. Light intermittent touch and a continuous copious stream of sterile water were used to prevent overheating. Following the formation of the preliminary socket, the tissue flaps were repositioned while the donor tooth was elevated.

The buccal surface of the crown of the donor tooth was acid-etched to prepare a site to receive a direct-bond bracket for stabilization. Full thickness buccal and lingual flaps were then elevated along the donor tooth and adjacent (neighboring) teeth. A releasing incision in this flap was made at the medial buccal line angle of the tooth mesial to the donor tooth allowing easy access and visualization of the entire buccal plate of bone covering the root of the donor tooth. On anterior teeth, care was taken not to elevate tissues buccally or lingually at recipient sites. The buccal plate was then reduced close to but not into the periodontal ligament space using a cross cut fissure burr. The tooth was then delivered buccally using appropriate forceps taking care not to disturb the former site of crestal gingival attachment or to traumatize unnecessarily that portion of the periodontal ligament remaining on the tooth. Any remaining buccal bone was removed with an osteotome using hand pressure. The donor tooth was then tested for fit in the
surgically prepared socket. In most cases, a perfect fit had not been achieved and the donor tooth was returned to the donor socket and the gingival flaps re-opposed while adjustments were made in the recipient socket to ensure passive fit with the tooth minimally out of occlusion (Hale, 1954, 1956; Tam, 1956; Costick, 1962; Guralnick and Shulman, 1962). Following socket adjustments and final placement of the donor tooth into the recipient surgically prepared socket, buccal and lingual tissue flaps were contoured surgically along their free edge to conform to the donor tooth. Tissue flaps were repositioned and sutured to ensure a maximum of buccal and lingual attached gingiva. The exposed bone in the donor site was recontoured and the soft tissues were closed and sutured.

Transplanted teeth were re-etched using a minimum of etchant to prevent contamination of the newly juxtaposed cervical tissues. Siamese-twin edgewise orthodontic brackets (0.018 x 0.022 mm slot) were fixed to transplanted teeth at a height that allowed slots to fit onto the former space retainers.

Seven days after transplantation, transplanted teeth were isolated with rubber dams and conventional endodontic therapy was performed using powdered calcium hydroxide as root canal filling (Andreasen, 1972; Knight, Gans, and Calandra, 1964; Andreasen and Harting-Hansen, 1966). Splinting was re-ascertained and the monkeys were maintained on soft diets during the experiment.

**Preparation of Histologic Material**

Monkeys were anesthetized with Ketamine and were sacrificed and fixed by perfusing heparinated formalin through the femoral artery followed by further perfusion of nonheparinezed formalin. The mandibles were
dissected and cut into appropriate control (untreated) and experimental segments and decalcified for 16-22 weeks in a formic acid-sodium citrate solution. Each experimental segment contained the transplanted tooth and one half of each of the adjacent teeth which served as control normal tissue.

Following decalcification, control and experimental mandibular segments were embedded in paraffin in a vacuum oven, sectioned longitudinally at 12 μm with a sliding microtome and stained with hematoxylin and Biebrichs Scarlet. Some of the stained material was photographed with phase light to exaggerate the fiber organization of the periodontal ligament. The schedule of operations, endodontic therapy, and post-transplantation healing periods are summarized in Table II.

RESULTS

RADIOGRAPHIC FINDINGS

Normal Teeth

Fig. 1 (right) is a radiograph of a normal mandibular second molar. Note the lamina dura which is continuous around the alveolus. Interseptal bone is present as well as characteristic trabecular pattern and a well defined crestal bony ridge.

Recipient Sites

Radiographs of healed recipient sites 71 days after extraction show extensive remodeling of the tissues. Fig. 1 also illustrates a loss of the lamina dura and a re-establishment of crestal bone over the former alveolus. In addition, examination of Fig. 1 shows the re-establishment of a trabecular pattern in the former extraction site similar to that in the adjacent areas of the mandible.
<table>
<thead>
<tr>
<th>Monkey</th>
<th>Tooth</th>
<th>Healing Time Post Extraction</th>
<th>Healing Time Post Transplantation</th>
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<tr>
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<td>PM</td>
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Transplanted Teeth

