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The Mechanical Properties of Bone Allografts and
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Joia Stapleton Mukherjee

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THE EFFECTS OF STERILIZATION AND STORAGE PROCEDURES
ON THE MECHANICAL PROPERTIES OF BONE ALLOGRAFTS

By

Joia Stapleton Mukherjee

A THESIS

Submitted to
Michigan State University
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ABSTRACT

THE EFFECTS OF STERILIZATION AND STORAGE PROCEDURES ON THE MECHANICAL PROPERTIES OF BONE ALLOGRAFTS

By

Joia Stapleton Mukherjee

The purpose of this research was to study the effect of freeze-drying and gamma irradiation, used for storage and sterilization, on the mechanical properties of bone allografts. The particular grafts used in this study were Cloward plug cancellous bone allografts used in the fusion of cervical vertebral bodies. The cylindrical grafts were subjected to diametrical compression and load deflection curves were generated. The parameters of stiffness and peak load were obtained from the linear elastic portion and the break over point of the load deflection curves. The failure mode of the grafts was also recorded as either plastic or brittle failure.

Rehydration of the freeze-dried grafts was essentially complete after two hours of rehydration when the grafts assumed a plastic mode of failure. Prior to two hours of rehydration all grafts failed in the brittle mode. Irradiation of the freeze-dried grafts tended to increase both the stiffness and the incidence of brittle failure.

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I. Introduction

Bone, unlike tissue of the body's organs, is an ideal transplant material. It does not undergo the close scrutiny of the body's immune system as does soft tissue because the immunogens in bone can be destroyed without destroying the material. When transplanted, a bone graft is eventually replaced by new bone material for which the implant has laid the framework. Transplant bone is used mainly for reconstruction and joint fusion. When a joint is fused surgically, the surgeon will introduce a piece of cancellous bone, a bone graft, between the two links of a joint and stabilize the area. The transplanted bone graft then provides a matrix for new bone to be deposited and the joint will eventually fuse. The incorporation of the transplanted bone, however, may take up to six months (41). During that time it is necessary for the graft to function as an autonomous unit in adequately bearing the physiological loads placed on it. Therefore, the transplantation of human bone is as much a mechanical problem as it is a physiological problem.

This research program focused on the Cloward plug (14) bone graft. One of the first standardized and widely used grafts, the Cloward plug is used in the surgical fusion of two vertebrae which is necessary when an intervertebral disk becomes degenerated by injury or disease. The plug is used specifically in anterior cervical spinal fusion. The surgical procedure for this fusion, developed by Cloward in 1958 (14), is done with the patient in a supine position. The surgeon enters the

neck region anteriorly, exposing the vertebral bodies of the spinal column in the cervical region. The degenerated disk is then removed and a hole is bored between the vertebrae while a clamp maintains the disk space (Figure 1). A graft with the same diameter as the hole is inserted between the vertebrae and the clamps removed. After insertion, the graft is subjected to compression by vertebrae, musculature, and the weight of the head. If the disk space is compromised, impingement of the nerves which pass between the spinal cord and the peripheral nervous system results causing pain or lack of function. Thus, fracture of the graft in this area could produce neurological damage. The Cloward plug bone graft must be capable of bearing the compressive loads transmitted between the inferior and superior vertebrae and maintain the disk space over the period of incorporation of the graft into the boney matrix. Therefore, it is desirable to test typical grafts in compression to determine their load bearing capacity and their stiffness.

Currently, there are two types of bone grafts, allogenic and autogenic. Autogenic are those grafts which are taken from the healthy tissue of the patient in need of a bone graft and transplanted to the desired area of reconstruction or fusion. The problems associated with autogenous bone grafts include the destruction of healthy bone and the necessity of two surgical incisions (41). Allogenic bone grafts, harvested from tissue donors postmortem and stored in tissue banks, obviously solve these problems; however, the allogenic grafts must be sterilized and stored without damaging the integrity of the graft. Methods for preservation and sterilization of human transplant tissue are lyophilization (freeze-drying) and gamma irradiation. It is important to understand how these procedures alter the mechanical

