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A BIODEGRADABLE AND PROTEOLIPID BONE REPAIR COMPOSITE
(U) ARMY INST OF DENTAL RESEARCH WASHINGTON DC
J O HOLLINGER 10 NOV 83

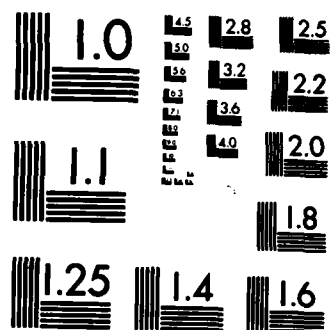
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Request approval for the submission to the *Medical Device and Diagnostic Industry* of the enclosed manuscript entitled "A Biodegradable and Proteolipid Bone Repair Composite" by Jeffrey O. Hollinger.

1 Incl
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JEFFREY O. HOLLINGER
LTC, DC
Chief, Physiology and Biochemistry Branch

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TO Chief, Physiology/Biochemistry FROM Cdr, USAIDR DATE 15 November 1983 CMT 2

Request for publication of manuscript has been approved.

Thomas P. Sweeney
THOMAS P. SWEENEY
COL, DC
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A Biodegradable And Proteolipid Bone Repair Composite

INTRODUCTION:

Many different types of materials have been used for the purpose of repairing, replacing, or augmenting bone. Autografts are the favored modality of treatment used by orthopedic and maxillofacial surgeons.¹ Unfortunately, there are numerous disadvantages associated with autogenous grafting, such as unpredictable results, failure rates ranging from 13% to 30%, inability to recover sufficient autogenous bone, technical inconvenience, and trauma to the patient as a consequence of a second surgical site.^{2,3} Allografts and alloimplants also are plagued with many problems.^{4,5} Derivatives of bone, such as demineralized bone and collagen gels have been employed with mixed results. The use of ceramics for bone repair or augmentation has been described extensively.^{6,7} This class of agents has a limited orthopedic utility. Ceramics may be useful for the treatment of some types of alveolar bone loss in periodontal disease. Biopolymers known as poly- α -hydroxy acids (a class of polyesters) have garnered considerable attention in the medical and dental fields in the past ten to fifteen years. The poly- α -hydroxy acids known as polyglycolic acid (PGA) and polylactic acid (PLA) were initially formulated and described as biodegradable suture materials.^{8,9,10} They are commercially available as Dexon[®] and Vicryl[®]. Different formulations of the PLA and PGA also have been used experimentally for osseous repair and reconstructive procedures.^{11,12,13,14,15,16}

Considerable attention has been focused on the type II matrix vesicle for initiating calcification.^{17,18,19} Intensive investigation of the structure of the matrix vesicle has revealed that its trilaminar membrane possesses a high content of acidic phospholipid.^{20,21} It has been shown that a protein-acidic

phospholipid complex similar to that of the matrix vesicle can induce hydroxy apatite formation both *in vitro* and *in vivo*.^{22,23,24} The formulation of the protein-acidic phospholipid complex that was used by Hollinger²⁴ has a paste-like consistency that is unsatisfactory for most types of bony wound applications, such as when rigid fixation is required or when expansive discontinuity defects must be repaired. It was the purpose of this study, therefore, to develop a composite bone repair material that would have utilitarian application. The combination of the biodegradable polyesters of PLA and PGA with a protein-acidic phospholipid component were conceived for such a purpose.

METHODS and MATERIALS:

The experimental bone repair materials were prepared according to the flow diagrams (1, 2, 3, and 4). Bone repair agents were evaluated according to Tables I and II. All tissues were prepared for histomorphometric assessment described by Hollinger¹⁴ using an Image Analysis System (IAS) (diagram 5). The possible number of bony wound healing measurements that could be generated for this study are presented in Table III.

DATA ANALYSIS:

Data from the analyses of the same variable (i.e., bony trabecula) from the same treatment and temporal groups were combined, yielding a pooled mean value based upon 2,000 measurements. A standard deviation and a standard error of the mean were computed for each set of the 2,000 measurements for each value according to the formulae^{25,26} The pooled mean for each variable (based upon 2,000 fields) plus and minus its standard error of the mean was then used to construct a histogram. A trio of sets (i.e., A_3 , B_3 , C_3) representing treatment and temporal groups were arranged along the abscissa and the corresponding units or percentage appeared along the ordinate.

A two-way analysis of variance was used to examine and to test the effects of (1) the treatment groups (differences between the three treatment groups analyzed over the six time periods) and (2) time periods (differences between the six time periods averaged over the three treatment periods). Effects having an observed significance level (p-value) equal to or less than 5% (based on the appropriate F-test value which was derived by performing the two-way analysis of variance) were considered to be statistically significant. Differences between the pooled means of any two treatment groups were tested by partitioning the overall difference between treatment group variability, with variability being equivalent to the sum of squares. Specific comparisons were then made between treatments.

