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# Collagen and Chondroitin Sulfate Xenogeneic Implants in Primates: A Sequential Histopathologic Study

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COLLAGEN AND CHONDROITIN SULFATE XENOGENEIC IMPLANTS  
IN PRIMATES: A SEQUENTIAL HISTOPATHOLOGIC STUDY

by

Raymond Alister Skinner, D.D.S.

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 in Oral Biology

May

1975

## DEDICATION

To my mother and father, Frances and Raymond Skinner, who's help was invaluable in the completion of my formal education. To my father's confidence and aid, without which my goals would not have been attained.

To my wife, Mary Sue, for her long struggle, patience, love and understanding, throughout these past ten years of my formal education.

To my daughter, Kristen Marie, the most precious happening of my graduate education.

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## CHAPTER I

### INTRODUCTION

Osseous defects are generally found in periodontal disease. These defects are sometimes amenable to treatment by subtractive (osteotomy and osteoplasty) and/or new attachment procedures. These procedures do not lend themselves to treatment of advanced periodontal osseous lesions. The reattachment procedure is not predictable when extensive bone destruction is present. When osseous resection techniques are employed to recontour the bony architecture, there is a net loss of vitally needed support of the functioning dentition.

The search for an ideal graft or implant material for osseous augmentation has been sought for the past 50 years. Since the results of these efforts have only, for one reason or another, been partially promising, the search still continues.

Various viable substances have been implanted into periodontal lesions at the time of surgery in an effort to induce the formation of additional amounts of new bone.<sup>1-7</sup>

These substances have shown promising results but they are of limited use in routine office procedures due to a number of reasons: (1) lack of predictability, (2) added patient insult and discomfort, (3) lack of sufficient quantities of material, and (4) specialized storage techniques which complicate treatment. What is needed is a bio-material which has a comparable or higher degree of bone formation, but presents no additional patient insult or discomfort, easily obtained in large quantities, and has no special storage problems. It also must be acceptable to the host tissue.

Collagen and chondroitin sulfate constitute the organic matrix of bone.<sup>8</sup> These substances are easily obtained in large quantities, have no special storage problems, and are acceptable to the host.<sup>9-12</sup>

Collagen is of particular significance as a bio-material because it has been developed in the course of biologic evolution, rather than by our scientific skills.<sup>13</sup> It has proved itself to be a strong, malleable, and versatile combination of amino acids. It is the supporting stroma for tissues (including bone) of animal as diverse as dinosaurs and man.

The question remains whether collagen and chondroitin sulfate can successfully be employed in periodontal therapy.

The purpose of this investigation is to determine, on a histologic level, the sequential healing phenomena of implanted collagen and chondroitin sulfate in two-walled osseous defects, and to observe any histomorphologic changes in the rhesus monkey periodontium.



## CHAPTER II

### REVIEW OF THE LITERATURE

#### A. XENOGENEIC COLLAGEN AND CHONDROITIN SULFATE

Collagen is a biomaterial which has evolved over time as the main structural component of animal tissues. Collagen has adapted, through evolution, into a strong, versatile combination of amino acids. It is a three-stranded helix in the form of a fibrous protein noted as the principle constituent of skin, tendon, bone and other connective tissues. There exists a similar pattern in the main structural molecule of collagen, in various species of animals.<sup>14</sup>

Neuman<sup>15</sup> demonstrated that the circulating body fluids are supersaturated with respect to bone mineral (hydroxyapatite) and new crystals do not form spontaneously. According to one hypothesis of the calcification mechanism, the first apatite crystals are formed by "epitaxy"<sup>16</sup> and not by precipitation. The most likely crystalline-like substance to induce crystallization from serum is the

collagen fibril. Mineralization of cartilage and new osteoid must induce crystals to form where none pre-existed. Neuman performed many experiments using collagen, in otherwise stable solutions of calcium and phosphate ions, which produced hydroxyapatite crystals. Collagen behaved as a surface catalyst for aggregation of ions in crystal formation.

In 1963, Rubin and his associates<sup>17,18</sup> concluded that the interaction properties of tropocollagen are markedly altered by treatment with the proteolytic enzyme, pepsin. This treatment liberates terminal or near terminal short polypeptides whose composition of amino acids are strikingly different from pepsin resistant triple-helix body of the collagen itself. These non-collagenous polypeptides may be the macromolecule which participates in the following: (1) biological control of fibrogenesis (2) pathological alterations in the so-called collagen diseases (3) in the aging phenomena and (4) immunological properties.<sup>9</sup>

Schmitt et al. and others<sup>9,10,18</sup> with the use of purified calfskin tested the antigenicity of collagen. These studies revealed that the action of certain enzymes, most notably pepsin and protease, was at the site of

antigenicity of the collagen. Even though non-purified collagen itself is a poor antigen, tests were conducted to measure the antibody concentration by compliment fixation of serum from sensitized rabbits.<sup>19</sup> The serological reaction which was demonstrated can be reduced or abolished by enzyme action on the structural component of collagen, the tropo-collagen molecule. This leaves 95 percent of the molecule, the triple-helix body, undamaged as demonstrated by electron microscopic examination. Enzyme action, therefore, does not destroy the native molecular structure of collagen, but leaves its immune capability non-existent.

Hiatt and Solomons<sup>20,21</sup> have established that a pepsin treated citrate extract of ox bone is a powerful potentiator of new bone formation. The results of Hiatt's work on mongrel dogs with periodontal disease revealed rapid proliferation of repair cells which accelerated healing of bony lesions. This accelerated healing included connective tissue, bone and cementum. He also demonstrated that greater amounts of new bone formation could be expected in the healing lesion.

Cutright et al. and Peacock<sup>22,23</sup> have shown that collagen is well tolerated by host tissues with no necrosis.

The collagen implant acted as a matrix. In each case, the collagen was utilized as a scaffold or template for the lodging of fibroblasts while they produced their own fibrous network or stroma. When tissue is removed, either surgically or by trauma, morbidity is reduced if this tissue can rapidly and successfully be replaced by the body. This provides a protective covering and/or to correct a tissue defect. This type of material then could act as a space occupier giving the body a chance to heal a bony defect.

Recently, Hunt<sup>24</sup> evaluated the histological response of microcrystalline collagen in tooth extraction sites in cats. Twenty adult male cats were utilized as the experimental animals. The lower first molars were surgically sectioned and removed. One site served as the control while the opposite extraction site was loosely packed with approximately 6 mg of regenerated bovine microcrystalline collagen powder. The cats were sacrificed at intervals of one and three days and every week up to and including the eighth week. Histologically, no excessive inflammatory response was noted in either group. The collagen implant accelerated the early phases of the healing process in which it appeared to act as a scaffolding for the ingrowth of the new tissue.

The accelerated healing included connective tissue and bone; there was evidence of greater amounts of new bone formation.

DeVors,<sup>25</sup> in 1973, studied the effectiveness of implanting untanned and quantitatively cross-linked, reconstituted calfskin collagen in surgically created mandibular defects. The collagen was implanted into rabbit mandibles which were sacrificed and examined. It was shown in this study that the greater the degree of cross-linking, the longer the time of degradation and resultant replacement by new bone. He postulated that by controlling the degree of cross-linkages, a reconstituted collagen graft can be made which will have a predictable rate of degradation and replacement by bone.

The use of collagen that emerges from these studies is that of a fibrous protein which has a structure with a biologically important end-region which seems to tag the collagen of individual animals. This biomaterial, collagen, can be purified.<sup>10</sup> Its end-region, removed by proteolytic enzymes, accounts for important reactive and antigenic sites<sup>9,10</sup> of the molecule. Many forms of collagen have been used; all showing some degree of success as an inductor, template, scaffolding, and/or space occupier.

By solubilizing animal skins and treating them with proteolytic enzymes, large amounts of collagen can be produced (altering antigenicity) at the same time. This material has a vast potential as the raw building block for a variety of medically useful materials.<sup>26</sup>

Burwell and Cushing<sup>27,28</sup> felt that the osteogenic inductor was from the necrosing bone grafts or marrow. Burwell thought that the osteogenic substance when liberated, induced osteoblastic differentiation in primitive cells, derived from its surviving portions. But Cushing concluded that the inductive signal came from products of the necrosing marrow. The great potential of this material to form bone appeared to be due to the availability of source cells lining the vascular sinusoids which have the propensity to differentiate into osteoblasts.

Moss,<sup>29,30</sup> in 1958, and 1960, demonstrated that some extractable substance (probably not chondroitin sulfate as such) from the organic ground substance of bone is most probably the inductor of osteogenesis. He states that competent connective tissue cells possess an intrinsic ability to respond to an extrinsic inductive stimulus. The undifferentiated cells once stimulated toward osteogenesis, has little choice but to follow this pathway.

Further research by Goldhaber<sup>31</sup> on the problem of bone induction implies the presence of an osteogenic inducing substance. He utilized allogeneic skull bone of immunized neonatal mice for implantation subcutaneously and within a millipore diffusion chamber. New vital bone, free of any homograft reaction, was found on the host side of the filter. The control bone implanted freely was found to be necrosed, surrounded by inflammatory cells, with no evidence of new bone formation. The formation of new bone found on the host side of the diffusion chamber certainly lends support to the existence of a cell-free inducing factor.

Urist,<sup>32</sup> in 1965, stated that new evidence in favor of the theory of bone induction can be gathered from the implantation of acellular, decalcified, bone matrix. This decalcified bone yields new bone in an amount proportional to the volume of the implant; the percentage of positive experimental results was equal to or greater than ninety per cent. It was observed that an implant of decalcified bone was degraded by the host inflammatory connective tissue cells including histocytes and foreign body giant cells. The histocytes were observed to be more numerous than any of these other cells. The ingrowth of new cells

and organizing tissue in the host bed is also induced to differentiate. It was also theorized that the inductor cell is a descendant of a wandering histocyte; the induced cell is a fixed histocyte or perivascular young connective-tissue cell.

Urist, McLean, Strates and their associates<sup>33-36</sup> stated that recent observations have, more than ever before, demonstrated that the proteins of the organic matrix of bone and dentin contain the precursor of the inducing substance.

Urist<sup>33</sup> coined the phrase, "the bone induction principle," which is a cellular differentiation phenomenon from which a tissue substrate exerts a physio-chemical effect upon competent mesenchymal cells to stimulate their differentiation into osteoblasts capable of both osteogenesis and further induction (autoinduction). The bone induction principle can be demonstrated by simple tissue implantation in ectopic sites of animals. The inductor factor is a specific entity only in the sense that it is more consistently demonstrated and more active in an implant of decalcified lyophilized bone matrix than any other tissue with the exception of bone marrow.



The organic matrix of decalcified bone has two chief components,<sup>34,37</sup> collagen and chondroitin sulfate; the most prominent, being fibrillar in nature, is collagen. The bone induction principle is intimately related to bone collagen, probably because of its high density, low permeability, and relative insolubility.<sup>35</sup> This matrix also includes, between the fibers, a protein-bound polysaccharide, chondroitin sulfate.

It is obvious from these studies and the observations of others<sup>38-42</sup> that demineralized bone may effectively be used to "induce" the formation of new bone.

Sobel and his associates<sup>11,12,43,44</sup> proposed that chondroitin sulfate, together with collagen or other substances, may be responsible for initiating calcification. He demonstrated<sup>11</sup> that synthetic chondroitin sulfate collagen complexes exhibit similar behavior as that of rachitic cartilage with regard to calcifiability. Results obtained with implants of chondroitin sulfate or collagen and chondroitin sulfate show collagen complexes homologous to the actual calcification mechanism, and support the hypothesis that they are involved in calcification.

Burger and Sobel,<sup>12</sup> in 1961, studied the repair of bony defects in the calvaria of albino rats. This histologic and roentgenographic study showed an accelerated rate of bone repair in the defects, in which chondroitin sulfate was used. The chondroitin sulfate was in combination with other substances: gelfoam, demineralized bone, fresh homogeneous bone, and collagen. When these substances were used alone, the healing rate was less profound as compared to their combination with chondroitin sulfate. None of the repair rates were equal to the demineralized bone-chondroitin sulfate combination; although, collagen-chondroitin sulfate showed the second most profound healing rate of bony defects in rats.

Narang, Lebrin and their associates<sup>45,46</sup> have recently demonstrated that implantation of decalcified, lyophilized bone allografts of both the cortical and cancellous types resulted in new bone formation and a gain in the attachment level, too. Lebrin observed no apparent evidence of graft rejection for as long as two years following implantation. There seems to be little doubt to date that these organic matrices do cause new bone formation.

Cartilagenous materials also have been used, with varying success, in the treatment of periodontal osseous defects in humans and animals. Cartilage has an organic structure composed of collagen and sulfated mucopolysaccharides (chondroitin sulfate).<sup>37</sup>

Urist and McLean<sup>47</sup> reported in 1951, on the effects of implants of fibrocartilagenous callus, epiphyseal cartilage, articular cartilage, external ear cartilage and other tissues to the anterior chamber of the eye. Using ear cartilage, they found that no bone was produced in the eye within thirty days. They reported that if they had followed these transplants longer, they too may have induced new bone formation. The transfer of the other cartilages were usually successful in inducing new bone formation.

Lacrox,<sup>48</sup> in 1956, using several ectopic sites in rabbits, has shown that the central portion of a vital epiphyseal cartilage was capable of causing the formation of bone. This bone demonstrated an encircling layer of membranous bone termed the "perichondral ossification ring." He considered the cartilage as an osteogenic inducer.

Schaffer<sup>49</sup> demonstrated in 1956, that the use of cartilage, fresh or preserved in alcohol, showed promise in

the repair of periodontal osseous defects in rhesus monkeys. In 1958, he<sup>50</sup> repaired human periodontal defects with allogeneic cartilage. This cartilage was obtained from the noses of other patients following submucous resections and stored in refrigerated seventy per cent ethyl alcohol. The implant was well tolerated by the host tissues. There was no apparent foreign body reaction as observed clinically. The results of the grafts were determined by measuring the pockets before and after implantation. Some new attachment coronal to the pre-existing attachment was achieved in 60 of the 70 implants. New bone coronal to the old was evident in 28 of the implants, as judged by roentgenographs.

Collagen obtained from the scleral portion of the eye was used by Klingsberg<sup>51</sup> in the repair of periodontal osseous defects in humans. This method proved to be advantageous in the repair of destroyed bone. Biopsy specimens prepared for histological study revealed a rapid ingrowth of connective tissue which appeared to bind the graft to the bone and gingiva. Re-entry at post operative intervals up to two years showed a lasting restoration of the bone defects after scleral grafting. The sclera (collagen and its cementing substance, chondroitin sulfate) persisted, but along its

edges osteoblasts were present, suggesting that the sclera was being transformed into new bone. This would lead one to surmise that the host site has cells, which given the chance, are competent of induction themselves. This collagen may be a "space occupier" which allows the prepared host site to react in a positive manner. Sclera, has to be preserved very carefully and trimmed, in order to remove the choroid coat and Tenon's capsule.

If either of the preparation techniques are not followed carefully, the implant appears to fail.