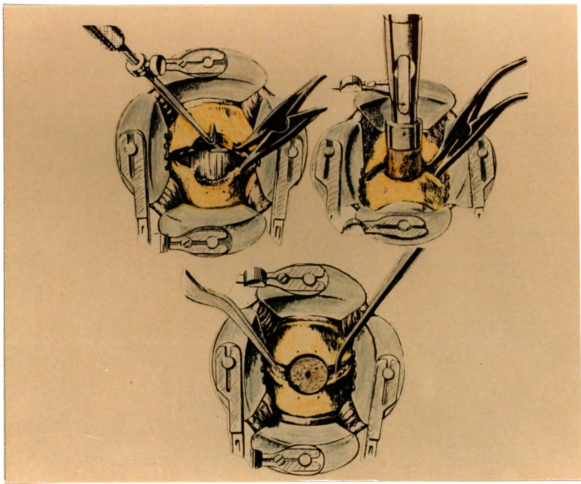


Figure 1: The implantation of a Cloward plug between two vertebral bodies in the cervical spine.

properties of bone if it is to be used successfully as a transplant material. Freeze-drying serves to preserve tissue for storage as well as prevent the growth of bacteria. Cancellous bone, however, contains 20-35% water by volume (18), therefore, change in water content would be expected to change the behavior of the tissue. Gamma irradiation is used in preserved bone grafts to kill bacteria and to denature proteins that would cause rejection of the graft by the recipient (16). However, irradiation may cause damage to the ultra - structural matrix of the bone (3). Thus, it is necessary to study the effects of both freeze-drying and gamma irradiation, common methods of preserving and sterilizing bone.

This investigation was conducted to determine the mechanical properties of freeze-dried bone grafts and compare these properties to the mechanical response of fresh bone and to examine the effects of gamma irradiation on the preserved grafts. For both sets of parameters, rehydration time and irradiation effects, the grafts were tested in compression with test grips geometrically analogous to the inferior and superior vertebrae (Figure 2).

Finally, a finite element model was used to generate values for Young's modulus and peak principle stress and compare these well defined mechanical parameters with the experimental data for bone mineral density, peak load and stiffness.