A radiograph of an experimental pre-molar just after transplantation to a surgically prepared site is presented in Fig. 2. Note the size of the surgical cavity around the tooth and the lack of a lamina dura or organized trabeculae in the adjacent bone. Comparing Fig. 2 to Fig. 4 reveals that extensive remodeling has taken place after 49 days of healing. The establishment of a lamina dura, oriented interdental trabeculae, and very minimal root resorption are evident in Fig. 3. Crestal bone has been re-established but at a lower level. Root resorption though minor in some cases (Fig. 3) was more advanced in other cases (Fig. 4) where there had been advanced intraradicular resorption. Fig. 5 radiographed at 112 days is taken from the same tooth as seen in Fig. 4 at 49 days of healing after transplantation. Comparison of the radiographs in Figs. 4 and 5 indicates that some healing of resorbed root areas and bone remodelling has occurred during the additional two months. The lamina dura, horizontal trabecular pattern and crestal bone are well defined at 112 days post-transplantation. Comparison of Fig. 5 with Fig. 1 shows that the re-organized area around the transplant resembles the area around the mandibular second molar control seen in Fig. 1.

HISTOLOGIC FINDINGS

Control Periodontal Ligament

The histology of the normal periodontal ligament of an untreated tooth lateral to the transplantation site is illustrated in Fig. 6. This section from the mid-alveolar area contains cell rests of Malassez, blood vessels, and the typical oblique orientation of fiber bundles as
they course from bone to cementum. There are stellate osteoblasts along bone surfaces and round cementoblasts along cemental surfaces. The apical attachment of a normal control tooth is seen in Fig. 7. Note the smooth conical root, and layers of cellular cementum, and the well defined vasculature.

**Extracted Tooth**

Some periodontal soft tissue remains attached to the donor tooth Fig. 8. This periodontal soft tissue contains cementoblasts, collagen fibers, and cell rests of Malassez.

**Healed Recipient Site**

The histological appearance of a healed recipient site is illustrated in Fig. 9. A well defined cortical plate which forms the alveolar crest and the lateral walls of the mandible has been formed. Also visible in the center of the photomicrograph is the more open, less dense medullary area of the mandible which contains fatty marrow. This is the portion of the mandible which surrounded the apex of the surgical socket. There is a layer of soft tissue and attached gingiva overlying the bone. It is evident that any outline or remnants of the previous socket and periodontal ligament are absent.

**Sharpey's Fibers**

Supracrestal gingival fibers are shown in Fig. 10. This area healed uniformly and consistently. The large number and orientation of fibers can easily be identified. Vascularity in this region and the orientation of supracrestal fibers as they course up and over the crestal bone is seen in Fig. 10. Complete healing in the lateral portions of the periodontal ligament can be seen in Figs. 11 and 12. Of particular interest are the Sharpey's fibers running from their insertion in the
new cementum of the tooth across to their insertion in the new
cortical bone of the alveolus. The characteristic arrangement of
large bundles of Sharpey's fibers entering the bone and the smaller
bundles entering the cementum can be seen in Figs. 11 and 12.

Complete healing in apical portions of the ligament is demonstrated
in Fig. 13. However, healing similar to that seen in Fig. 13 was less
consistent in lateral areas then supercrestal areas. Sharpey's fibers
can be identified coursing from the large apical cap of cellular cementum,
in the lower left, to the bone, in the upper right (Fig. 13). The more
oblique orientation of Sharpey's fibers in the apical region is evident.

Width of the Healed Periodontium

Normal periodontal width was re-established in the healed attach-
ment areas (see Fig. 6 and Figs. 11 and 12). On the other hand, Figs.
14 and 15 display atrophic, relatively acellular and narrower periodontal
attachments seen near areas of ankylosis. Of interest in Figs. 14 and
15, is the orientation of the bone-cementum interface. In these in-
stances of poor healing there seems to be little or no interaction of
collagen fibers into bone or cementum. The apical areas show variable
periodontal width (Fig. 16). The root apices often had blunted pro-
files (Fig. 16) with much wider bands of poorly organized amorphous
apical connective tissue (contrast to Fig. 13).

Vasculature

The re-establishment of a vasculature is one of the early and con-
sistent findings in healed portions of the ligament. Fig. 12 depicts
the prominent vascular network laterally, while Figs. 13 and 16 show its
distribution apically. In atrophic portions of the periodontal ligament
(Fig. 14) a vasculature network is almost entirely absent.
New Bone and Cementum Deposition

Osteoblastic activity was observed very early (Fig. 17, 16 days post transplantation). By 23 days after transplantation (Fig. 18), a nearly continuous layer of cells with irregular nuclei lined all bony surfaces. Figure 18 also illustrates the increased number of osteoblasts in rear marrow spaces. In healing areas, cementoblastic activity was also prevalent as early as 16 days. Layers of newly formed cementum lined by cementoblasts were seen laterally (Fig. 19) and apically (Fig. 20), but the deposition of cementum was not as consistent as that of the deposition of bone 16 days after transplantation.