In view of the evidence of bone induction suggested by the literature, a study of both chondroitin sulfate and purified collagen seems an appropriate parameter in studying bony defects in the periodontium. The study concerns itself with the histomorphologic changes in the rhesus monkey periodontium in which an experimental surgical defect was created and implanted either with chondroitin sulfate or purified collagen.

## CHAPTER III

### MATERIALS AND METHODS

#### A. EXPERIMENTAL DESIGN

Four adult rhesus monkeys (*Macaca mulatta*), three males and one female, were utilized as experimental models in this study. The experiment covered a one-hundred day interval utilizing the first fourteen days as a quarantine and tuberculosis inoculation period. This period allows the monkeys to acclimate to the Loyola animal care facility and a definite parameter of health to be established. Throughout the experimental period, the animals maintained their physical parameters as recorded during their quarantine, and appeared to remain in good health during the remaining eighty-six days of the experimental period.

The oral condition of each animal was noted. They demonstrated slight marginal gingivitis with varying amounts of materia alba, plaque and calculus. The gingiva was firm in consistency and pink in color. The sulcus depths were

examined and noted to be within acceptable limits, one to three millimeters (Figure 1).

The maxillary and mandibular left quadrants were utilized as experimental sites except on monkey number one. This monkey had an unacceptable mandibular left quadrant due to exfoliated deciduous teeth and partially erupted permanent teeth. The maxillary right quadrant was used as a replacement. Thus, five maxillary quadrants and three mandibular quadrants were included in the study. A total of three two-walled surgically created osseous defects per quadrant were created. One was utilized as a control and the other two were corrected by Xeno-implants of chondroitin sulfate and purified collagen. The control site preparation and correction (i.e., curettage only, no implants) was accomplished at the same time as the preparation of the analogous implanted defects within the same quadrant.

The one-hundred day schedule of the experiment was planned to allow for a 14 day quarantine and a 86 day experimental period. This allowed two-walled osseous defects to be created thirty days before the initial implantation. The predetermined schedule was so designed to allow the sacrifice of the control, chondroitin sulfate and collagen

implants on 0, 3, 7, 14, 21, 28, 42, and 56 days, post-operatively. Therefore, each time sequence allowed a control experimental defect and defects corrected by chondroitin sulfate and collagen.

Fifteen minutes prior to the surgical procedures, the monkey received an intramuscular injection of 8 mg Sernylan\* for sedation (Figure 2). When long procedures were encountered, an additional dose of 5 mg Sernylan was given intramuscularly, as needed for sedation. Local anesthetic Xylocaine 2%\*\* with 1:100,000 epinephrine was used in the area of surgical intervention.

Throughout the course of each surgical procedure, strict conditions of asepsis were maintained for the operator and the monkeys' protection. All pertinent clinical observations were recorded, and Kodachrome slides were obtained.

#### B. GENERAL PREPARATION

Full mucoperiosteal flaps from the mesial of the first premolar to the distal of the second molar were utilized for

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\*Parke, Davis, and Co., Detroit, Michigan

\*\*Astra Pharmaceutical Products Inc., Worchester, Mass.



both the creation and the correction of the defects (Figure 3). An intrasulcular incision was carefully performed with a #15-C Bard-Parker blade and reflected with a small periosteal elevator (#7 was spatula). Care was taken so the tissue would not be perforated. This scalloped incision preserved the interdental papillae for better primary closure. Tissue tags on the inner surface of the flap were removed with a small tissue scissors. Gracey curettes were used to remove all interproximal granulation tissue and to thoroughly plane the exposed root surfaces.

#### C. PREPARATION OF THE DEFECTS

The sites chosen for the creation of the osseous defects were the interproximal osseous septae distal to the first premolar, second premolar and the first molar. These areas were readily visible and presented no access problems. There was an adequate amount of interproximal bone in which the osseous defects were prepared, and this area affords protection postoperatively due to the contour of the crowns and the interproximal contacts between adjacent teeth. The control defects were placed on the distal of the first

premolar, and the chondroitin sulfate and collagen was placed in the distal of the second premolar and molar, respectively.

Surgically created two-walled osseous defects were made in the selected sites. A straight fissure bur in a slow speed dental handpiece was used to penetrate the interproximal alveolar crest, along the distal root surface, apically to a depth of 3 mm. The root surface was notched to allow a reference point for histologic examination. The depth of the defect was carefully monitored with the use of a calibrated periodontometer\*. All the defects had a mesial wall of cementum and/or dentin and a distal wall of bone. The two-wall defects therefore consisted of a mesial wall of cementum and/or dentin and a distal and lingual wall of bone (Figure 4). During the osseous excision, isotonic saline irrigation was utilized in order to reduce the amount of heat caused by the surgical bur.

#### D. INTRODUCTION OF CHRONIC IRRITANTS

Wooden toothpicks served as a chronic irritant in each defect. They were trimmed to fit from the apical portion

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\*HU-FRIEDY--Michigan probe

of the defect to below the contact. This afforded a wedging effect for retention of the toothpick. The wood, therefore, acted as a route of direct communication from the oral cavity to the defect, allowing bacteria and their toxins ingress. Thus, the osseous defect would simulate a chronic defect seen in human periodontitis (Figure 5).

The buccal full mucoperiosteal flap was repositioned and secured to its original position with interrupted, vertical mattress, interproximal 5-0 Ethaflex\* sutures. These sutures also helped hold the wedged wooden irritants in their position. The suture was tied on the lingual surface interproximally, and the ends were cut short (Figure 6).

#### E. POSTOPERATIVE CARE

Immediately following the surgical procedure, a intramuscular injection of 600,000 units of Combiotic\*\* (Penicillin and dihydrostreptomycin) was given (Figure 2). The Combiotic gives a 72 hour antibiotic coverage to the monkey. The animal received no food for 24 hours, post surgically, a

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\*Ethicon-Johnson and Johnson

\*\*Pfizer

soft diet for the following 72 hours, and a normal diet thereafter.

#### F. REMOVAL OF IRRITANTS

Seven days post surgically, the monkey was sedated with 8 mg Sernylan for removal of the wooden irritants and the sutures. Clinically inflamed gingiva was noted at this time.

#### G. PROCUREMENT OF IMPLANT MATERIAL

The collagen was obtained through the courtesy of Dr. Howard S. Kramer, Jr.\* It is an antigenically altered<sup>9</sup> collagen extracted from enzyme-solubilized calfskin collagen. It was exposed to  $3.3 \times 10^6$  rads of Gamma ( $\gamma$ ) irradiation to increase the cross linkages between the collagen molecules, therefore, decreasing its solubility.

A large piece (3.5 x 6.25 cm) of antigenically altered collagen was scored, on the large flat surface, with a

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\*Chief of the Department of Oral and Maxillofacial Surgery, Highlands General Hospital, Oakland, California.

#15 Bard-Parker blade. This was done under a two power magnification to obtain a small particle size (Figure 7). The obtained particulate was then soaked in 70% ethyl alcohol for 3 hours and rinsed three successive times with sterile distilled water. Drying was accomplished at (43° C) for one hour. The resulting particulate was then transferred into a sterile test tube for storage until needed. The above procedure was accomplished under conditions of asepsis.

A very clean mixture of chondroitin sulfate isomers were obtained from Sigma Chemical Company\*. The mixture consisted of a mixed isomer of 99% pure chondroitin sulfate from whale and shark cartilage. The approximate percentage of chondroitin sulfate "A" or "B" was 13% and that of chondroitin sulfate "C" was 73%\*\* (personal communication with John Marx, Sigma Chemical Co.). The remaining 13% was the other isomers of chondroitin sulfate.

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\*P.O. Box 14508, St. Louis, Missouri

\*\*John Marx, Sigma Chemical Company

#### H. CORRECTION OF THE DEFECTS

The same surgical procedures outlined previously were used. Exactly 30 days postoperative of the creation of the two-walled osseous defects, the osseous defects were exposed for surgical implantation or curettage in the case of the control. All the previously made two-walled osseous defects were meticulously curetted to remove chronic inflammatory tissue and the root surfaces were thoroughly planed.

The control defect on the distal of the first premolar was left debrided only and no implant material was placed.

Chondroitin sulfate was placed in the debrided defect distal to the second premolar. When it mixed with blood, the chondroitin sulfate became a gelatinous mass which presented some difficulty in placement. The collagen was placed in the debrided defect distal to the first molar. It packed well with little difficulty in placement. Hemorrhage was controlled by adequate suction and gauze squares.

With the surgical correction of the defects completed, the full mucoperiosteal flap was repositioned and held firmly in place with sutures (as previously described). The same post operative antibiotic and diet instructions as previously described were given.

Seven days after the surgical correction of the osseous defects, the animal was again sedated for suture removal. The monkey was then maintained until the next procedure or until the scheduled sacrifice.

#### I. COLLECTION OF SPECIMENS

On the day of sacrifice following sedation, the animal was given a intracardial injection of a lethal dose of Totaltox\*.

Block sections were obtained with the aid of a Stryker saw. The specimens were washed with distilled water and placed in pre-tagged jars containing 10% formalin for fixation. The volume of formalin used was 20 times that of the specimen.

#### J. PREPARATION FOR HISTOLOGIC EXAMINATION

Following adequate fixation (2 weeks) each specimen was decalcified in formic acid and sodium citrate, (50%/50%

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\*Chicago Veterinary Supply, Chicago, Illinois

solution) trimmed, embedded in paraffin, sectioned at 10 microns in a transverse buccolingual plane, and stained with hematoxylin and eosin (H and E). The degree of decalcification was determined by radiographs taken at weekly intervals after an initial 4 week period. The slides from each experimental site were stained with hematoxylin and eosin, and a representative histologic section was selected for detailed histologic analysis.



## CHAPTER IV

### FINDINGS

#### A. CLINICAL OBSERVATIONS

The gingival tissues of the experimental animals were characterized by a slight marginal chronic gingivitis at the beginning of the study (Figure 1). The gingiva was firm in consistency and pink in color. The sulcus depths were examined and noted to be within acceptable limits, one to three millimeters. Seven days following creation of the two-walled osseous defects and placement of the irritants, the gingiva demonstrated marked inflammation. The marginal and papillary gingiva was edematous, erythematous, and displayed hemorrhage upon manipulation. Thirty days post-operatively, when the osseous defects were corrected, decreased gingival inflammation was noted. Although, this inflammation of the gingiva appeared to be greater than normal.

The architecture of the osseous defects had been changed by resorption as observed at the time of defect correction,

by implantation or curettage. Additional bone resorption was consistently observed, when compared to the original osseous defect. The two-walled osseous defect was characterized by loss of mesial and distal cortical plate giving the defect a large "V" shape.

After defect correction, the tissue showed moderate inflammation of the marginal gingiva and papillae as compared to adjacent areas. The tissue inflammation progressively improved and appeared normal within 7 to 14 days.

## B. HISTOLOGIC OBSERVATIONS

### 1. INTRODUCTION

Epithelial changes, as observed during the experimental period, were inflammatory in nature. This demonstrated the effect of surgical insult and normal chronic gingivitis that is found in the monkey.

Connective tissue changes were also chiefly inflammatory in nature, characterized by edema, vascular dilation, inflammatory cell infiltration and progressive maturation of granulation tissue being re-organized to near normal fibrous connective tissue.

Histologic changes (control, chondroitin sulfate and collagen) of the alveolar bone all demonstrated osteogenesis. With the re-establishment of the periodontal triad, the following was noted: (1) bone, (2) cementum, and (3) intervening attachment by fibers. The cementum and new periodontal ligament in the collagen implanted defect appeared to be more extensive than in the control.

## 2. TWO-WALLED CONTROL

### a. Epithelium

Defects were created in the monkeys as previously explained. Two 0 day control specimens were obtained, one in which the defect had not been flapped or curetted, and the other defect which was flapped and curetted prior to sacrifice.

#### 0 Day

This area was not flapped or curetted. Keratinized stratified squamous epithelium and the sulcular epithelium was normal. The epithelium was closely adapted to the tooth surface and demonstrated a normal epithelial attachment near the cemento-enamel junction.

0 Day (Figure 8)

This area was flapped and curetted just prior to sacrifice. All of the control defects (3-56 days) had been flapped and curetted prior to their respective sacrifice date. The keratinized stratified squamous epithelium was not adherent to the tooth. The sulcular epithelium was thin (normal).

3 Day

Keratinized stratified squamous epithelium with the sulcular epithelium demonstrating intercellular edema, some degeneration and a fibrinopurulent exudate.

7 Day

Keratinized stratified squamous epithelium and the sulcular epithelium was normal (thickness).

14 Day

Keratinized stratified squamous epithelium. There was slight cellular edema and some desquamation

of the epithelial cells. The epithelial attachment extends to the defect and was adherent to the exposed dentinal notched surface.

21 Day

The block section representing the 21 day was destroyed due to active eruption of a succeeding tooth in the area of defect.

28 Day

Keratinized stratified squamous epithelium with a long epithelial attachment extending into and was in contact with the dentin.

42 Day

Keratinized stratified squamous epithelium. The epithelial attachment terminates on newly formed cementum which covered the defect on the dentinal surface.

56 Day

Keratinized stratified squamous epithelium:

The epithelial attachment was attached to cementum coronal to the defect.

## b. Connective Tissue

0 Day

This area was not flapped or curetted. The connective tissue showed slight to moderate plasma cell infiltration. Small spicules of bone and/or cementum were seen in the connective tissue. There was an inflammatory infiltrate of plasma cells around these spicules. There seemed to be new bone forming around these spicules.

0 Day (Figure 8)

This area was flapped and curetted. The full mucoperiosteal flap was detached. The connective tissue showed both slight edema and plasmacytic infiltration. Small spicules of cementum and/or

bone were seen in the detached flap. Hemorrhage was seen near the detached flap. The inflammatory reaction was equivalent to that which pre-existed.

### 3 Day (Figure 9)

The full mucoperiosteal flap was detached. The connective tissue was partially destroyed. There were loosely arranged connective tissue fibers in the defect. Superimposed on this connective tissue was a fibrinopurulent exudate, with some polymorphonuclear leukocytes and some fibrin.

### 7 Day

The connective tissue showed a slight plasma cell infiltrate and appeared normal.

### 14 Day

The connective tissue was unremarkable, showing old collagen fibers connecting to newly formed fibers in the defect area. Connective tissue healing by fibrogenesis was mostly completed.

21 Day

The block section representing the 21 day was destroyed due to active eruption in the area of the defect.

28 Day

The lamina propria of the flap was composed of mature connective tissue and it formed a junction with the new connective tissue, produced by healing, in the defect.

42 and 56 Day

The lamina propria showed slight edema and slight plasma cell infiltration (normal). It showed mature collagenous fibers extending from the gingiva to the cementum.

c. Alveolar Bone and Defect

0 Day (Figure 10)

This area was not flapped. The defect was filled with fibrous connective tissue, and showed an



absence of cementum where the notch in the tooth was made. The alveolar crest showed both immature bone and lamellated bone with active osteoblastic activity.