RESULTS:

Statistical and Histomorphometric:

The statistical evaluation based upon the analyses of 180,000 possible histomorphometric measurements is summarized in Table IV. Histograms I-IV represent a sample of the histograms derived from the data for each of the variables that were analyzed with the IAS.

Histological:

The overall trends in healing frequently displayed only subtle visual differences when viewed histologically. Photomicrographs of selected stages of repair can be observed in Figures 1-6.

All treatment groups displayed the typical patterns of osseous wound healing. There was often equivocal evidence that histologically evaluated wound healing might have been superior for one type of treatment class than in another; however, when histomorphometric assessments and statistical analyses were performed, contrasts were extracted that frequently proved to be

significantly different.

Histological evaluation seemed to indicate that the patterns of healing consisted of more reparative elements in the copolymer-proteolipid (group A) treated sites at an earlier period in time than either the plain copolymer (group B) or control (group C) sites. Further, group B treated wounds appeared to possess more of the elements of osseous repair at the same time periods than group C treated wounds. It did not appear that the presence of the composite implant material deterred bony healing, which centripetally manifested. There was no callus formation and no evidence of an adverse inflammatory response engendered from the implants from either groups A or B.

DISCUSSION:

Materials such as bone grafts and implants, collagen gels, ceramics, bone derivatives, and biopolymers are some of the many agents which have been employed by orthopedic and maxillofacial surgeons for initiating osseous repair or for replacing bone. Failure to achieve beneficial results with these materials has not been necessarily a consequence of imprudence; but rather, due to deficiencies inherent to the repair and replacement agents. A combination of the biopolymers PLA and PGA in conjunction with the particular proteolipid described, appears to offer considerable promise as an alternative to the more common, conventional materials.

Hollinger²⁴ prepared a specific proteolipid for implantation into endochondral bone defects in rats. The healing response elicited by the proteolipid exceeded that of the control sites. Hollinger²⁴ suggested that the material established a unique chemical environment conducive to calcium and phosphate precipitation, nucleation, and subsequent crystal growth. Moreover, the implication was that the locally introduced proteolipid was tantamount to surrogate

extracellular matrix vesicles, the structure whose limiting membrane is heavily endowed with an acidic phospholipid component. Their extremely critical function in the calcification scheme has been described at length.^{27,19,28}

The breakdown of the biopolymers of PLA and PGA occurs by nonspecific hydrolytic scission that results in the generation of lactic acid and glycolic acid residues.^{29,30,31} The lactic acid becomes incorporated in the TCA cycle and is excreted by the lungs as CO_2 . Glycolic acid dimers, trimers, etc., are enzymatically degraded by esterases and carboxy peptidases³² and are converted to monomers of glycolic acid which either can be excreted in the urine or enzymatically converted by glycolate oxidase to glyoxylate.²⁹ This moiety reacts with glycine transaminase and the glycine that is produced can be used for synthesis of serine, which can be employed in the TCA cycle after transformation into pyruvate.^{33,34}

Hollinger³⁵ has speculated that the positive bone healing response engendered in experimental animals from the copolymer of PLA and PGA may be a consequence of several factors. The linear polyester macromolecular structure could act as a matrix, trellis, or foundation upon which bony reparative elements may be consolidated. Further, a possible consequence of the degradation of the copolymer could be that the pH of the local environment was altered and the potential inhibitors to calcification (proteoglycans, glycosaminoglycans) were debilitated and rendered ineffectual. The pH changes and the organic monomeric acid residues interaction with host organic matrix could function as a mechanism engendering release from the matrix of certain polypeptides, such as bone morphogenetic protein and human skeletal growth factor.^{36,37} These factors have been speculated as being agents capable of increasing both osteoblast progenitor cell proliferation and subsequent bone formation rate.

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TABLE I

Organization of Treatment and Temporal Groups

Treatment group	A	B	C
(Each treatment group had 10 animals per temporal group)	(50:50 PLA: PGA-DPI-L)	50:50 PLA: PGA -	Control
<u>Temporal group</u>			
(Number of days post-treatment that animals were sacrificed)			
3	10	10	10
7	10	10	10
14	10	10	10
21	10	10	10
28	10	10	10
42	10	10	10
Total number of animals per treatment group	60	60	60

PLA:PGA = Polylactic acid: Polyglycolic acid

DPI-L = Diphosphoinositide - Lysozyme

TABLE II

Organization of Data for the Variable Trabecular Diameter (D-TRAB)
from Treatment Group A, Temporal Group 3 Days