Ankylosis

Ankylosis was seen on several teeth and appeared to be most prevalent on premolars. Ankylosis appeared most frequently on cervical areas and especially on buccal surfaces. Fig. 21 illustrates a typical view of ankylosis with the more cellular bone blending into the cementum and creeping out laterally along the tooth surface.

Cervical Healing

The location of healed junctional epithelium was variable, it occurred from just below the cementoenamel junction (Fig. 22) to extensive migration onto the most apical extent of the root (Fig. 23). Interestingly, apical migrations of the epithelium attachment were often limited to the level of the first major area of cemental root resorption (Figs. 22 and 24).

Root Resorption

Varying degrees and kinds of root resorption were seen on most
specimens. In agreement with the finding of Andreasen (1972), surface resorption was the most prevalent. Fig. 22 is an example of this type of resorption occurring just below the attachment of the junctional epithelium. Replacement resorption appeared at all levels of the tooth (Fig. 21) and Howship's lacunae were found on apical portions of two implants. Inflammatory resorption though less common was seen most often in cervical areas (Fig. 25). The hard tissue mass in the right corner of Fig. 25 is cementum ankylosed to bone. Interestingly, resorption is taking place selectively on the cemental surface.

Rests of Malassez

Epithelial rests of Malassez present on donor teeth survived transplantation and were found frequently in the healed transplanted teeth. Cell rests were most commonly seen in supra-crestal areas (Fig. 26) and in apical areas (Fig. 27). There were fewer epithelial rests found along lateral surfaces of transplants and their location was sporadic and variable. Cell rests were most often associated with well organized healed areas (Fig. 12). In contrast, the rests were not associated with atropic areas adjacent to areas of ankylosis. The morphology and orientation of cell rests appear normal in the healed ligament where they were close to cemental portion of the periodontal ligament.

DISCUSSION

Origin of Cells During Periodontal Regeneration

There has been much discussion about the origin of the cells in tooth supporting structures that appear in transplanted or explanted teeth. The regenerated periodontal ligament and alveolar bone could originate from (1) transplanted dental tissues or (2) the surrounding
stroma at the host site. Investigators have generally interpreted their data from these two alternatives. Hoffman (1960) for example, suggested an inductive influence of the transplanted tooth on the host tissue. Ten Cate and his co-workers (1971, 1975a, 1975b) and Andreasen (1980 a,b, 1981a, 1981b) on the other hand, take the view that dental tissue transplanted or replanted into the host are the source of the newly formed periodontal tissues. Al-Talabani and Smith (1978), Barrett and Reade (1981), Yoshikawa and Kollar (1981), and Atkinson (1972) worked with much younger dental tissue and ascribe much of the regenerative capabilities to the inductive nature of these tissues and to the plasticity to form a variety of dental supporting structures in experimental combinations.

Much of the confusion derives from the use of a variety of animal species, varying ages of transplanted tissues, ambiguous cellular markers and labels, and immune incompatibilities between donor tissue and host extraoral transplantation sites. All of these confounding elements in experimental design have left the question of the origin of the new tissues completely open.

In this study, adult teeth were transplanted into surgically prepared sockets on the contralateral side of the mandible thus avoiding many of the experimental problems described above. There was no histological evidence in healed extraction sites (serving as the transplantation site) of any remnants of the periodontal ligament that could have survived after the former tooth was extracted. The extensive healing time and the thorough surgical destruction of the tissue when a new experimental socket was formed effectively removed any existing periodontal tissues which might have remained in this area. Thus, any
tissues in the transplant having a periodontal developmental history had to be of donor-tooth origin.

Functional periodontal attachments were formed often in these transplanted teeth. We conclude, therefore, that donor teeth supplied the cells in the regenerated ligament. The presence of epithelial rests of Malassez consistently found in association with transplanted teeth adds support to our contention that donor tissue was the source of new attachments; epithelial rests could have only come from the donor tooth. Others have noted that epithelial cells survive transplantation (Loe and Warchaug, 1961; Sherman, 1968; Groper, 1970; Sprouge, 1980) and have ascribed an inductive or directive role to these cells during periodontal reorganization.