0 Day (Figure 11)

This area was flapped and curetted. The crest of the alveolar bone demonstrated that it had been removed leaving a defect. There was an amorphous eosinophilic material composed of degenerating bone fragments.

3 Day (Figure 9)

The alveolar bone below the defect showed the periodontal ligament to be attached to the cemental surface of the root. The defect was filled with loosely arranged fibrous connective tissue. Superimposed on this tissue, superficially and occlusally, was a fibrinopurulent-exudate. The notch portion of the defect extended into the pulp of the tooth. There was a degeneration of the pulp in this exposed

area with occlusion of the exposure by formation of irregular dentin closing it. Cementum was being apposed on this defect wall.

7 Day (Figure 12)

The defect was filled with granulation tissue characterized by dilatated capillaries, a few polymorphonuclear leukocytes and many proliferating fibroblasts accompanied by mitotic activity. There was slight residual fragments of bone and/or cementum within the defect.

14 Day (Figures 13 and 14)

A thin layer of cementum was forming on the dentinal surface of the defect. The defect, itself, showed proliferating and maturing fibroblasts with collagen fiber bundles being formed. Some patent capillaries were seen. The small fragments of bone and/or cementum present in the healing wound were not distinguishable. The crest of the alveolar bony defect demonstrated new bone apposition. The new bone was growing into the defect and showed

active osteoblastic activity. There seemed to be a fibrous connection between this new bone and the cementum appearing as a periodontal ligament.

21 Day

The block section representing the 21 day was destroyed due to active eruption in the area of the defect.

28 Day (Figure 15)

The defect notch was covered by new cementum which was artifactually separated from the dentin.

Young collagenous fiber bundles, which still showed greater numbers of fibroblast and fibrocytes than the connective tissue of the flap, were attached to the new cementum. There were multiple foci of new bone in the new connective tissue of the defect. Continuous with the old bone is newly apposed bone which continued to grow into the defect.

42 and 56 Day (Figures 16 and 17, respectively)

There was new bone apposition on the old, alveolar bone proper, bone. Young dense collagenous fibers extended from the new bone to the new cementum. These fibers appeared to be forming the new periodontal ligament. There was also active osteoblastic activity seen on the alveolar crest.

3. CHONDROITIN SULFATE TWO-WALLED IMPLANT

a. Epithelium

0 Day (Figure 8)

Keratinized stratified squamous epithelium and the sulcular epithelium was normal. In the flapped defect, the epithelium was not attached and in the unflapped defect, it was.

3 Day

The oral surface keratinized stratified squamous epithelium and the sulcular epithelium showed slight ridges of epithelium into the flapped defect.

7 Day

The keratinized stratified squamous epithelium demonstrated a plexiform hyperplasia. The sulcular epithelium was normal. There was an artifactual detachment of the epithelial attachment from the cementum. Also, the epithelium had grown into the defect between the connective tissue and cementum.

14 Day

Keratinized stratified squamous epithelium demonstrated mature epithelial ridges. There was an epithelial attachment on the cementum extending into the defect.

21 Day

There was a keratinized stratified squamous epithelium with an intact sulcus. The epithelial attachment was short and it attaches to the cementum.

28 Day

There was a keratinized stratified squamous epithelium which showed slight intercellular edema and sharp, elongated, immature epithelial ridges. The epithelial attachment on the dentin and cementum was along the notched portion of the defect, half way into the defect.

42 Day

The keratinized stratified squamous epithelium showed a normal sulcus. The epithelial attachment terminated on old cementum coronal to the notch.

56 Day

The keratinized stratified squamous epithelium showed a normal sulcus epithelium. The epithelial attachment extended just into the notch portion of the dental defect.

b. Connective Tissue

0 Day (Figure 8)

The connective tissue showed both slight edema and a plasmacytic infiltration. Small spicules of cementum and/or bone were seen embedded between the collagen fibers and defect.

3 Day (Figure 18)

The full mucoperiosteal flap was detached. The connective tissue demonstrated normal connective tissue. However, the part of the flap attached to the tooth showed a fibrinopurulent exudate on the surface with some loss of collagen apparently by collagenolytic activity.

7 Day

There was a granulomatous reaction within the connective tissue of the papilla as illustrated by proliferation of fibroblasts and many capillaries. There was, therefore, considerable capillaries and many fibroblasts as the organization of the clot

continued. The preoperative collagen fibers had undergone a dissolution. Also, some fragments of dentin were seen.

14 Day (Figure 19)

The connective tissue showed old collagen fibers clearly marked by a junction with the new fibers. Fibrogenesis of the connective tissue healing appeared almost complete.

21, 28, 42, and 56 Day

The connective tissue was repaired. The mature collagenous fibers joined by interlacing with the new, relatively young, collagenous fibers in the defect was evident. There was a fairly cellular matrix with fibroblasts and many dilated capillaries were in the tooth defect. These collagenous fibers extended to and connected with the newly formed cementum on the surface of the dentinal defect.



c. Alveolar Bone and Defect

0 Day (Figure 11)

The defect in the alveolar bone demonstrated that it had been ground away, as small particles of bone were present. The alveolar crest showed both young immature bone and lamellated bone, both with osteoblastic activity. There also was amorphous eosinophilic material which appeared to be degenerating bone fragments.

3 Day (Figure 20)

The defect was filled with a fibrinopurulent exudate and showed some amorphous material which looked slightly eosinophilic. This represented the chondroitin sulfate implant. Apical to the defect there was the residual alveolar bone with periodontal ligament fibers attached to the old cementum.

7 Day (Figure 21)

There was new bone formation in the defect. Generally the defect demonstrated fibroplasia with

resorption of the chondroitin sulfate and fragments of bone and/or cementum. The injured alveolar bone of the defect showed resorption and was filled with granulation tissue. The remaining alveolar crest showed the periodontal ligament extending to the cementum of the tooth.

14 Day (Figures 19 and 22)

The osseous defect showed an interlacing junction of old (the flap side) and new fibrous connective tissue. Therefore, it was filled with fibrous connective tissue. The alveolar crest of the defect showed young cellular bone being apposed indicated by active osteoblastic activity. The quantity of new cementum on the root defect was apparently greater than in the control.

21 Day (Figure 23)

The osseous defect itself shows 2 elements:  
(1) eosinophilic amorphous material, dentin and bone fragments surrounded by osteoclasts. The osteoclasts

and this material were surrounded by fibrous connective tissue. (2) New bone formation in the defect. There was a continuity between the old alveolar bone and the new bone formed on it. The new bone extended into the root defect. Fibrous connective tissue extending from the new bone to the new thin cementum on the dentin, of the dental defect, formed a new periodontal ligament.

28 Day (Figures 24 and 25)

The osseous defect was filled with relatively young collagenous fibers and a cellular matrix with many capillaries. These young collagenous fibers extended to and connected with the newly formed cementum (artificially separated) in the notch part of the dental defect. On the crest of the old alveolar bone was seen apposition of young lamellated bone. It also showed a dense layer of osteoblastic activity. Extending from the new bone were dense collagenous fibers which were attached to the newly formed cementum artificially separated from the

dentinal surface of the dental defect and formed a new periodontal ligament.

42 Day (Figures 26 and 27)

The dental defect demonstrated newly formed cementum apposed on the dentin. The osseous defect showed newly formed bone on the crest of the old bone. A well organized periodontal ligament with patent capillaries, between the cementum and new bone, was formed. The lamina propria of the flap was firmly attached to the periosteum of the alveolar bone.

56 Day (Figure 28)

This area resembled the findings seen at day 42 except within the periodontal ligament were a few islets of newly formed bone. The new cementum was artifactually separated from the dentin.

d. Chondroitin Sulfate Implant

0 Day (Figure 29)

The aqueous solubility of the chondroitin sulfate made it impossible to obtain an ideal section demonstrating the chondroitin sulfate 0 day implant. Therefore, we utilized a smear of aqueous chondroitin sulfate stained with aldehyde fuchsin. The stain was positive for chondroitin sulfate.

3 Day (Figures 18 and 20)

The implant material (chondroitin sulfate) was in contact with the surrounding alveolar bone, periodontal ligament, tooth and overlying flap. It was surrounded by a fibrinopurulent exudate which fills in the remaining portion of the defect.

7, 14, 21, 28, 42, and 56 Day

The implant of chondroitin sulfate is not visualized but was replaced by new fibrous connective tissue which showed progressive maturation.

## 4. COLLAGEN TWO-WALLED IMPLANT

## a. Epithelium

0 Day (Figure 8)

The oral keratinized stratified squamous epithelium and the sulcular epithelium were normal.

3 Day

The oral keratinized stratified epithelium and the sulcular epithelium were normal. The flap was detached.

7 Day

The oral keratinized stratified squamous epithelium showed ulceration and a fibrinopurulent exudate and contained some fragments of bone being sequestered by the exudate.

14 Day

The oral keratinized stratified squamous epithelium had its epithelial attachment on the dentinal surface of the dental defect.

21 Day

The oral keratinized stratified squamous epithelium was normal. The epithelial attachment was attached to the surface of dentin of the dental defect and in some areas was artifactually separated.

28 Day

The oral keratinized stratified squamous epithelium showed slight intercellular edema.

42 and 56 Day

The oral keratinized stratified squamous epithelium was normal and reflected into a normal sulcus. The epithelial attachment was adherent to the old cementum just above the dental defect.

## b. Connective Tissue

0 Day (Figure 8)

The connective tissue showed slight edema and a slight plasmacytic infiltration (normal) and

contained a few spicules of cementum.

### 3 Day

The full mucoperiosteal flap was detached. The flap connective tissue showed mature dense collagenous fibrous bundles showing a slight inflammatory infiltrate and contained fragments of dentin. The portion of bone to which the periosteum still was attached showed defects infiltrated by a fibrinopurulent exudate and contained fragments of bone. The fibrous connective tissue coronal to the defect area showed a loss of collagen; replacing it was a fibrinopurulent exudate with some residual fragments of the host collagen. There was vasodilatation with red blood cells seen and a few polymorphonuclear leukocytes.

### 7 Day (Figure 30)

The connective tissue in the defect was largely replaced by ulceration and its fibrinopurulent exudate. The granulation tissue demonstrated patent capillaries, proliferating fibroblasts and fragments of bone.



14 Day

There was normal appearing connective tissue attached to the newer connective tissue of the defect. The granulation tissue had interlaced with the adjacent normal fibrous connective tissue of the flap.

21 Day (Figure 31)

There were dilated capillaries and old collagenous fibers in the connective tissue. There was a junction between the old fibers of the flap, and the new connective tissue forming in the defect. The connective tissue in the defect was more cellular and showed fibroblasts, young connective tissue fibers and some eosinophilic material which may have been remnants of the implanted collagen or remnants from the creation of the defect.

28 Day

The lamina propria showed young fibroblasts and dilated capillaries. Deep to this was mature

dense collagenous fiber bundles extending to the original flap. Some fragments of striated muscle also were seen.

42 and 56 Day

The lamina propria of the attached gingiva showed dense mature collagenous fibers attached to the new and old bone.

c. Alveolar Bone and Defect

0 Day (Figure 11)

The defect on the alveolar bone demonstrated that it had been ground away. The alveolar crest showed some young immature bone and lamellated bone showing osteoblastic activity. There was also an amorphous eosinophilic material which may be degenerating bone fragments.

3 Day (Figure 32)

The notched dentinal portion of the defect was free of cementum. There was a slight fibrinopurulent

exudate seen within the defect. The remaining area of the defect has scattered particules of implanted collagen.

7 Day (Figure 33)

The defect was filled with granulation tissue showing active fibrogenesis and osteoblastic activity. At the alveolar crest, new bone was forming. This osteoblastic activity was surrounded by loose connective tissue which was differentiating to osteoblasts. There were multinucleated giant cells in the granulation tissue; these may be residual osteoclasts that are removing the implant or fragments of bone.

14 Day (Figure 34)

The defect in the tooth showed irregular new cementum formation. There were highly cellular fibroblasts showing delicate collagenous fiber bundles and patent capillaries filling the defect. At the junction of young and older connective tissue of the flap, residual fragments of collagen implant

were seen with giant cells around them. The collagen appeared to be removed by a collagenolytic process. The residual alveolar bone showed no evidence of new bone on it. Essentially, there was a fibroblastic response.

21 Day (Figure 31)

On the surface of the notched part of the defect was a thick new layer of cementum. The old crest of bone showed new bone apposition. From this new bone there was an extension of collagenous fibers to the newly formed cementum, re-establishing a periodontal ligament.

28 Day (Figure 35)

The dental defect was filled with mature fibrous connective tissue containing dilated capillaries and showing some perivascular histocytes. On the surface of the old bone, newly formed bone was seen; newly formed cementum on the old cementum was also seen. Newly formed collagenous fibers in the defect were attached to

this cementum and in some areas were artifactually detached from it. No new bone formation was seen in the connective tissue in the dental defect. There was a residual inflammatory process in the area of the dental defect which had not yet been resolved. There were some perivascular plasma cells in the residual inflammatory process.

42 Day (Figure 36)

The dentinal notch was lined by newly formed cementum and artifactually separated from it. In the defect was fibrous connective tissue with islets of newly formed bone and fragments of dentin and/or bone. New bone was also seen on the crest of the old alveolar bone. There was active osteoblastic activity. It appeared that the collagenous fiber bundles extended from the newly formed bone to the new cementum, re-establishing a periodontal ligament. On the labial surface of the old bone there was some evidence of osteoclast resorption, indicated by reversal lines in the bone, which was covered by normal periosteum.

56 Day (Figure 37)

There was a thick band of newly formed cementum, artifactually separated from the dentin, in the dental defect and attached to this newly formed cementum were mature dense collagenous fiber bundles. These collagenous fibers have patent capillaries filled with blood. Newly formed bone was arising and extending coronally from the old bone. The dense collagenous fibers attached to the cementum extended and attached to the newly formed bone, re-establishing a mature periodontal ligament. There was still osteoblastic activity on the labial surface of the bone.

## d. Collagen Implant

0 Day (Figure 38)

The collagen would not remain within the defect which made it impossible to obtain an ideal section demonstrating the collagen 0 day implant. Therefore, we utilized the particulate of purified collagen imbedded in paraffin. Ten micron sections were made

and stained with Mallory's stain. This showed a positive stain for collagen.

3 Day (Figure 32)

A slightly fibrous mass of acellular purified collagen was within the defect. The fibers were pale and eosinophilic.

7 Day (Figure 33)

There was a residual of the implanted collagen still present. The proliferative granulation tissue seemed to be replacing the implant.

14 Day (Figure 34)

A small residual amount of the collagen implant was seen between the junction of the new and older connective tissue.

21, 28, 42, and 56 Day

The implant of purified collagen was not visualized but was replaced by new fibrous connective tissue which demonstrated progressive maturation.

## CHAPTER V

### DISCUSSION

This study demonstrated that the particular implants used, purified antigenetically treated collagen and a mixed isomer of chondroitin sulfate, are well tolerated by the host. This material was successfully implanted in osseous defects of the rhesus monkeys with no rejection phenomena elicited by the host. Two-walled osseous defects in rhesus monkeys demonstrated repair by regeneration and reattachment following implantation with collagen or chondroitin sulfate; however, the control also healed. The implanted defects seemed to show a delayed sequence as compared to the control.