<u>Number of animals</u>	<u>Pooled number of fields per animal</u>
10	200
<u>Total number of fields/set</u>	<u>Possible number (range) of variables</u>
2,000	1-9
Trabecular diameter in μm	
2,000 measurements of D-TRAB per set A_3	
Computer derivation of standard deviation (A) for D-TRAB	
Result = $A_3 \cdot \text{D-TRABts}$	

Key:

- T = Temporal group (3, 7, 14, etc.) in days
- Tx = Treatment group (A, B, C)
- Set = (Tx) (T) = $A_3, B_3, C_3, A_7, \text{etc.}$
- s = Standard deviation

TABLE III

Example of Possible Number of Variables
from Treatment Groups A, B, and C

Temporal groups (T) in days	3	7	14	21	28	42
Pooled number of fields	200	200	200	200	200	200
Number of animals per T	10	10	10	10	10	10
Total number of fields	2,000	2,000	2,000	2,000	2,000	2,000
						Grand total
						12,000

Explanation:

1. There were ten animals per temporal group (T).
2. There were 200 fields measured per animal; therefore, 2,000 fields were measured per T ($200 \times 10 = 2,000$).
3. The possible range of derived variables was from one to nine with a mean of five; therefore, 10,000 pieces of information could be computed per T ($2,000 \times 5 = 10,000$).
4. The term set can be defined as a treatment group (Tx) at a particular time (that is, a T of 3, 7, 14, 21, 28, or 42).
5. A grand total (average) of 180,000 measured values could, therefore, be derived ($10,000 \times 18 = 180,000$).

TABLE IV

Results of the Two-Way Analysis of Variance of Sum of Squares

Variable ▲	<u>Time derived values</u>		<u>Sum of squares</u>		<u>Treatment derived values</u>	
	F-test	p	Treatment	Time	F-test	p
VV	65.08 ●		24,782	192,511	21.32 ●	
Treatment ▲ ▲						
<u>contrasts</u>						
A vs. B		**				**
A vs. C		**				**
B vs. C		**				**
D-TRAB	179.93 ●		2,322	38,603	27.02 ●	
A vs. B		**				**
A vs. C		**				**
B vs. C		**				**
TH-OS	127.79 ●		205	1,958	33.34 ●	
A vs. B		**				*
A vs. C		**				**
B vs. C		**				**
VV-OS	85.03 ●		10.2	205.7	10.21 ●	
A vs. B		**				**
A vs. C		**				**
A vs. C		**				*

TABLE IV (Con't.)

Results of the Two-Way Analysis of Variance of Sum of Squares

<u>Variable</u> ▲	<u>Time derived values</u>		<u>Sum of squares</u>		<u>Treatment derived values</u>	
	F-test	p	Treatment	Time	F-test	p
OBI	53.99 ●●		5,706	123,364	6.85 ●	
Treatment ▲ ▲						
<u>contrasts</u>						
A vs. B		**				**
A vs. C		**				**
B vs. C		**				*
OB%	53.99 ●●		157	4,480	6.85 ●	
A vs. B		**				**
A vs. C		**				**
B vs. C		**				*
OCI	7.55 ●●		6.8	1,857.3	2.43 ●	
A vs. B		**				
A vs. C		**				
B vs. C		**				
L-TOT%	247.51 ●●		0.51	280.22	1.02 ●	
A vs. B		**				
A vs. C		**				
B vs. C		**				

TABLE IV (Cont.)

Results of the Two-Way Analysis of Variance of Sum of Squares

<u>Variable</u> ▲	<u>Time derived values</u>		<u>Sum of Squares</u>		<u>Treatment derived values</u>	
	F-test	p	Treatment	Time	F-test	p
VV-MAR	495.59 ●●		9,815	512,756	23.73 ●	
<u>Treatment contrasts</u> ▲ ▲						
A vs. B		**				**
A vs. C		**				**
B vs. C		*				**

Key: ▲ = Variables described in section III. J. ▲▲ A = Copolymer plus proteolipid

** = $p < 0.01$

B = Copolymer

* = $p < 0.05$

C = Control

●● = F confidence level 99%

● = F confidence level 95%

(VV) = Volumetric density of bone

(OCI) = Osteoclast index

(D-TRAB) = Mean trabecular diameter

(L-TOT%) = Fraction of trabecular surface exhibiting resorptive lacunae

(TH-OS) = Mean width of osteoid

(VV-MAR) = Volumetric density of marrow

(VV-OS) = Volumetric density of osteoid

(OBI) = Osteoblast index

(OB%) = Fraction of total trabecular surface covered by osteoid



Fig. 1. Copolymer-proteolipid (Group A) treated wound site at three days. Bone trabeculae (*) hapazardly oriented and rimmed with osteoid and osteoblasts.