Previous studies by Ten Cate (1975b) and Yoshikawa and Kollar (1981) indicate that cells of dental origin can also form bone. Since new attachments and bony alveoli were found in healed transplants in this study, we conclude tentatively that the new alveolar bone seen in this study was formed from pluripotential and cells carried along with donor teeth. These cells retain their osteogenic potential and contribute to the healing process (Roberts, Mozsary, and Klinger, 1982). Labelling experiments, in the future, should provide an unambiguous solution to this question.

While it may be argued that the newly formed walls surrounding surgically prepared sites provided laminae durae by growing toward the transplanted tooth, the presence of bone in furcation areas of molars and premolars suggests a more direct role of donor periodontal cells. Our observations suggest that the lamina dura is formed under the direction and in close proximity to the transplanted tooth and pos-
sibly from the tissues associated with the transplant. This notion is further supported by radiographic evidence of a re-established normal trabecular bony pattern and the maintenance of a regular periodontal space in these areas. This interpretation is reasonable in light of the report (Freeman, Ten Cate and Dickinsen, 1975) of gomophosis formation by teeth transplanted to parietal bone and is also in agreement with Line, Polson, and Zander (1974). Growth of bone from the socket walls would have resulted in an almost total ankylosis in the furcation areas and certainly should have resulted in marked deviations of the periodontal width in the furcation.

The concept that alveolar bone arises from osteogenic donor periodontal tissue sheds light on the question of periodontal regeneration. We suggest that the periodontium has in a real sense healed from the root side out and that the surgical socket has closed inward to meet the newly formed lamina dura, ligament and cementum.

Further support for the donor-tissue origin of the periodontal apparatus comes from the work of Roberts and his collaborators (1974, 1975, 1982) and Gould, Melcher and Burnette (1980). Roberts investigated parathyroid stimulation of the periodontal ligament while Gould et al. (1980) examined the repair of the periodontal tissues after injury. These workers agree that the rapid increase in cellularity associated with stimulated tissues arises from a rapid differentiation of cells from perivascular areas and from the undifferentiated fibroblasts in the periodontal ligament. Roberts, Mozsary and Klinger (1982) confirmed that osteogenic cells are derived from undifferentiated cells of the periodontal tissue by proliferation of cells that are normally blocked in G1 or G2 phases of the cell cycle (Gelfant and Smith, 1972). Thus,
the potential for repair by the donor periodontal tissue from the remnants of tissue left after extraction is present and could be the source of the regenerated periodontal tissue in healed teeth in this study.

Other work suggests that the periodontal ligament possesses the capacity to inhibit both osteogenesis and root resorption (Loe and Warchaug, 1961; Andreasen and Harting-Hansen, 1966; Sherman, 1968; Hodges, 1969; Melcher, 1969, 1970, 1976; Barkakow, Austin and Cleaton-Jones, 1977). Donor teeth retained the cemental portion of the ligament which in this view would have retarded healing in the mandibular bone while a new attachment apparatus was regenerating from this same donor tissue.

Experimental traumata inflicted by our procedures could have decreased the amount of periodontal tissue actually transplanted. This reduction in periodontal tissue would reduce or eliminate the inhibitory influences of donor tissue resulting in ankylosis. Melcher (1970) contends that ankylosis results because a homeostatic state is interrupted while Line et al. (1974) feel that in areas where intervening periodontal tissue is lost ankylosis occurs because the periodontal space is infiltrated with cellular elements from adjacent bone marrow. Line et al. (1974) feel that bone marrow precursor cells infiltrating the periodontal space deposit ankylosis bone. However, these two ideas are not mutually exclusive and may represent two statements of the same mechanism. The uniform width of the periodontal space in healed areas in contrast to the wider apical regions and narrow atrophic tissue associated with ankylosis regions might all be explained by altered homeostatic influences that maintain the periodontium (Melcher, 1970) or inhibit
mandibular osteogenic stem cells. A critical consideration in tooth transplantation studies then may be to protect, to retain and to augment healthy periodontal tissue capable of maintaining this homeostasis and enhancing regeneration.

The pattern and degree of ankylosis seen in this study can be related to the position on the teeth that were most likely to be damaged by the surgical techniques; it is similar to that reported by Andreasen's (1980 c, d) replantation studies. The ankylosis seen cervically can be related to tissue damage inflicted by forceps during extraction and the buccal ankylosis may be related to surgical burr and osteotome trauma when buccal bone was removed from the donor teeth.