The sequential healing description of the gingival epithelium demonstrated its potential for swift and extensive regeneration. Rapid regeneration and repair were also seen within the connective tissue of the mucoperiosteal flap. Dominant cellular activity was demonstrated by fibroblasts and endothelial cells.



The clinical findings demonstrated that the experimental defects created in this study simulate those of chronic periodontitis, but the histologic healing of the control defect demonstrated that the flaw was the experimental model. One might think a disease endogenous to man should be studied in man and not his primeval cousins who, through evolution, possibly are not affected with the disease. Specifically, the complex and often unknown etiologic factors of "natural" periodontal disease are not present in the experiment animal; therefore, the repair proceeded without the influence of these complicating factors. The healing then, is accomplished without the presence of a significant etiologic factor--a point which must be kept in mind at all times.

The question of osteogenesis, and what causes it, is an area of prime importance in present and future periodontal research. Bone induction properties of grafts or implants are a major area of research. In particular, the source of the osteoblast is the real subject of interest. The osteoblasts which are induced to produce new bone have three possible origins: They may arise from pre-existing osteoblasts,<sup>52</sup> from periosteum, from endosteum lining the marrow

cavities,<sup>53</sup> and perivascular undifferentiated mesenchymal cells.<sup>54</sup> These three sources can act simultaneously.<sup>54</sup>

There is evidence that undifferentiated mesenchymal cells are capable of transformation into functional osteoblasts in a wide variety of experimental situations.<sup>53</sup> The initiating factor in bone formation is not elucidated as yet.

Collagen and chondroitin sulfate were implanted because of their vast potential as raw building blocks in animal bodies. The possibility, therefore, of these materials being inductors for osteogenesis exists. The principle organic matrix of bone (collagen and chondroitin sulfate) is so interrelated to the phenomena of bone induction that it is of extreme importance to see if the principle constituents of bone could be involved directly or indirectly in osteogenesis.

Urist<sup>32</sup> hypothesized that collagenolytic activity by histocytes or other cells, causes dissolution of the organic matrix which yields an "inductor substance" for new-bone formation. This new bone calcifies rapidly. Collagenolytic activity induced by collagen may have the same effect on osteogenesis.

The ingrowth of new cells and organizing tissue in the host bed can also be induced to differentiate. It could, therefore, be hypothesized that host compatible implanted material acts as a "space occupier" which delays healing of a defect. This allows a greater proliferation of granulation tissue with its increased numbers of undifferentiated mesenchymal cells, as compared to non-implanted defects. This would allow a greater potential for osteogenesis by virtue of an increased pool of potential progenitors of osteoblasts.

From death comes life. This is the age old cycle of nature; whether death supplies the food for life or the initiator of life matters little. Nature's phenomena, that life is interdependent and death allows life to continue, is a concept which Cushing<sup>28</sup> touched upon when he observed bone induction which was signaled by necrosing marrow. Both marrow and bone matrix induce osteogenesis, possibly a material common to both is the initiator. Collagen and/or chondroitin sulfate could be the initiator; since the implants of chondroitin sulfate and collagen were compatible to osteogenesis in the monkey, they may be implicated in the phenomena. It would follow that a combination of both materials should be tried.

Although bone can be successfully regenerated, it is only part of the triad of periodontal support. Reconstruction of new cementum, periodontal ligament and supporting alveolar bone must be achieved simultaneously. Therefore, the problem is not only one of repairing bony deformation, but rather, one of formation of a total new attachment apparatus. Cementogenesis did occur in the implant defects and the control, demonstrating the implants compatibility to both. The cementum produced in the implanted defects appeared to be more profound and fibrogenesis seemed to be of a higher order than in the control.

We must at all times keep in mind that the problem of osteogenesis in human periodontal disease is a very complex order of body function with many possible initiating factors. This multifaceted problem is not easily paralleled in an animal model. In the last analysis, therefore, only human studies will offer valid and sufficient insight into osteogenesis.

It is important in research, to standardize the materials being used so that sound conclusions can be obtained. Therefore, this purified collagen and a purified isomer of chondroitin sulfate "A" or "C" should be used in follow-up

experiments. As suggested by the present literature, these purified materials should be used in combination with each other. This correction in the experimental design, along with the only valid experiment model, man, would help to elucidate the actual value of these basic building blocks of tissue. In retrospect, therefore, this experiment should have been planned to utilize purified materials for standardization and implanting them in man, the only applicable model of periodontal disease.

Sclera is the body's own "almost pure collagen." It has dense collagenous fibers with very few widely scattered cells. When prepared properly, there are no glands, nerves or blood vessels. This material would, therefore, be of value in periodontal therapy as pointed out by Klingsberg. Like marrow, sclera has problems of specialized storage and limited availability. Purified antigenetically treated collagen has no storage or availability problem; therefore, it would be a more ideal material for implantation.

## CHAPTER VI

### CONCLUSIONS

1. The experimental defects employed in this study on rhesus monkeys did not emulate those of chronic periodontitis in man, and therefore, represent a dubious experimental model for healing studies.
2. The two-walled osseous defects corrected by purified collagen and a mixed isomer of chondroitin sulfate were accepted by the host site and did not impede osteogenesis.
3. These materials possibly act as a "space occupier" which inhibits the apical migration of the epithelial attachment and allows for a greater number of undifferentiated mesenchymal cells, the progenitors of osteogenesis.
4. The evidence to date seems to indicate a further study is needed, using a mixture of purified collagen and chondroitin sulfate. This further study would be of extreme value, if man were used as the experimental model.

## CHAPTER VII

### SUMMARY

This study was undertaken to examine histologically, in monkeys, the sequential healing phenomena of created two-walled osseous defects, which have been corrected by purified collagen and a mixed isomer of chondroitin sulfate.

Four adult rhesus monkeys were used as experimental models and provided twenty-three specimens, from 0 to 56 days, postoperatively. Sixteen of these served as implant specimens, and seven served as control specimens in which the defects were corrected by curettage only.

Two types of implant materials were utilized. The collagen was obtained through the courtesy of Dr. Howard S. Kramer, Jr. The collagen is a purified, antigenically altered collagen extracted from calfskin. The material was exposed to  $3.3 \times 10^6$  rads of Gamma ( $\gamma$ ) irradiation to increase the cross linkages between the collagen molecules.

A mixed isomer of chondroitin sulfate was obtained from Sigma Chemical Company.

Histologic sections from each specimen were stained with H and E, a description of the sequential healing events were recorded, and the results discussed.

The implants were compatible with osteogenesis. Healing of the implanted defects seemed to be at a slower rate, as compared to the control. There was no real difference between the control and the implants. The two implant materials themselves did not differ in the healing events.

Collagen and chondroitin sulfate may have participated in osteogenesis, but due to a poor animal model, the results most certainly were obscured.

In view of the evidence of bone induction suggested by the literature, a study of both a mixed isomer chondroitin sulfate and purified collagen seems an appropriate parameter in studying bony defects in the monkey periodontium.



CHAPTER VIII

ILLUSTRATIONS



Figure 1. The preoperative appearance of the experimental area. Note the slight papillary and marginal gingivitis.



Figure 2. The preoperative sedation Sernylan and the postoperative antibiotic Combiotic which were used.



Figure 3. The osseous contour before creation of the two-walled osseous defects.



Figure 4. Experimentally created two-walled osseous defects (arrows) distal to the second premolar and first molar. A control defect (c) had been created distal to the first premolar.



Figure 5. Wooden irritants (arrows) stabilized in position by sutures.



Figure 6. Vertical mattress sutures (arrows) on the lingual of the maxillary arch.

late was obtained.

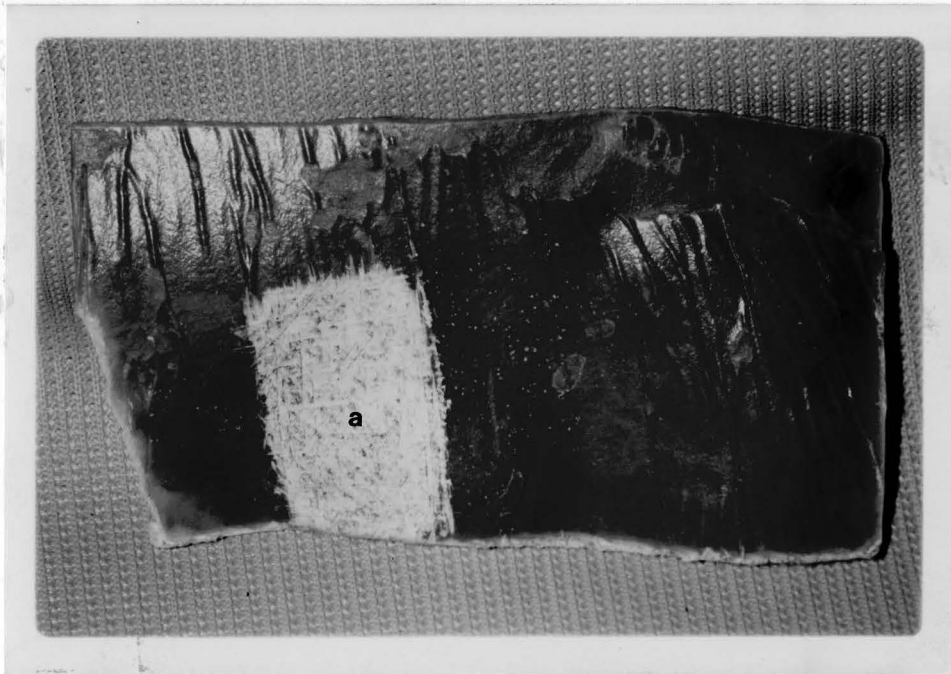


Figure 7. Purified antigenically treated collagen with the area (a) from which the particulate was obtained.



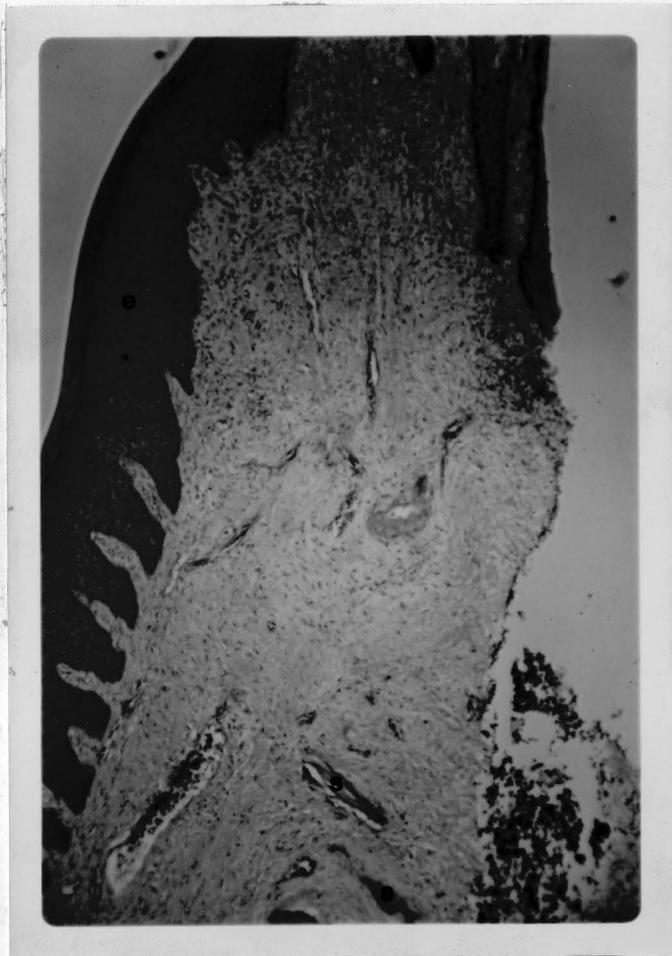


Figure 8. 0 day flapped control, 25x.

Keratinized stratified squamous epithelium (e) is not adherent to the tooth. Small spicules of bone and/or cementum (s) are seen in the connective tissue of the flap.



Figure 9. 3 day control, 40x. The defect has a new cemental (c) layer, loosely arranged connective tissue (ct) and blood vessels. The dentinal notch exposes the pulp (p) which has degenerated and newly formed dentin (d) occludes the exposure. Note the fibrinopurulent exudate (fe).



Figure 10. 0 day. This area was not flapped. 40x. Note on the dentin surface cementum is absent and the defect is filled with fibrous connective tissue (ct). Note spicules of bone and/or cementum (s) with osteoblastic (arrow) activity around these spicules.

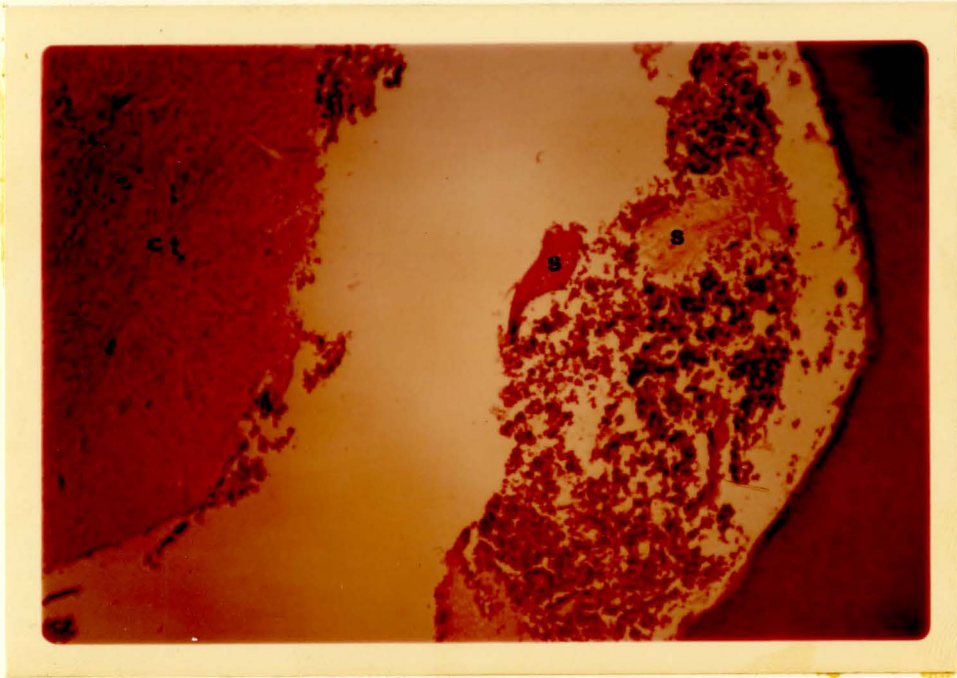


Figure 11. 0 day. This area was flapped and curetted. 40x. Note the spicules of bone and/or cementum (s) and the detached connective tissue (ct).