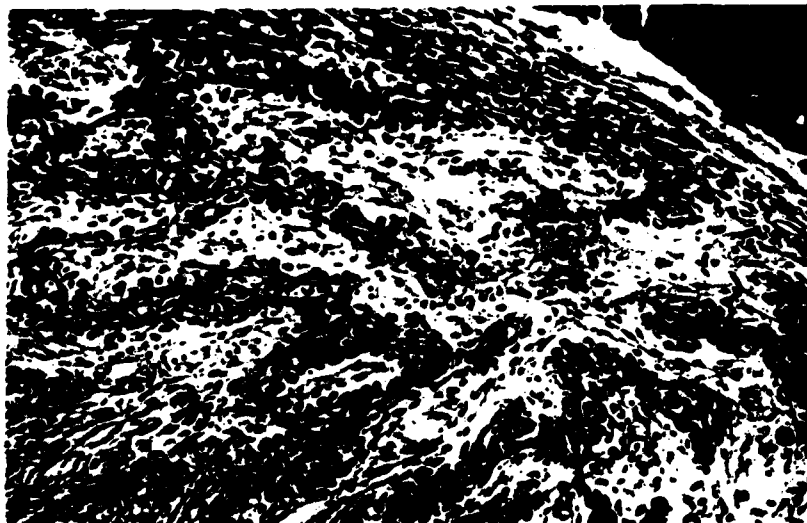


Fig. 2. Copolymer (Group B) treated wound site at three days. Area where osteoid being deposited and less calcified materials present than for Group A.

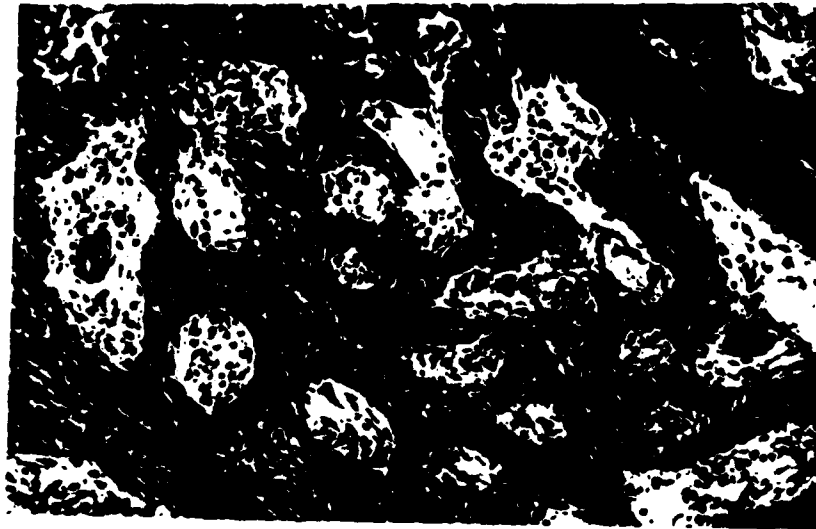


Fig. 3. Control (Group C) wound site at three days. Swirling pattern of fibrous connective tissue with numerous fibro blasts and occasional osteoid and bony trabeculae formation.

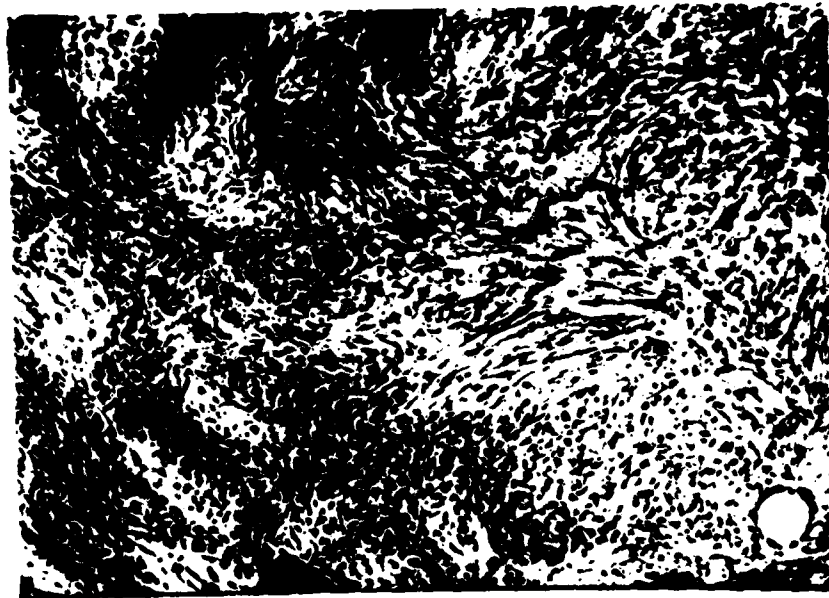


Fig. 4. Group A twenty-eight days with numerous trabeculae that are coalescing.