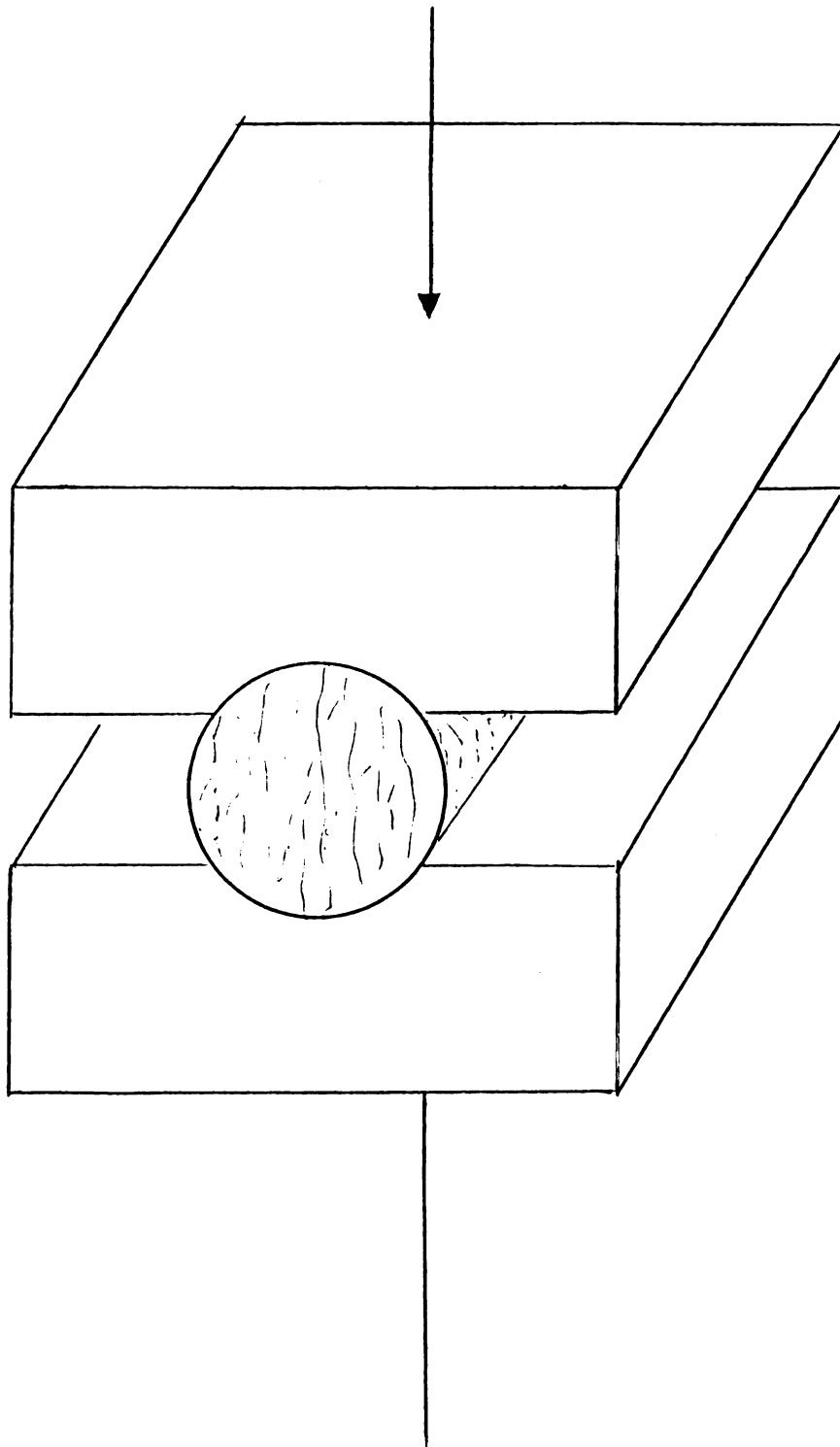


Figure 2: Cloward plug situated in test grips and placed in compression.

II. Survey of Literature

Since the first successful bone transplant in 1878, the idea of transplanting human bone gained attention in the orthopaedic world. In the 1940's when transplantation became accepted and refined, the first questions of tissue storage and tissue matching were posed by the medical community. Bush (9) suggested matching of blood type and Rh factor of the donor to the recipient was necessary. The "homogenous bone " could be stored at 3-5 degrees Centigrade for a maximum of three weeks. Bush suggested that the mechanism of the transplant is to serve as a supporting "trellis" for structure. About the same time Abbott (1) rebutted both the notion of tissue matching and the mechanism that Bush proposed. He claimed that bone is "so inert that it does not provide any type of foreign immune reaction." Secondly, Abbott's work, backed up by histological data showed that the transplants were replaced by new bone. This became the widely accepted mechanism for transplant incorporation and joint fusion.

In the 1950's a bone bank was established by the United States Navy to store bone for transplant. Campbell et.al. (10) studied different types of grafts, both autogenous and from banks. The study concluded that there was no difference in the fate of the two types of grafts and in fact they were " indistinguishable several months after implantation" as demonstrated by histological evidence. With the advent of bone banks and transplantation on a larger scale it was recognized by Carr et. al. (11) that over 10% of the grafts transplanted served as

vectors for bacterial infection and the grafts, therefore, needed to be sterilized. Carr's group proposed freezing the grafts at -70 degrees Centigrade after removal from the donor and subsequently freeze-drying the grafts for sterilization and long term storage such that only 1-5% residual water remained. The freeze-dried grafts would then be rehydrated by the surgeon pre-operatively. They concluded that freeze-dried bone was eventually vascularized by the body. Secondly, however, a high incidence of pseudoarthritis was noted two years post-operatively.

Proper sterilization was of primary concern since the freeze-drying technique improved storage time but did not reduce the occurrence of infection. Bright et.al. (3) studied different methods of irradiation sterilization and found gamma rays from Cobalt 60 to be the most penetrative without causing free-radical formation or "tissue activation". Two Megarads was given as a minimum to kill bacteria and four Megarads to kill viruses such as the polio virus.