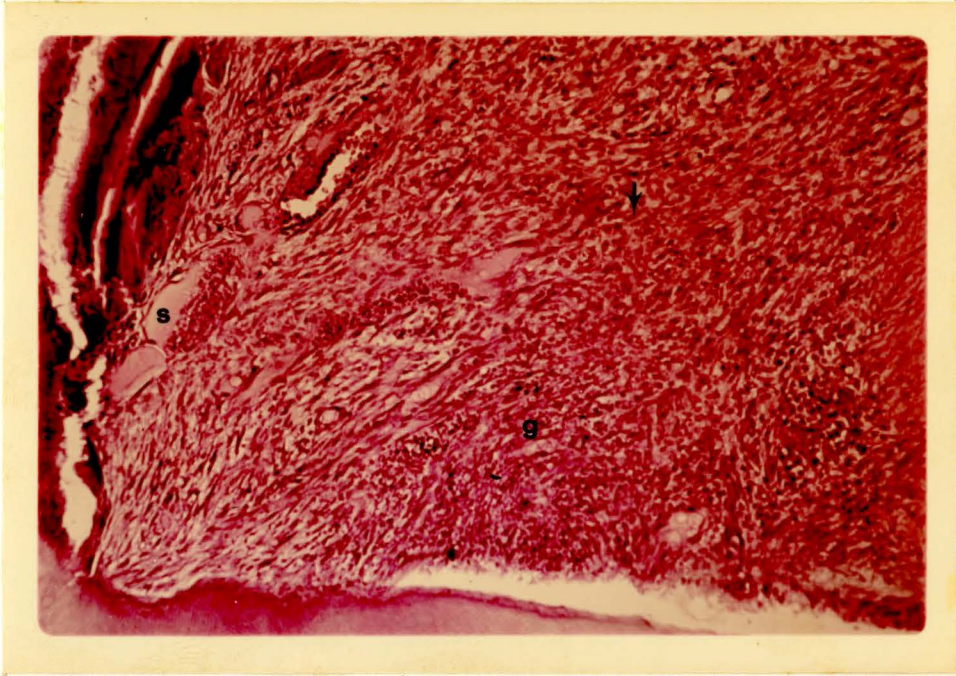


Figure 12. 7 day control, 40x. The defect is filled with granulation (g) tissue which is organizing and many fibroblasts (arrow) are seen. Slight fragments of bone and/or cementum (s) are within the dental defect.

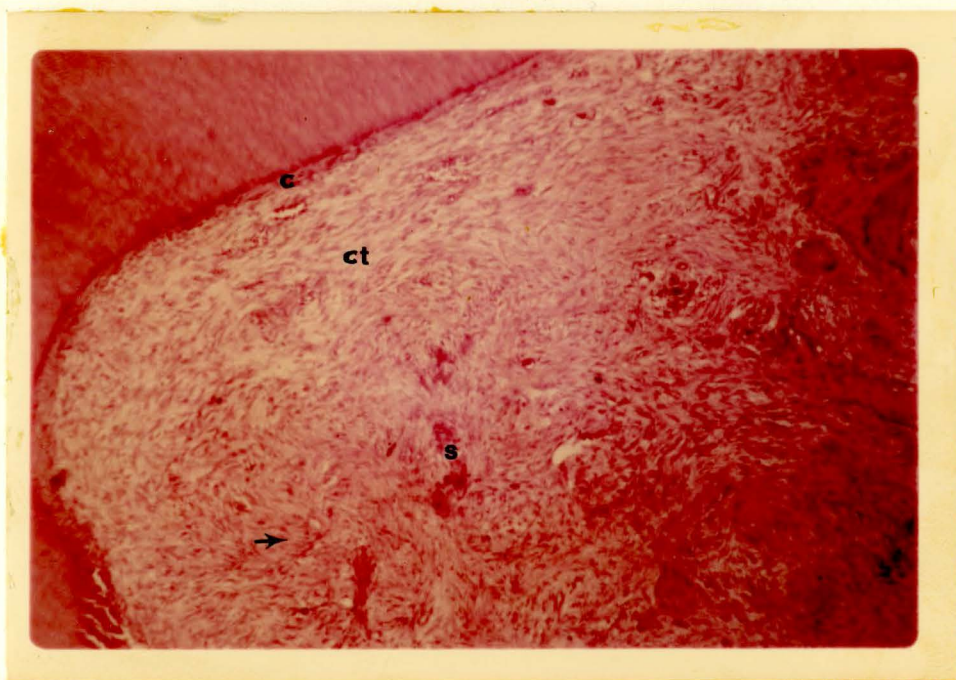
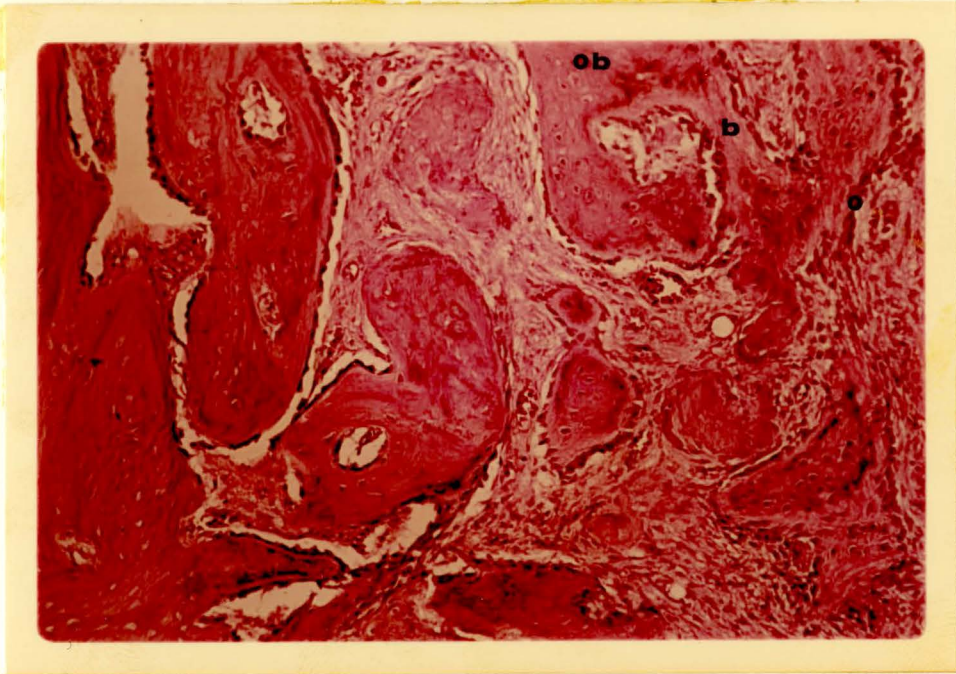


Figure 13. 14 day control, 25x. Note a thin layer of new cementum (c) covering the dental defect. In the connective tissue (ct) is seen young fibroblasts, new bundles of collagen (arrow). Fragments of bone and/or cementum (s) are also seen.



**Figure 14.** 14 day control, 40x. Note the new bone ( b ) apposition on old bone ( ob ) and the osteoblasts ( o ) activity on the periphery of the new bone.

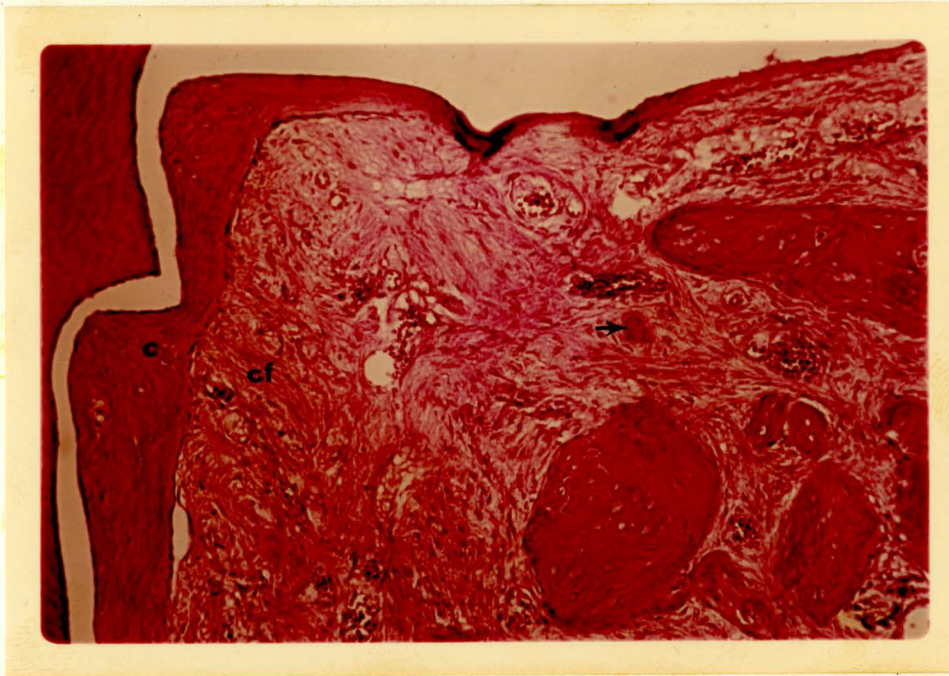


Figure 15. 28 day control, 40x. Note the new cementum (c) which is artifactually separated from the dentin and collagenous fibers (cf) which connect this cementum to the bone. Some foci of bone (arrow) formation are seen.



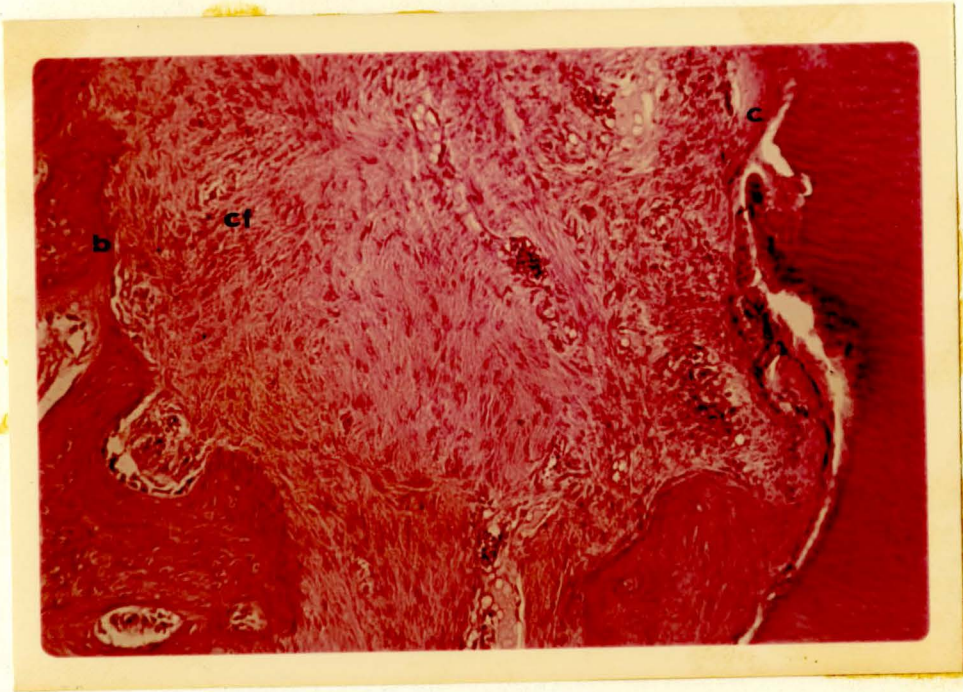


Figure 16. 42 day control, 40x. Note the new bone (b) apposed on old bone and young connective tissue (cf) fibers which extend from the new bone to the new cementum (c).

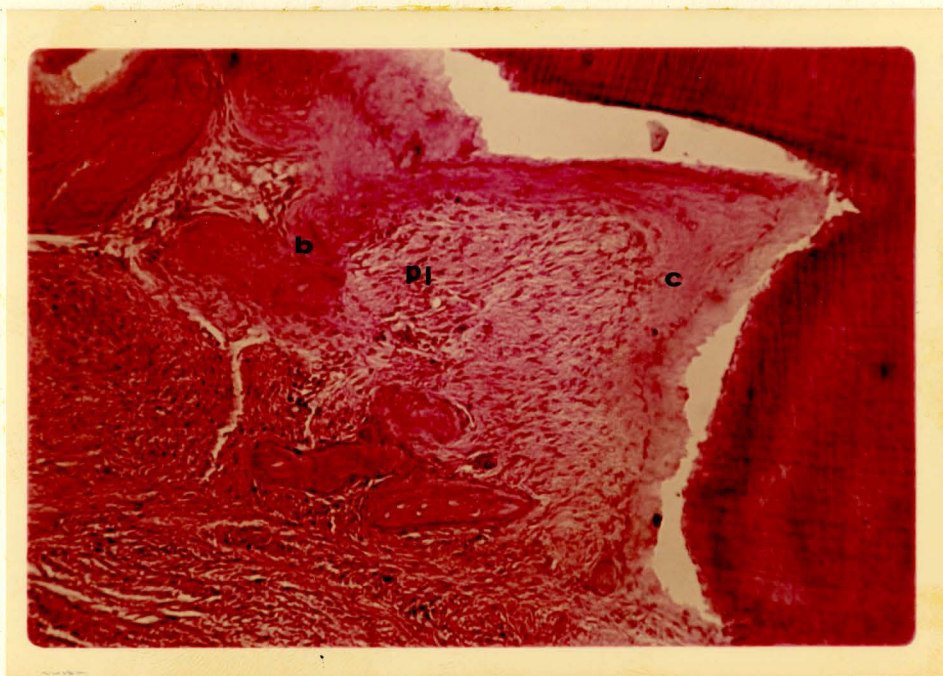


Figure 17. 56 day control, 40x. Note the newly formed cementum (c) artifactually separated from the dentinal surface. New bone (b) is extending into the dental defect and connective tissue fibers extend to this new bone and cementum forming a new periodontal ligament (pl).