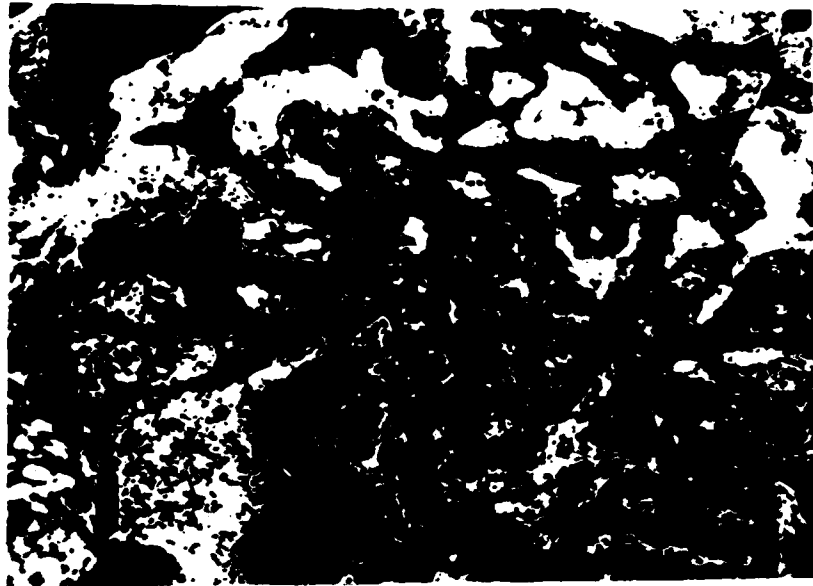


Fig. 5. Group B twenty-eight days demonstrating an area of active repair adjacent to the intact cortex.