**Cervical Reattachment**

Rapid re-establishment of a gingival and cuff fiber apparatus after transplantation was a consistent finding in the present study. However, the distribution was variable ranging from the cemento-enamel junction to the root apices. Three types of healing have been described: scar repair, collagen adhesion, and cemental repair (Daryategi, et al., 1980). All three types of healing were observed here but their location varied. We propose that the variability in the type of healing and the location of gingival cuff resulted from random surgical injury that stimulated cells of differing healing potential; possibly from the gingival tissues, or from the surgically prepared site, or from the vasculature. Cervical healing was more consistent than either lateral or apical healing suggesting that healing in the gingival area differs from healing elsewhere on the root and suggests a possibly different mode of healing response.
Andreasen (1980b) reported the establishment of a cervical periodontal ligament despite extended drying and removal of periodontal cell remnants. He suggested that the connective tissue adjacent to a cervical bony defect was capable of resorption while viable cells on the tooth were capable of inducing new bone formation (Andreasen, 1980b; Nyman and Karring, 1979). Andreasen (1980b; 1981b) concluded that there were different cell populations in the socket regions that differ in healing potential. Such a possibility would explain our variable results. The recent results of Noden (1983) suggest that dental tissues which are derived from first arch neural crest may be determined very early and may differ from other connective tissues in that region. Dental tissues, gingival connective tissue and mandibular bone may not be equivalent cell populations with respect to periodontal healing capacity.

Transplantation of adult teeth into surgically prepared sockets in the mandible provides an interesting and versatile model for examining the tissue interactions that occur during periodontal regeneration. This model may provide answers to the problem of the origin of periodontal attachments during healing.

SUMMARY

Periodontal healing in mature monkey teeth was assessed after transplantation into surgically prepared sockets in the mandible. Regeneration of a new periodontal attachment and variations in the healing process were examined histologically and radiographically. Teeth were extracted and the extraction sites allowed to heal. Later, after healing
was complete, periosteal flaps were elevated and sockets were prepared in the bony ridge. Contralateral teeth were then elevated and transplanted to surgically prepared sockets, stabilized with orthodontic brackets and allowed to repair their periodontal attachments. Endodontic treatment with calcium hydroxide was performed seven days after transplantation.

Healing was variable, but new periodontal attachments complete with new bone, cementum and Sharpey's fibers were formed in many areas. Ankylosis, inflammatory resorption and atrophic fibrous desposition in a narrow periodontal space were manifest in other areas. The theory that periodontal healing is due to the presence of donor ligament tissue and that healing occurs from the tooth side outward under the influence of the donor periodontal ligament remnants is supported by these findings. The variable data suggest that random damage incurred by donor tissue at the time of elevation and transplantation retards healing in damage-related areas of the periodontium. The finding that new attachments do form implies that with refinements in experimental protocol and surgical technique autogenous tooth transplantation into surgically prepared sites may be an effective potential clinical procedure.
Figure 1. Radiograph of the mandible showing a normal mandibular second molar *in situ*. Notice the continuous lamina dura and a normal trabecular bone pattern on the right. The healed recipient site showing extensive remodeling after 71 days with a well defined alveolar crest and trabecular pattern is seen on the left. 4X.

Figure 2. Radiograph of a transplanted premolar on day 0 of healing showing the size of the surgical defect, the lack of lamina dura, and the absence of organized trabecular bone. 4X.

Figure 3. Radiograph of a transplanted incisor after 97 days of healing. The re-establishment of a continuous lamina dura, crestal bone, interdental trabeculae, and minimum root resorption are evident. 4X.

Figure 4. Radiograph of a transplanted premolar in Figure 2 at 49 days of healing showing advanced inter-radicular resorption. 4X.

Figure 5. Radiograph of the transplanted premolar in Figure 2 and Figure 4 now at 112 days of healing. A better defined lamina dura, trabecular bone pattern, and crestal bone can be identified. There is apparent healing of the inter-radicular resorption seen earlier in Figure 4. 4X.

Figure 6. Photomicrograph of a section of the periodontal ligament of a normal tooth lateral to the transplantation site showing normal periodontal architecture and characteristic width. Cell rest of Malassez (R), blood vessels (V), osteoblasts (OB)
along the bone surface and cementoblasts (CB) along the cementum can be identified. 400X.

Figure 7. A photomicrograph of a section of the apex of an undisturbed tooth adjacent to the transplantation site illustrating the smooth conical shape, a cap of cellular cementum (CC), and a well defined vasculature. 40X.