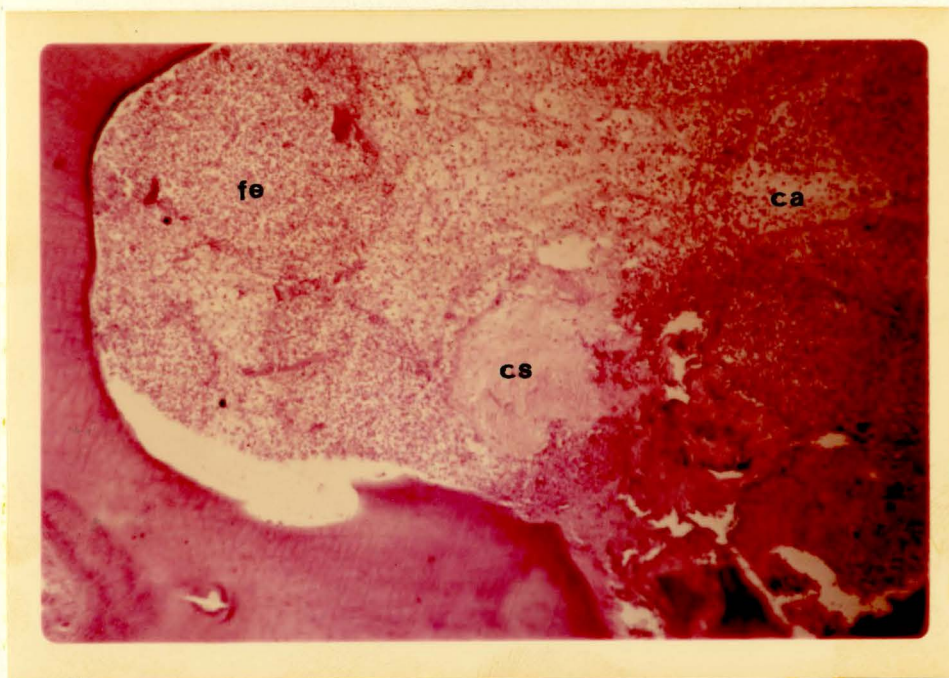


Figure 18. 3 day chondroitin sulfate implant, 25x.  
Note the dental defect is filled with a fibrino-  
purulent exudate (fe) and some areas of collageno-  
lytic activity are seen (ca). The chondroitin  
sulfate (cs) implant is seen as an amorphous  
eosinophilic mass.

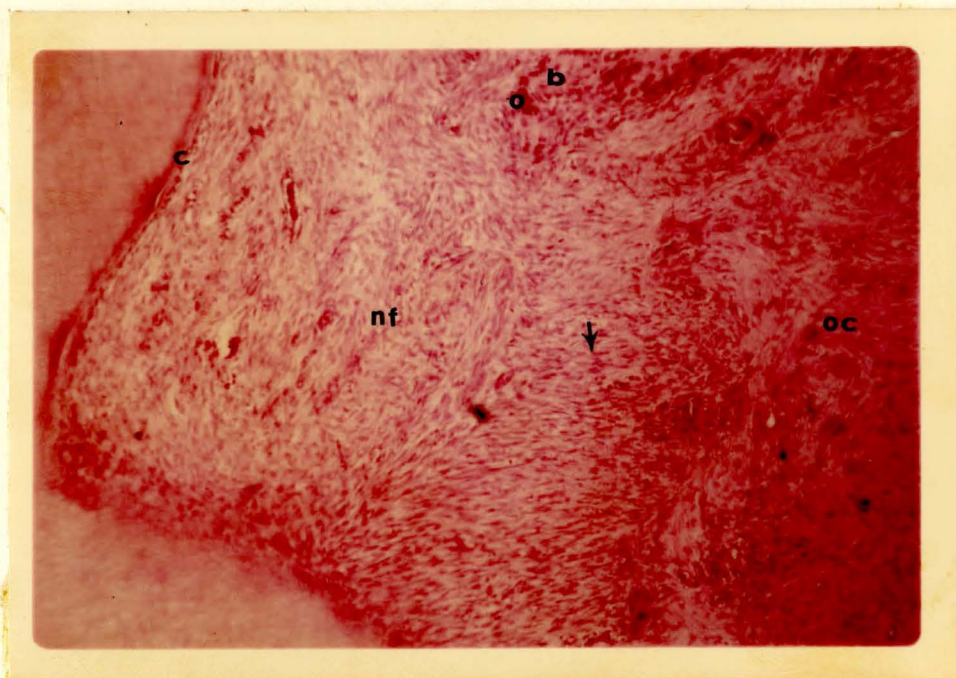


Figure 19. 14 day chondroitin sulfate implant, 25x. Note the connective tissue showing old collagen (oc) fibers clearly marked by a junction (arrow) with the new fibers (nf). New bone (b) formation is seen with osteoblastic activity (o) and also new cementum (c) is seen.

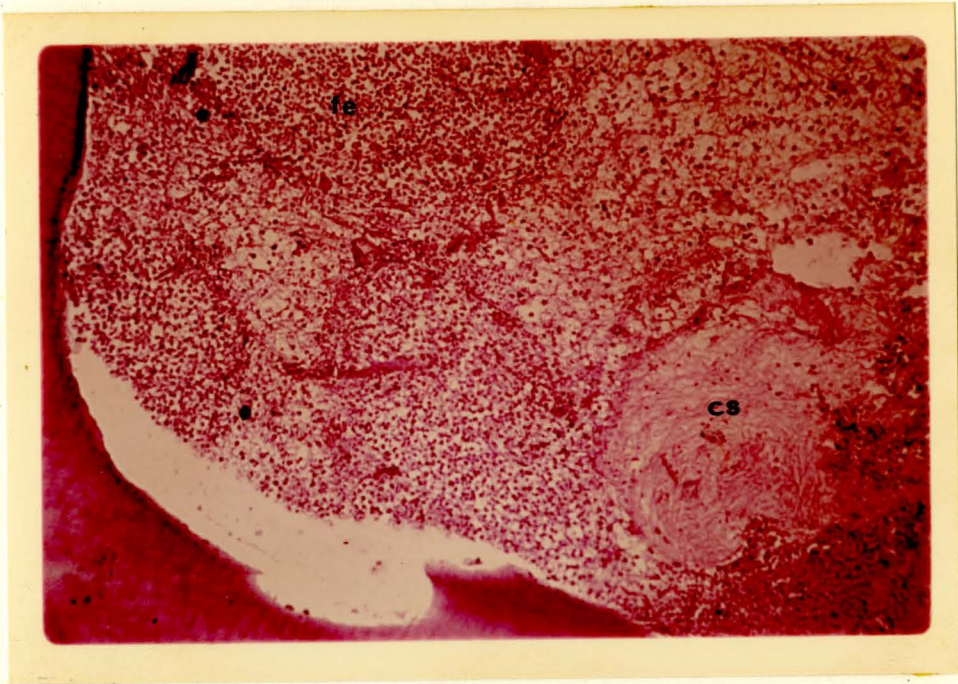


Figure 20. 3 day chondroitin sulfate implant, 40x. Note the dental defect is filled with a fibrinopurulent exudate (fe). The chondroitin sulfate (cs) implant is seen as an amorphous eosinophilic mass.

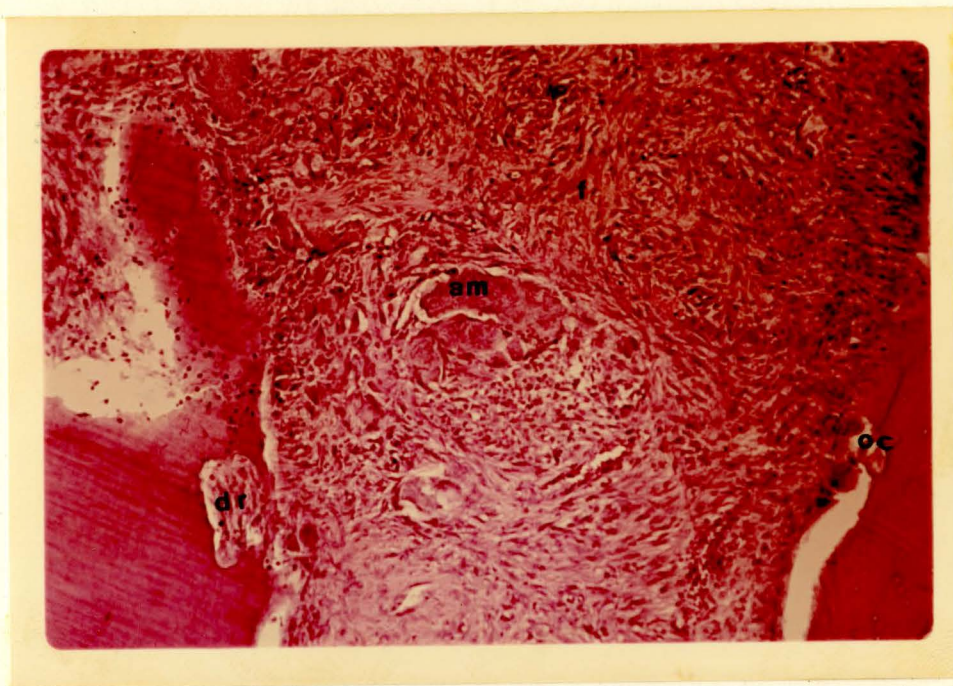


Figure 21. 7 day chondroitin sulfate implant, 40x. Note the amorphous material (am) which could be fragments of bone and/or cementum and a general fibroplasia (f) in the defect area. Osteoclastic (oc) activity is seen; dentin is also being resorbed (dr).

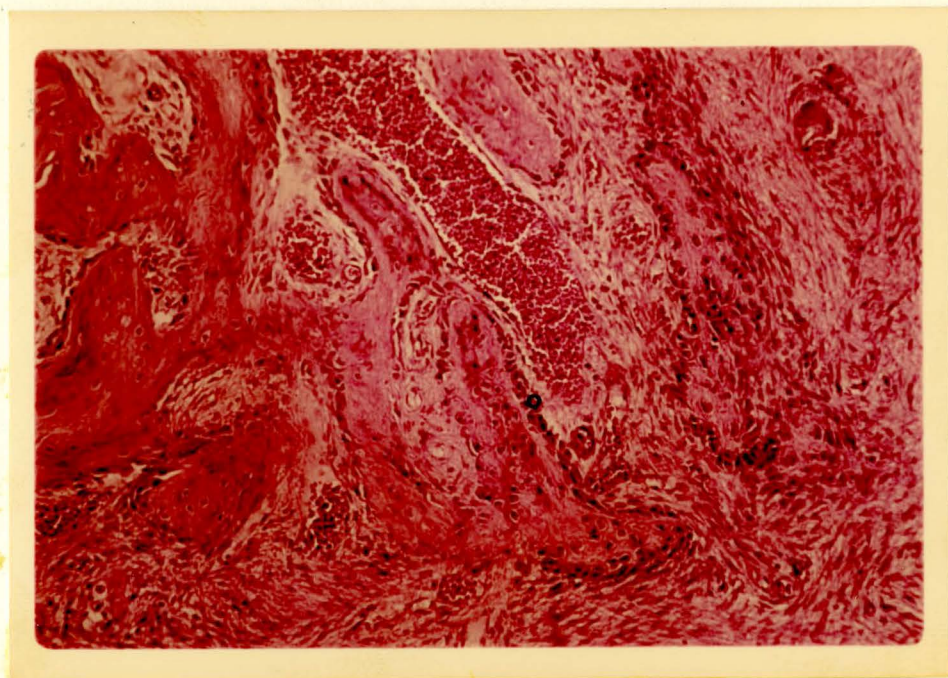


Figure 22. 14 day chondroitin sulfate implant,  
40x. Note the active osteoblastic (o) activity  
seen.

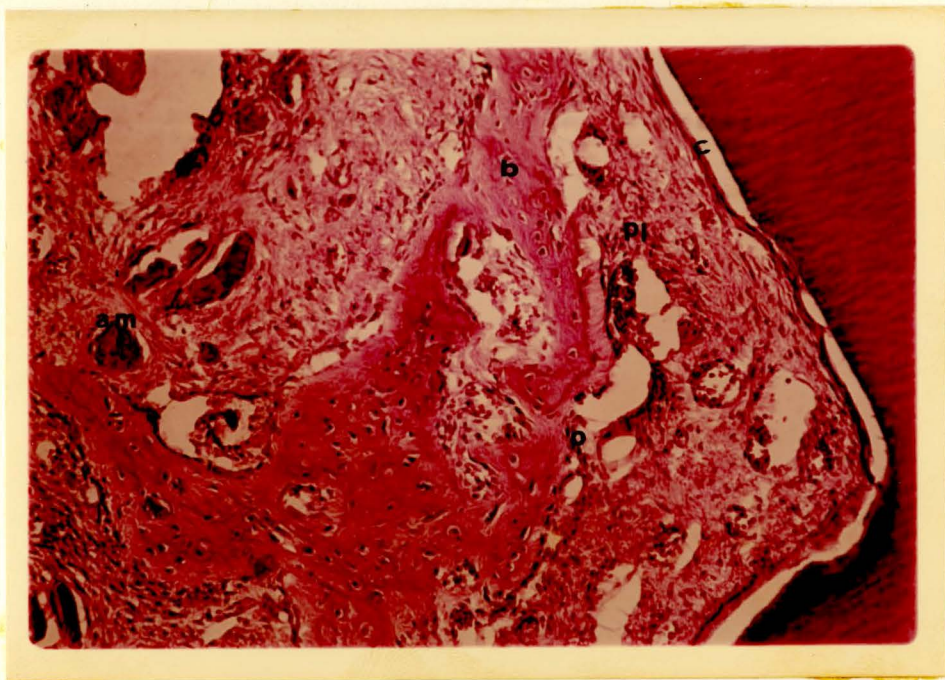


Figure 23. 21 day chondroitin sulfate implant, 40x. Note the new bone (b), with osteoblastic (o) activity, extending into the dental defect. There is a layer of cementum (c) artifactually separated from the dentin. A new periodontal ligament (pl) is seen attached to the new bone and cementum. Amorphous material (am) is seen which may be fragments of bone and/or cementum.



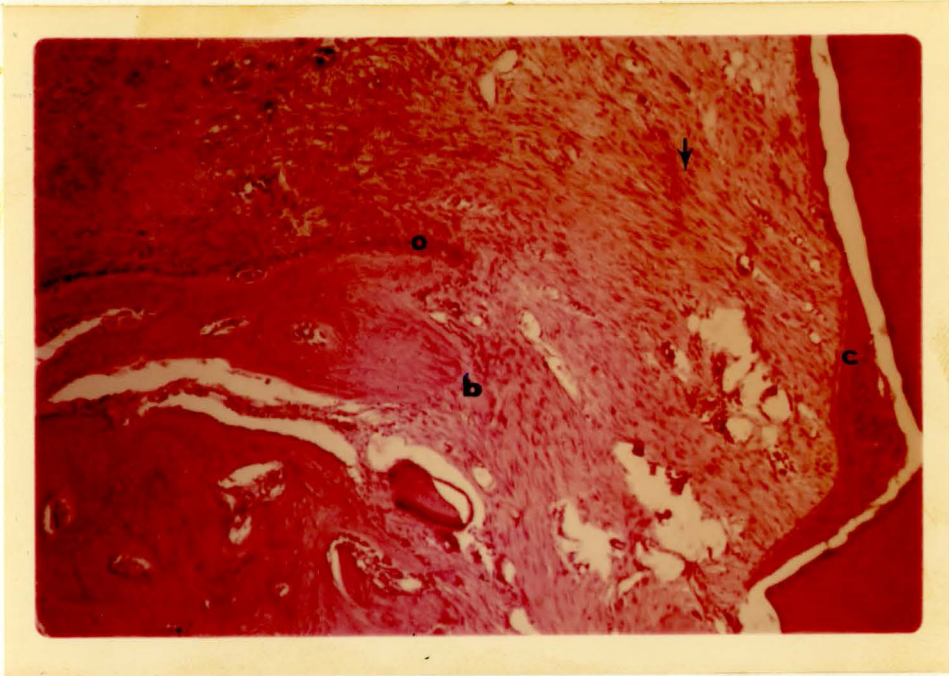


Figure 24. 28 day chondroitin sulfate implant, 25x. Note the defect which is filled with young collagenous fibers (arrow). These fibers extend and connect to the newly formed cementum (c) and bone (b) and a thick layer of osteoblastic (o) activity is seen.