With the applications of more sophisticated means of storage and sterilization, the initial bone used for transplant became subjected to a non-physiological set of conditions. Therefore, it became necessary to study the mechanical properties of the "new material" as well as the mechanical properties of the original bone to understand what differences and similarities exist between them. Triantafyllou (37) tested bovine cortical bone in three point bending under conditions of freeze-drying, irradiation and the combination of the two, all grafts rehydrated for two hours. He concluded that the breaking strength was decreased to 55-90% of fresh bone after freeze-drying, 50-75% of fresh bone after irradiation and 10-30% of fresh bone after freeze-drying and

irradiation. Bright et.al. (3) loaded cortical bone from human tibia dynamically. The test samples were freeze-dried and rehydrated for 1/2, 1, 2, 4, 6, 24 hour periods prior to testing. The freeze-dried bone was compared to a group of grafts frozen at -20 degrees Centigrade and thawed prior to testing. Elastic modulus and yield stress were all found to be elevated at 30 minutes of rehydration. They found that after one hour the elastic modulus returned to normal and after four hours of rehydration the yield stress returned to normal.

A more complicated problem is presented in the study of cancellous bone due to its anisotropic properties associated the arrangement of trabeculae, the volume fraction and mineral density of the bone. Carter (13) compared the mechanical behavior of cancellous bone to that of a two phase porous structure similar to the fluid filled materials in engineering, which reaffirmed the importance of returning water to the freeze-dried bone if it is to regain its initial properties after freeze-drying. Behrens (2) defined the regions of the load-displacement response as first an elastic deformation followed by irreversible collapse of the trabeculae. Gibson (18) attempted to quantify the material properties of cancellous bone by relating Young's modulus to the density or volume fraction of the material as well as its structural arrangement. Cancellous bone was defined by Gibson to be less than 70% solid by volume. He described the material as a cellular solid with plates or rods arranged in tandem which failed by buckling of the trabeculae. The maximum stiffness was reported along the longitudinal axis of the trabeculae and could be up to ten times stiffer in this direction. As the mineral density increased the plates become more become more contiguous and assume a "closed" arrangement. Stiffness

versus density curve shows the effect of "open" trabeculae configurations at low densities and "closed" configurations at higher densities. Turner and Cowin (38) also discussed the dependence of the elastic constants on porosity and trabecular orientation and derived a mathematical model to calculate the stresses in different directions.

Currently, there are many open questions concerning the integrity of cancellous bone and its use as grafting material. This study attempts to combine the research of general material properties of cancellous bone with the specific geometry, loading patterns, sterilization and storage of the Cloward plug bone allograft.

III. Experimental Materials and Methods

Human tissue samples obtained from the Michigan Regional Tissue Bank were used to study the compressive strength and change in mechanical properties of the Cloward plug bone allografts. To reduce variability in the data caused by sex or age of the graft donor, tissue was harvested from males between the ages of thirty-five and fifty-five. The cause of death of all subjects was sudden trauma, and no degenerative disease was observed which would effect the bone of the donor.

The grafts were taken from the condyles of the femur at six points corresponding to the tibia-femoral contact points at zero, forty-five and ninety degrees of flexion (2), (Figure 3). The plugs were drilled with a Black and Decker hole saw with a constant diameter of 14 millimeters. The length of the samples varied between fifteen and twenty millimeters and the cortical plate and cartilage ends were cut off with a Striker autopsy saw to insure the samples were cancellous bone throughout (Figure 4).

The first study was to determine the dependency of the mechanical properties on the rehydration time of the freeze-dried grafts. The grafts of six donors were freeze-dried so that the water content was less than two percent (21). The grafts were then rehydrated in physiological saline for either half-an-hour, two, six, twelve or eighteen hours. Two grafts from each donor were not freeze-dried but

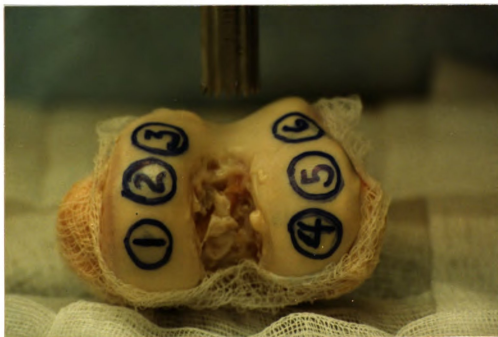


Figure 3: Condyles of the distal femur. Sites 1 and 4, 2 and 5, and 3 and 6 corresponding to 90, 45, and 0 degrees of knee flexion.