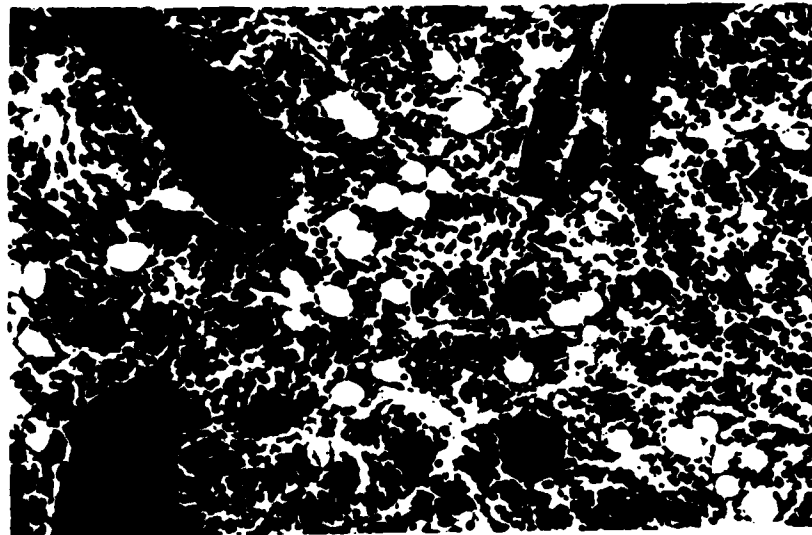
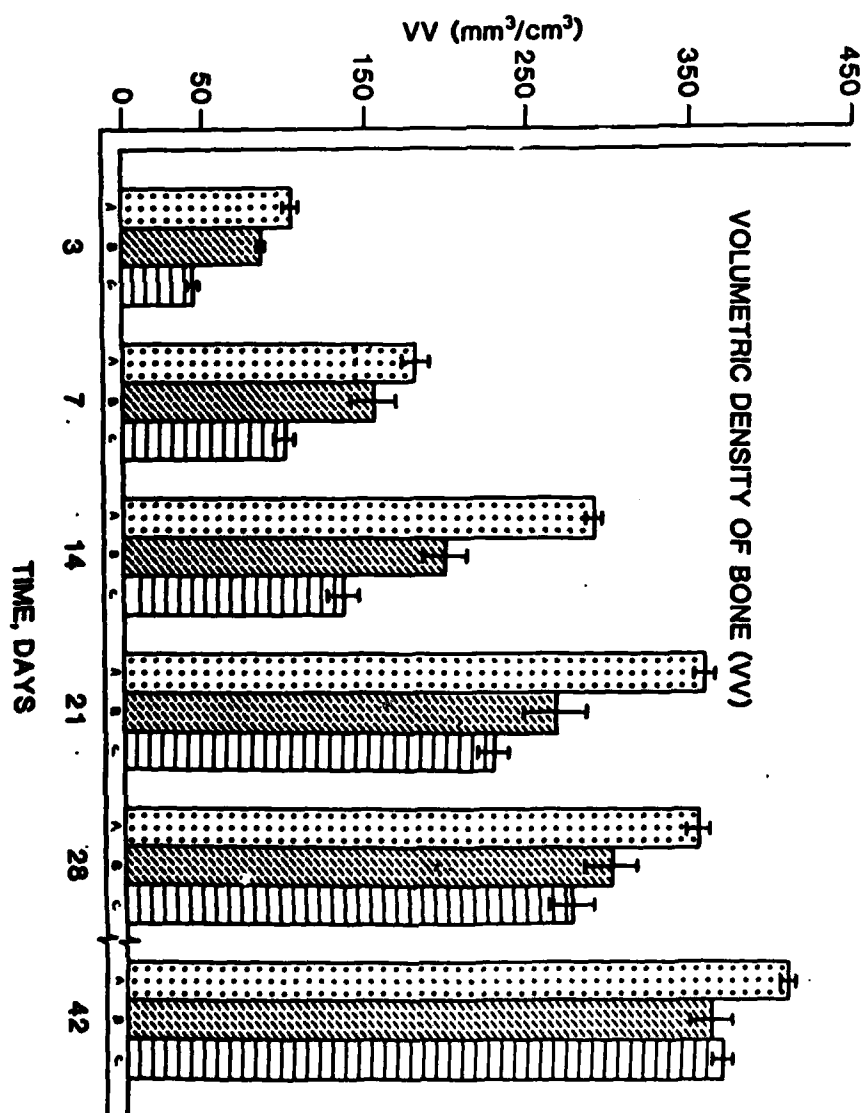
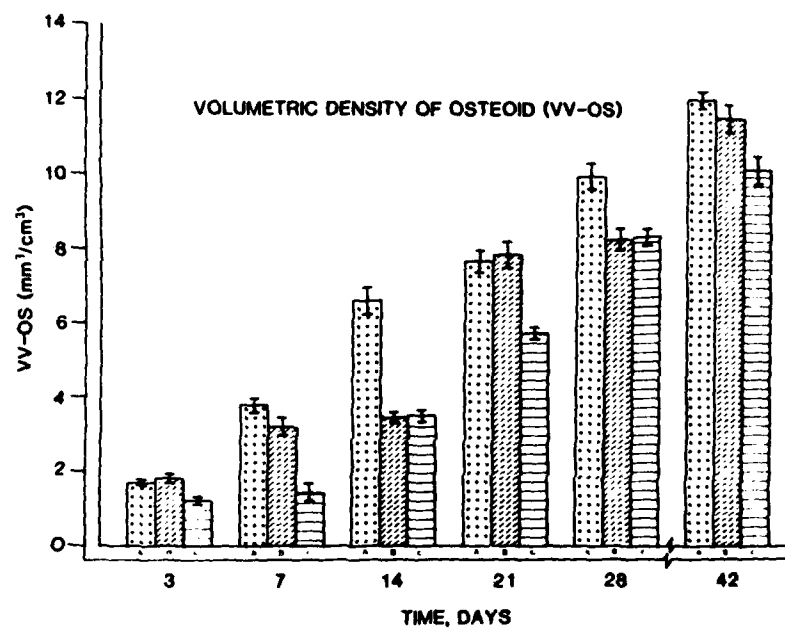


Fig. 6. Group C at twenty-eight days displaying normal elements of wound repair such as trabeculae osteoid, and osteoblasts.