Figure 8. A photomicrograph of donor soft tissue attached to extracted tooth. The soft tissue contains cementoblasts, collagen fibers and cell rests of Malassez. 40X.

Figure 9. A photomicrograph of the crest of the healed alveolar ridge at the recipient site after 173 days illustrating the absence of remnants of the previous periodontium, a well defined cortical crest, and the less dense medullary bone (MB) with fatty marrow. 40X.

Figure 10. A photomicrograph of a section of the supracrestal gingival attachment on a transplanted tooth showing the large number and orientation of supracrestal fibers as they leave the attachment in the cementum (bottom) and course up and over the bone shown in the upper right. 252X.

Figure 11. A photomicrograph of the lateral portion of the healed periodontal ligament of a transplanted tooth after 97 days showing normal architecture and width, the insertion of Sharpey's fibers (→) into the bone (B), and cementum (C). 252X.
Figure 12. A photomicrograph of a section of the lateral portion of the healed periodontal ligament of a transplanted tooth showing the normal architecture and width of the periodontal space with the insertion of Sharpey's fibers (--->) into the bone (B) and cementum (C). Also shown is the well defined vasculature (V) and the cell rests of Malassez (R) of normal morphology and location. 225X.

Figure 13. A photomicrograph of a section of the apical portion of the healed periodontal ligament illustrating the insertion of Sharpey's fibers (--->) into bone (B) and cementum (C) and a well developed vasculature. 400X.

Figure 14. A photomicrograph of a section of the atrophic, less cellular narrower periodontal space illustrating the orientation of fibers parallel to the cemental and boney surfaces. The reduced numbers of insertions and the greatly reduced vasculature can be seen. 400X.

Figure 15. A photomicrograph of a section of atrophic, less cellular and narrower periodontal space found in association with areas of ankylosis at 38 days after transplantation. The parallel orientation of the fibers and the reduced number of fiber insertions can be seen. Note also osteoblasts lining the narrow spaces (OB). 160X.
Figure 16. A photomicrograph of a section of a blunted apex of a transplanted tooth. A cap of cellular cementum (CC), a wide band of highly vascularized and poorly organized amorphous connective tissue can be seen. 40X.

Figure 17. A photomicrograph of a 16-day old periodontal ligament on the lateral portion of a transplanted tooth showing osteoblasts (OB) lining the bone surface of the attachment space and the absence of cementoblasts along the tooth surface. 252X.

Figure 18. A photomicrograph of a 23-day old lateral periodontal ligament on a transplanted tooth showing osteoblasts (OB) lining the surface of the bone and the marrow spaces. 252X.

Figure 19. A photomicrograph of a section of the lateral surface of a transplanted tooth 38 days after transplantation. A band of new cementum (NC) associated with a row of cementoblasts can be seen. 640X.

Figure 20. A photomicrograph of a section of the more apical surface of a transplanted tooth at 16 days after transplantation showing a band of cellular cementum (CC) lined by a layer of new cementum (NC) and associated with a row of cementoblasts (CB). 400X.

Figure 21. A photomicrograph of a section of the cervical area of a transplanted tooth showing replacement resorption or ankylosis with the more cellular bone fusing with and creeping
our laterally along the less cellular tooth surface. 252X.

Figure 22. A photomicrograph of a section just apical to the cemento-enamel junction of a transplanted tooth illustrating the surface resorption (SR) just apical to the well defined attachment of the junctional epithelium (---) which is just apical to the CEJ. 252X.

Figure 23. A photomicrograph of a section of the apical portion of a transplanted tooth indicating migration of the epithelial attachment (E) to the most apical extent of the root. 252X.

Figure 24. A photomicrograph of the cervical portion of a transplanted tooth showing the attachment of the junctional epithelium just coronal to an area of advanced inflammatory resorption. 160X.

Figure 25. A photomicrograph of a section of the cervical portion of a transplanted tooth illustrating advanced inflammatory resorption. Closer observation reveals that the cementum in the lower right has ankylosed to bone (---) and is being selectively resorbed. 160X.

Figure 26. A photomicrograph of the supracrestal portion of a transplanted tooth. Abundant cell rests of Malassez (R) can be seen near the tooth surface. 252X.
Figure 27. A photomicrograph of the apical portion of a transplanted tooth illustrating the abundant rests of Maiessez (R) near the tooth surface. 400X.
BIBLIOGRAPHY