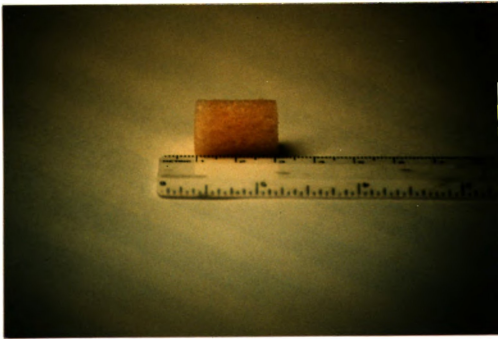
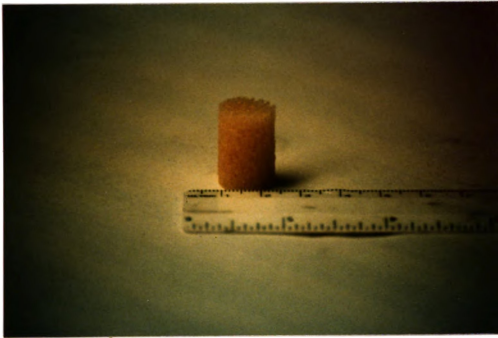


Figure 4: The Cloward plug bone allograft. $L=20\text{mm}$,
 $r=7\text{mm}$.

rather refrigerated after removal from the donor to be used as a non freeze-dried control group, designated as "fresh" bone.

The second part of this research was to examine the effects of irradiation on the grafts. There were two aspects of interest in the irradiation study: first, the effects of irradiation dosage on the mechanical properties of the grafts; second, being the effect of rehydration time or water content of the irradiated grafts. To determine the effects of irradiation dosage, three sets of freeze-dried grafts were prepared: two groups were irradiated at two and four Megarad dosages of Cobalt 60 gamma irradiation and the third group was not irradiated and served as the control group. All three groups, the two irradiated groups and the control group, were rehydrated for two hours in physiological saline prior to testing. Secondly, to determine rehydration time and water content effects on the irradiated grafts, the irradiation level of two Megarads was held constant and the mechanical properties of three sets of grafts were compared: frozen (not freeze-dried), freeze-dried bone rehydrated for two hours, and freeze-dried bone rehydrated for four hours.

The testing arrangement was designed to model the loading which the graft would be subjected to when it is placed between the vertebrae. The test grips were constructed from lucite with a four millimeter clearance between the upper and lower grips, equivalent to a typical intervertebral disk space (Figure 5). The grafts were measured and placed in the grips with the front end of the graft flush with the end of the grips. For uniformity, the major axis of orientation of the trabeculae was aligned with the direction of loading. A 10 X microscope was mounted to the front of the testing machine which permitted accurate

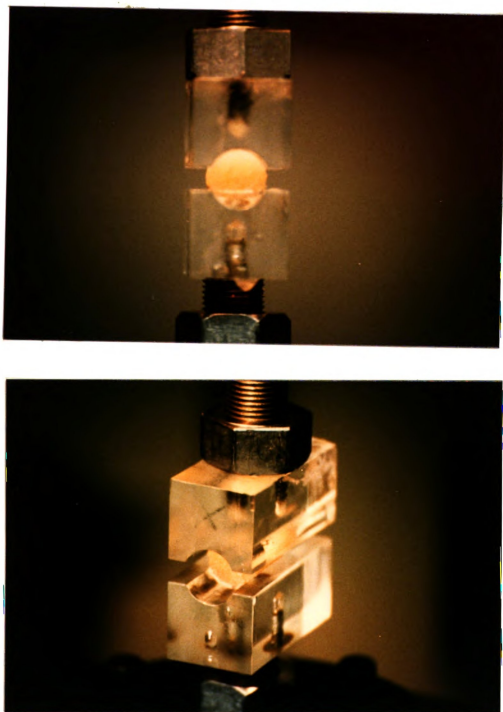


Figure 5: The gripping arrangement used to test the compressive strength of the grafts.

alignment of the grafts in the test grips and a photographic record of the structure of each sample (Figure 6).

An Instron servohydraulic materials testing machine was used for testing the grafts. Compression tests were run on stroke control with the maximum deflection, four millimeters, analagous to the complete loss of disk space - physiological failure of the graft. Samples were tested at room temperature and at a strain rate of three percent per second or four millimeters over a ten second period. Load and displacement data were monitored on a Nicolet digital oscilloscope and stored as voltages on floppy disks after each test run. These data were subsequently converted from voltage measurements of load and stroke to Newtons and millimeters by a program on a PDP 11/23 computer and plotted to generate a load deformation curve.