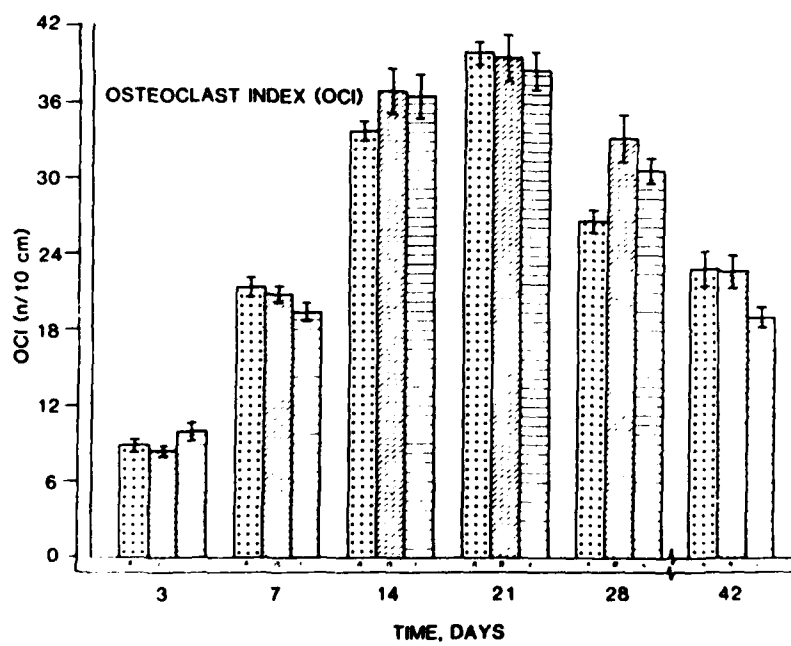
Histogram 1
Volumetric Density of Bone



HISTOGRAM II



HISTOGRAM III



Histogram IV
Volumetric Density of Marrow

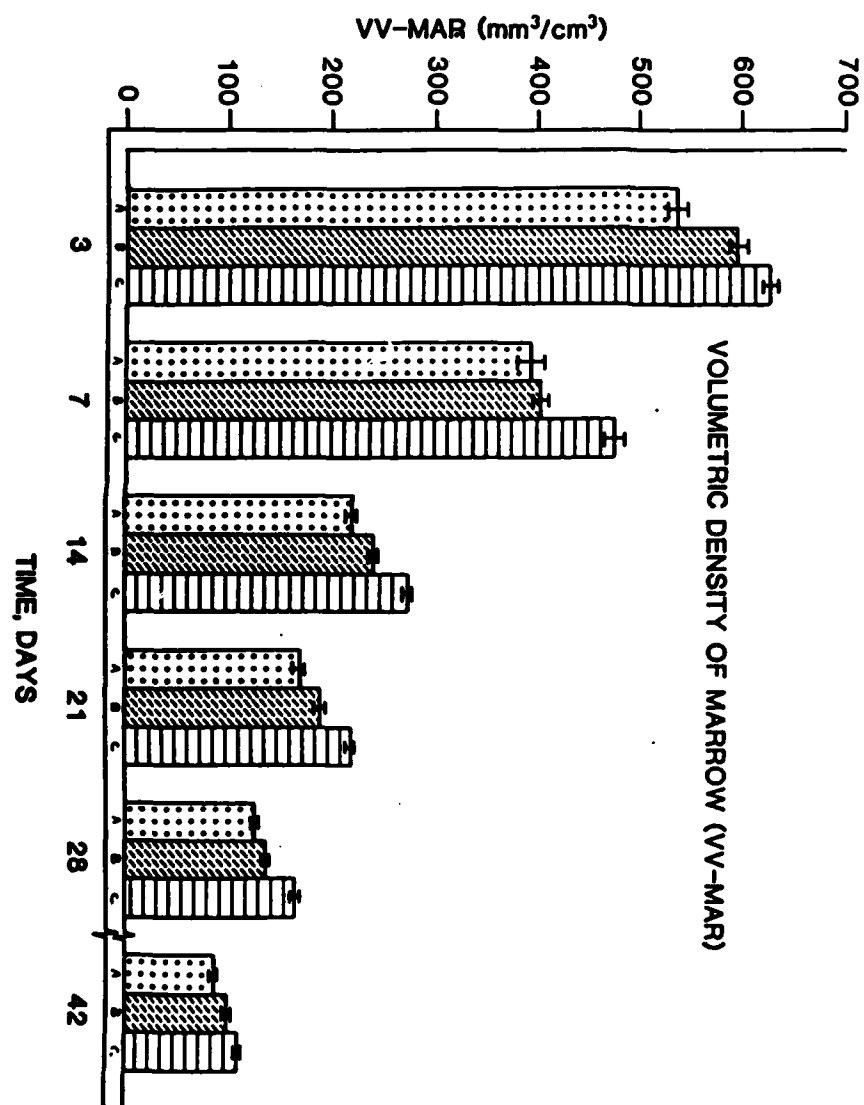
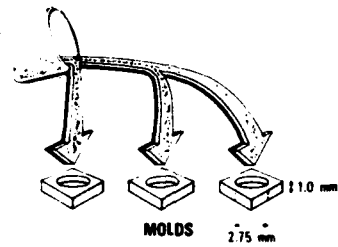


DIAGRAM I

(FABRICATION - CONTINUED)

SOUP POURED INTO TEFLON[®] MOLD.



MOLDS PLACED INTO LYPHOLYZER
24 HOURS X AMBIENT TEMPERATURE.

MOLDS PLACED IN
GLASS CYLINDER.



LYPHOLYZER

COPOLYMER IMPLANTS RETRIEVED
AND STORED IN DESICCATOR.