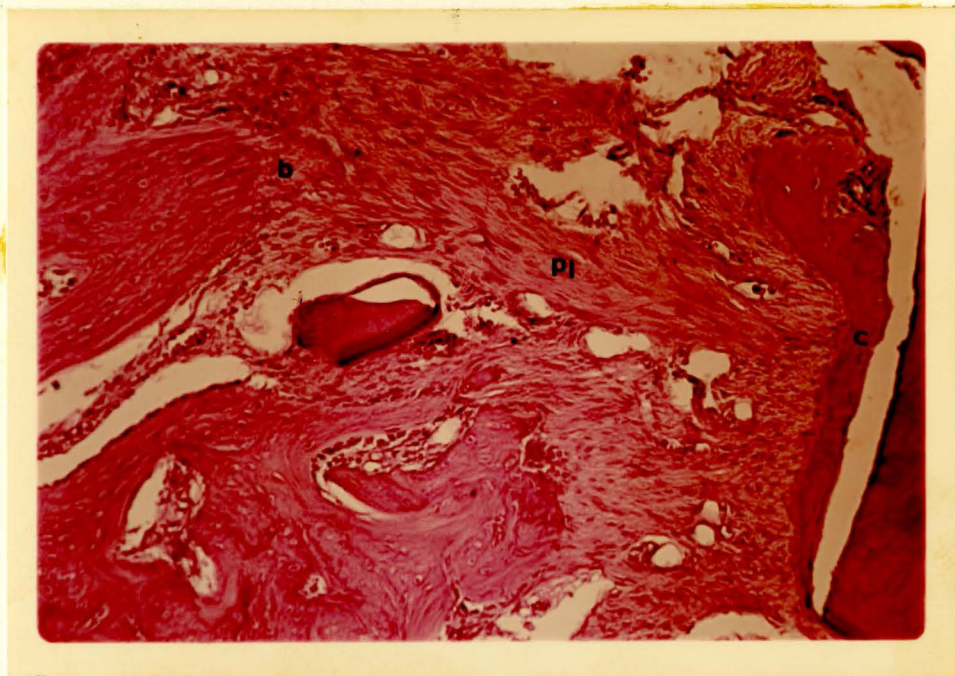


Figure 25. 28 day chondroitin sulfate implant, 40x. Note the dense newly formed periodontal ligament (pl). Newly formed bone (b) and cementum (c) is also seen.

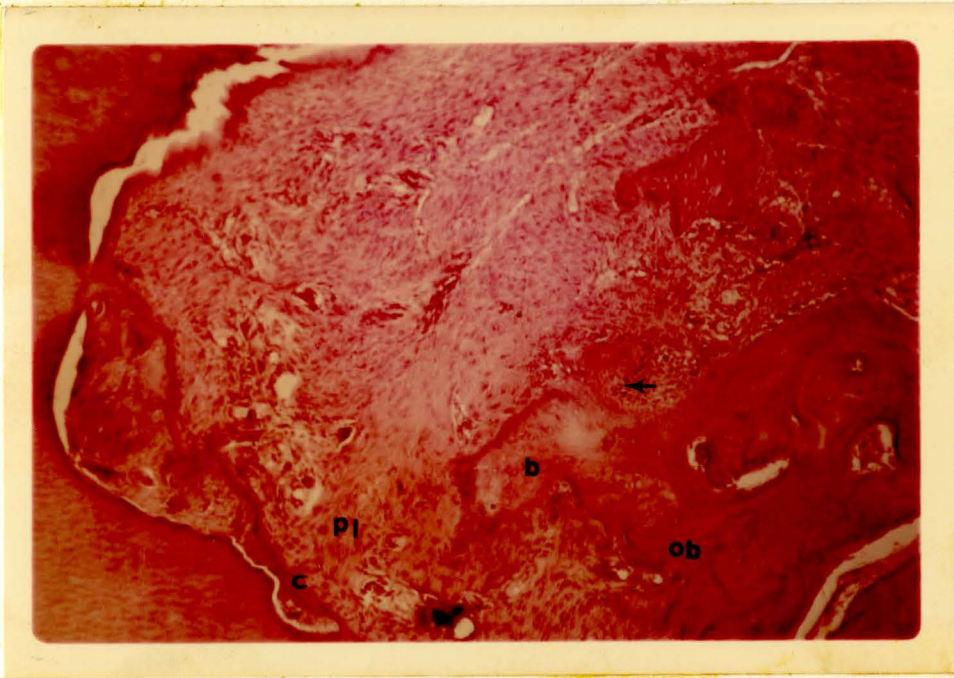


Figure 26. 42 day chondroitin sulfate implant, 25x. Note the newly formed bone (b) apposed on old bone (ob) with active osteoblastic activity (arrow). New cementum (c) is also seen. The periodontal ligament (pl) is seen between, and attached to, the new bone and cementum.

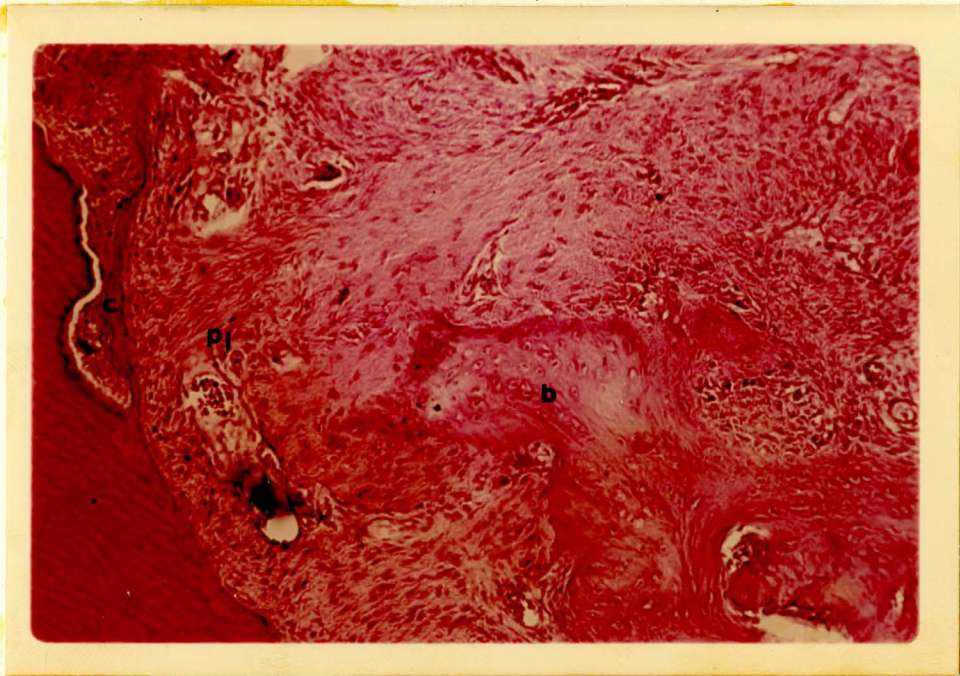


Figure 27. 42 day chondroitin sulfate implant,  
40x. Note the new bone (b), cementum (c) and the  
periodontal ligament (pl).



Figure 28. 56 day chondroitin sulfate implant, 40x. Note the newly formed cementum (c) which is artifactually separated from the dentin. There is new bone (b) formation with many osteoblasts (arrow). There is an islet of newly formed bone (ib) within the mature periodontal ligament (pl).

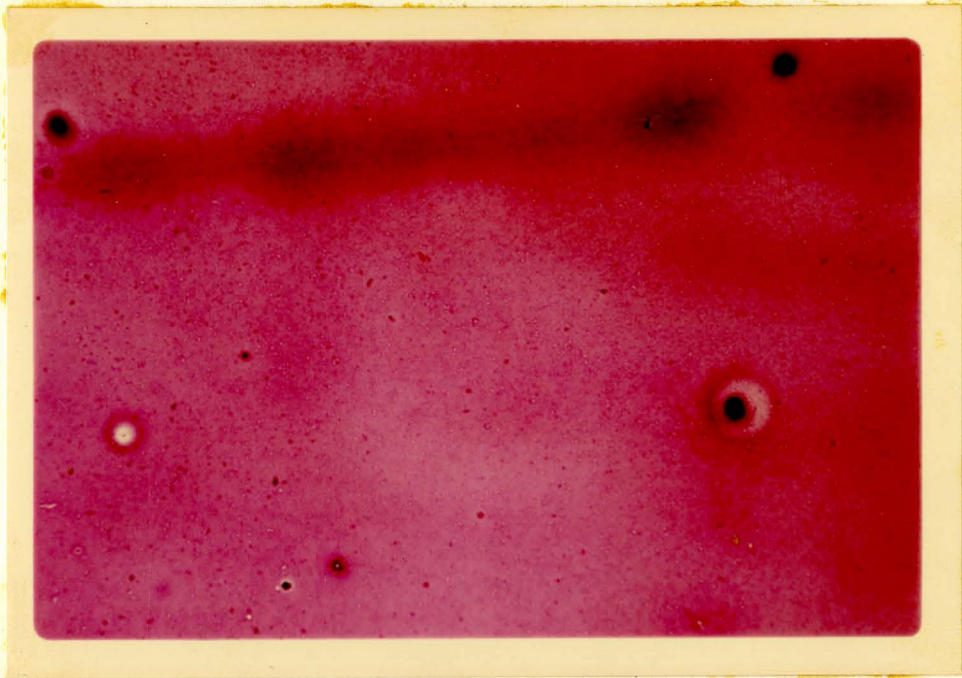


Figure 29. 0 day chondroitin sulfate smear, 25x.  
A smear of chondroitin sulfate stained with  
aldehyde fuchsin. The stain is positive for  
chondroitin sulfate.

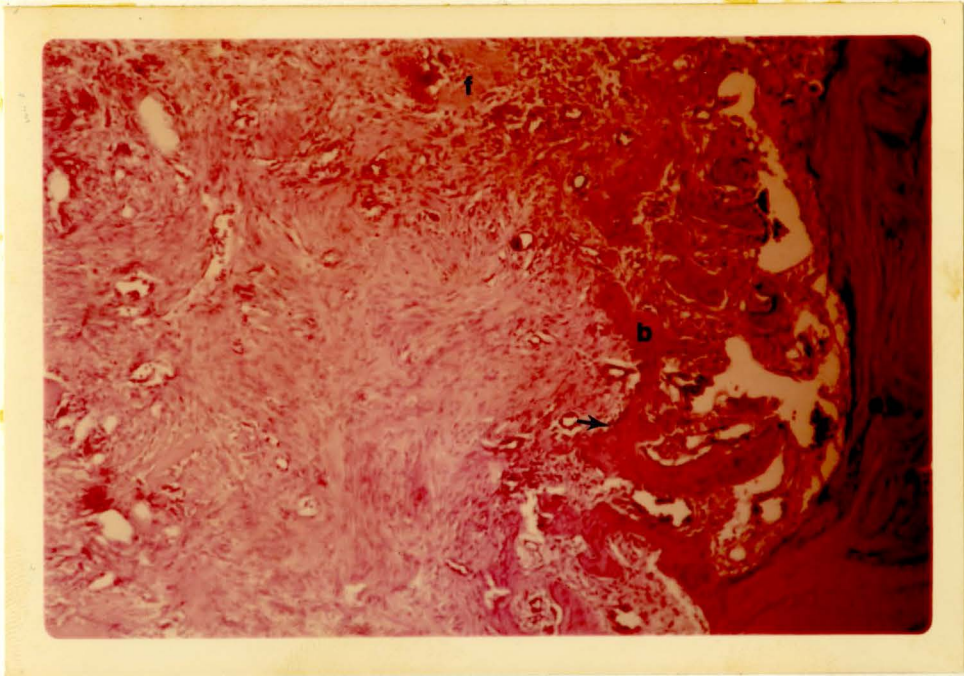


Figure 30. 7 day collagen implant, 25x. Note the old bone (ob) and the new bone (b) formation and osteoblastic activity (arrow). Fragments of bone and/or cementum (f) are also seen.

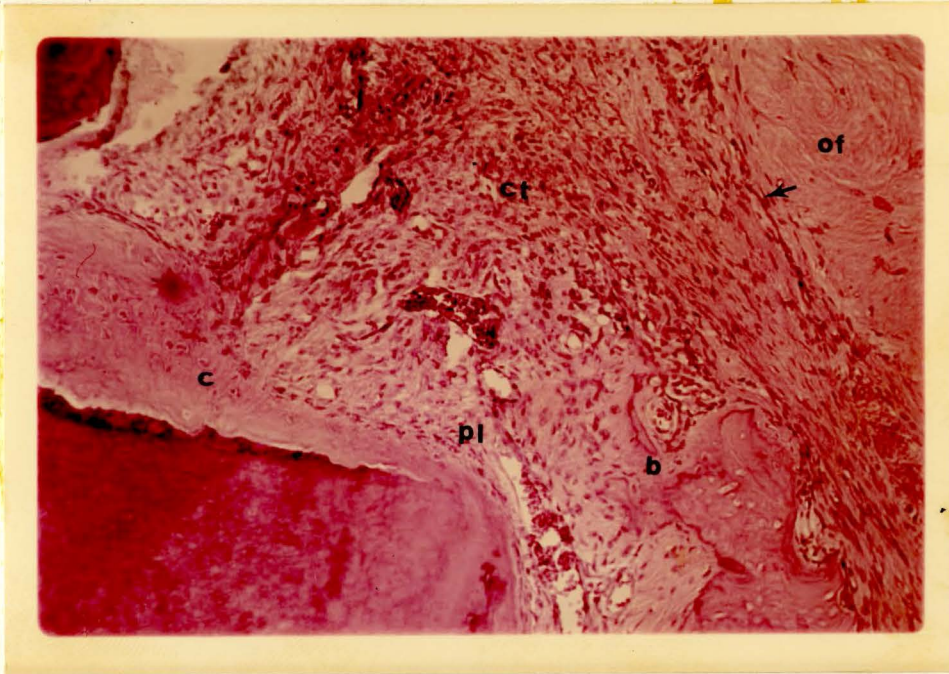


Figure 31. 21 day collagen implant, 25x. Note the junction (arrow) between the old collagenous fibers (of) and the new connective tissue (ct) in the defect. New bone (b) and a thick new cementum (c) is seen. Fibroplasia with many dilated capillaries is seen. There is a new periodontal ligament (pl) extending from the new bone to the new cementum.