DESICCATOR VACUUM PUMP

DIAGRAM II

FABRICATION OF COPOLYMER IMPLANT

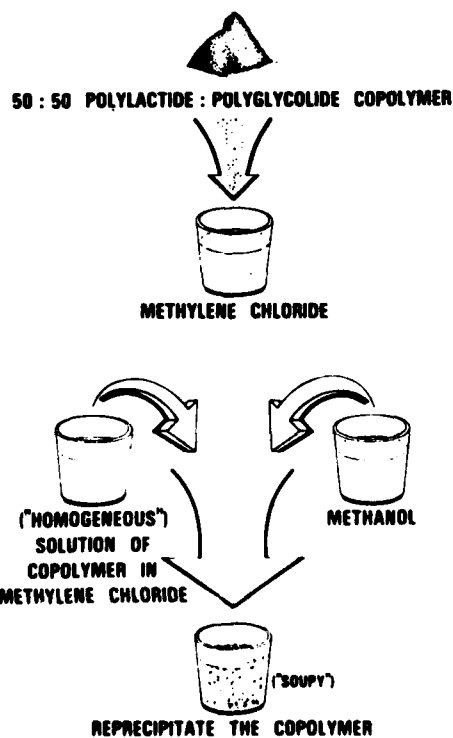


DIAGRAM III

IMPLANT PREPARATION

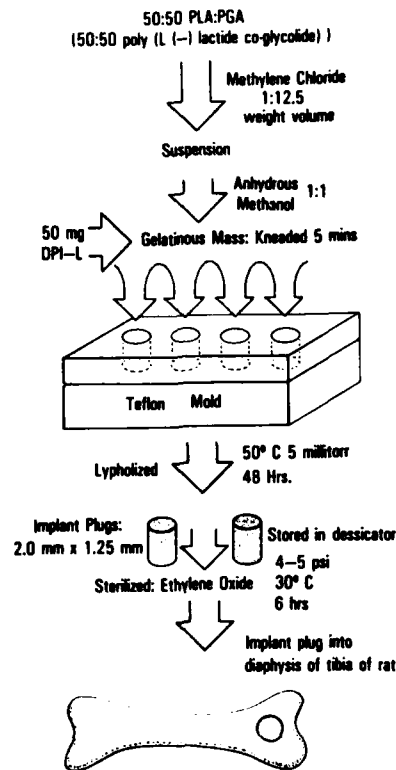


DIAGRAM IV

IMPLANT PREPARATION

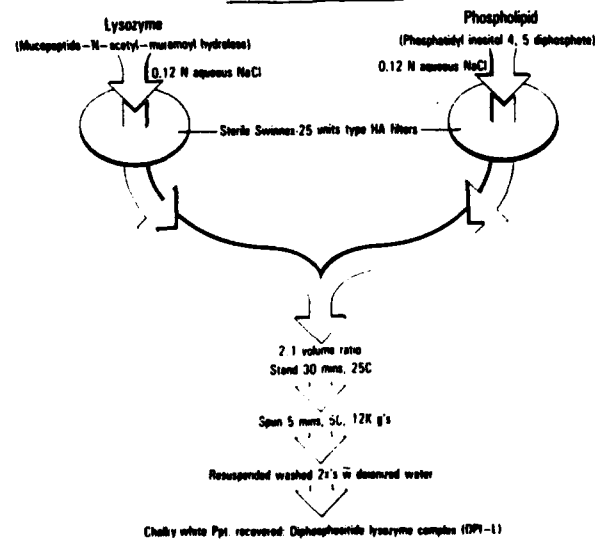
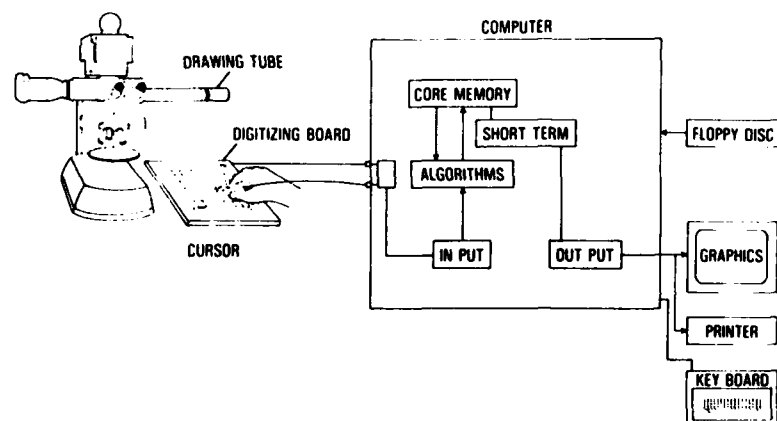


DIAGRAM V



CELLULAR STRUCTURE MAY BE TRACED ONTO A DIGITIZING TABLET WITH A LIGHT CURSOR WHILE SIMULTANEOUSLY OBSERVING THE TISSUE SPECIMEN THROUGH THE MICROSCOPE.

END

FILMED

DANIC