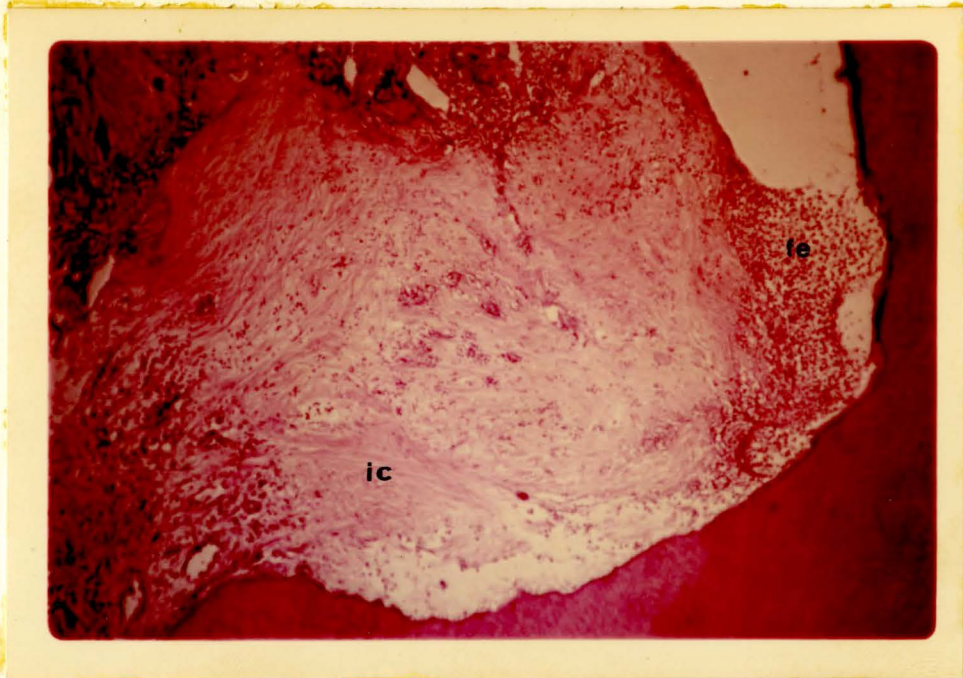


Figure 32. 3 day collagen implant, 25x. Note the dentinal notch is free of cementum. There is a slight fibrinopurulent exudate (fe) and scattered particles of implanted collagen (ic).

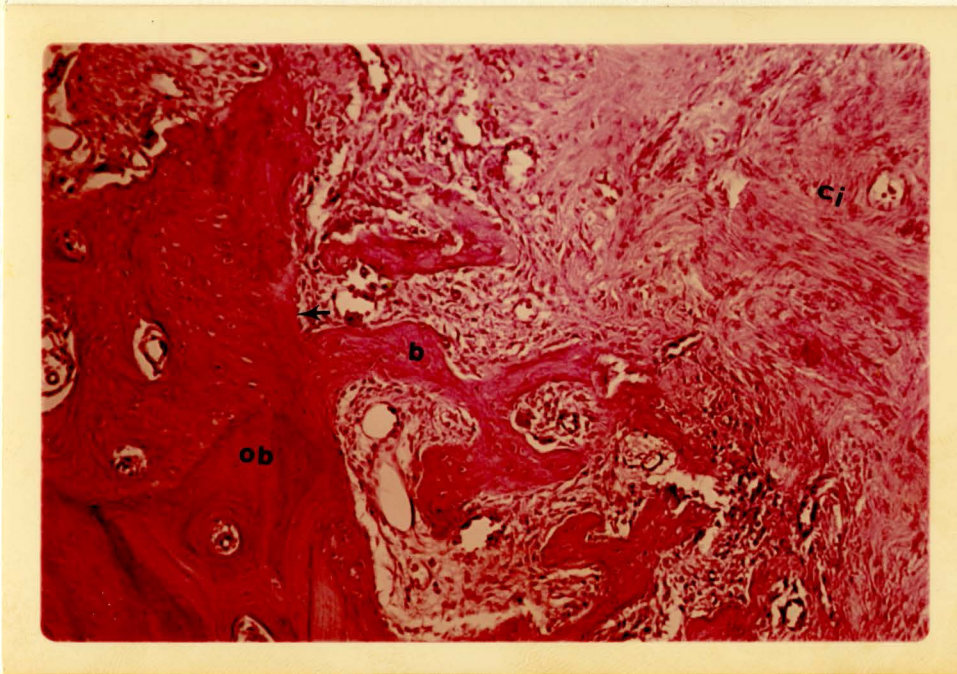


Figure 33. 7 day collagen implant, 40x. Note the new bone (b) with osteoblastic activity (arrow) is being apposed on old bone (ob). Some residual collagen implant (ci) is seen.

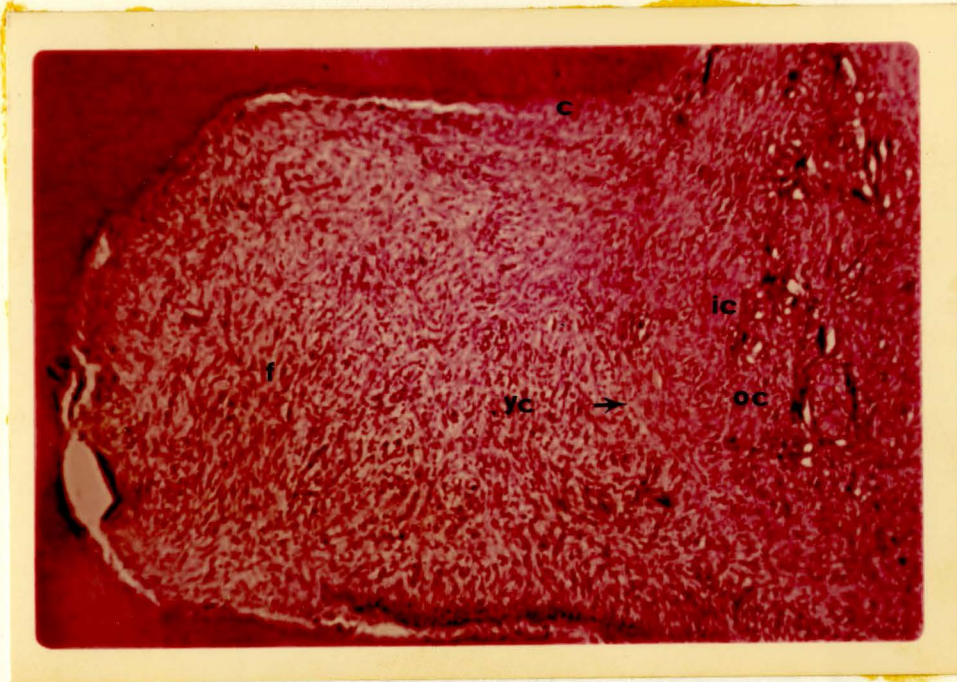


Figure 34. 14 day collagen implant, 25x. Note the young fibrogenesis (f) in the dental defect. The junction (arrow) between the young connective (yc) tissue and the older connective (oc) tissue. At this junction are seen residual fragments of implanted collagen (ic). Irregular areas of new cementum (c) are seen.



Figure 35. 28 day collagen implant, 40x. Note the new bone (b) being apposed on old lamellated bone (ob). Newly formed cementum (c) is seen on the old cementum (oc). Note also the new periodontal ligament (pl) between and attached to the new bone and new cementum.

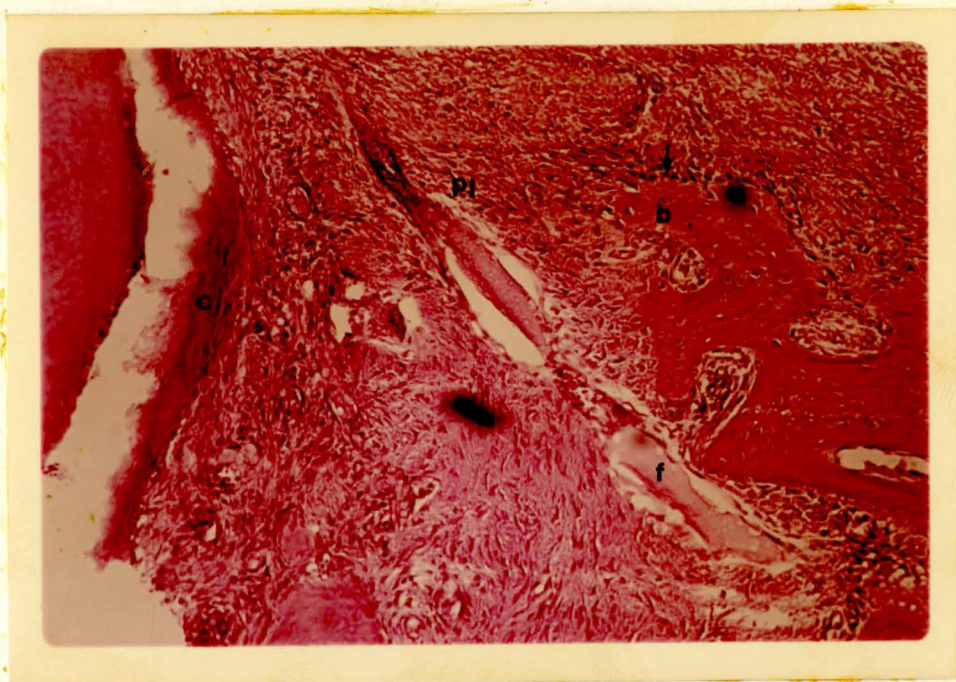


Figure 36. 42 day collagen implant, 40x. Note the newly formed cementum (c) artifactually separated from the dentin. Newly formed bone (b) and osteoblastic (arrow) activity is also seen. Note the new periodontal ligament (pl) between the new bone and new cementum. Fragments (f) of bone and/or cementum are also seen.

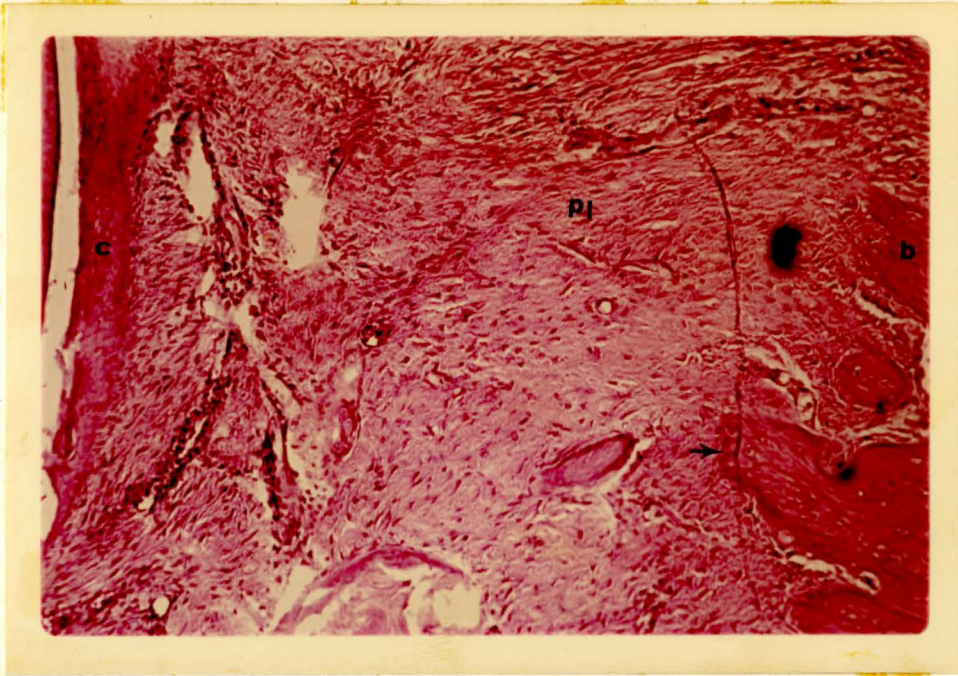


Figure 37. 56 day collagen implant, 40x. Note the thick layer of new cementum (c) and also the new bone (b) formation with osteoblastic (arrow) activity. There are mature dense collagenous fibers comprising the periodontal ligament (pl). This mature periodontal ligament is attached to the new bone and new cementum.

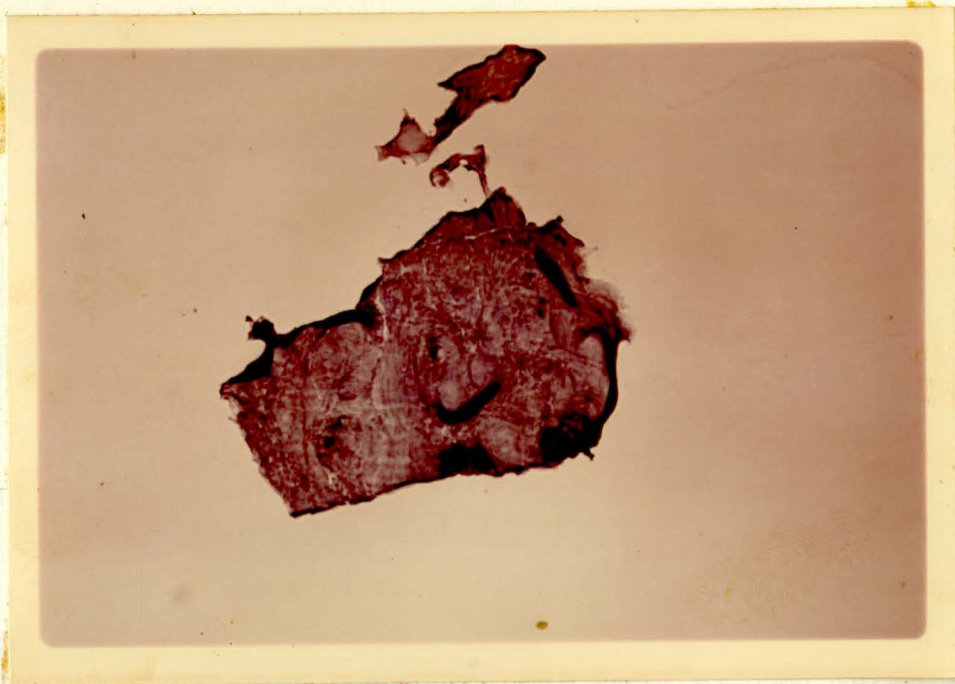


Figure 38. 0 day collagen implant, 25x. Note the particle of purified antigeneically treated collagen. This ten micron section was stained with Mallory's stain. The stain is positive for collagen.

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APPENDIX

GRAFT TERMINOLOGY

<u>Old</u>	<u>New</u>	<u>Definition</u>
Autograft	Autograft	Same Individual
Isograft	Synograft	Identical Twins
Homograft	Allograft	Within Same Species
Heterograft	Xenograft	Different Species

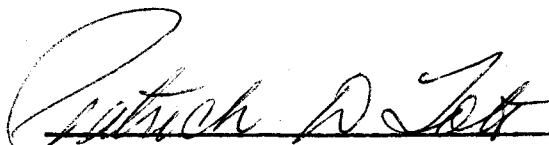
APPROVAL SHEET

This thesis, submitted by Raymond A. Skinner, has been read and approved by three members of the faculty of the Department of Oral Biology.

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.

5-7-75  
Date

  
Signature of Advisor