From each load deformation curve, stiffness and peak load were calculated graphically. Stiffness was defined as the slope of the linear portion of the curve. For consistency, peak load was defined as the intersection of a line parallel to the slope, 2% offset from it, with the curve itself (Figure 7).

After testing, all the samples were ashed in a furnace at 850 degrees centigrade for an hour and a half to drive off water and all organic material of the bone leaving only the mineral content (3). The samples were then weighed with a Mettler analytical balance and their mineral density calculated by dividing the ash weight of the sample by its volume. The peak load and slope values were normalized by the mineral density of each sample to remove variability due to graft donor.



Figure 6: The Cloward plug at 10 X, Showing the
verticle alignment of the trabeculae.

LOAD VS. DEFORMATION

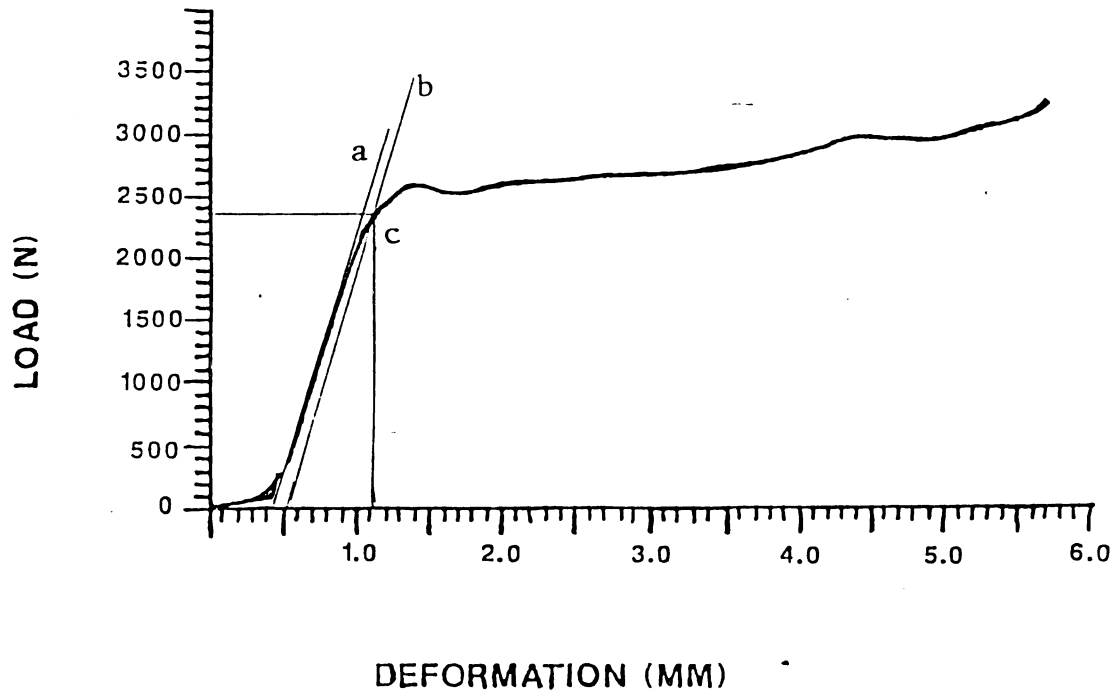


Figure 7: A typical Load vs. Deformation curve. Line A=linear elastic portion of the curve. Line B= 2% offset from the slope. Point C= the intersection of line B with the curve.

IV. Results

a. Experimental Results

In order to interpret the results of this study, it is necessary to define the modes of failure observed. In physiological terms, failure of the graft has occurred when the graft no longer maintains the intervertebral space such that the inferior and superior vertebral bodies make contact. Compression failure of the individual trabeculae of the graft resulting in the contact of the upper and lower test grips is referred to as plastic failure. A second type of failure was observed when the graft fractured macroscopically: this type of failure was called brittle fracture failure. Figure 8 shows the characteristic load vs. deformation curves for the two types of failure. For both the rehydration and the irradiation study, the parameters of fracture mode, peak load and stiffness were examined.

Dried bone, when placed in compression fails in a brittle mode (3). Thus, sufficient rehydration is necessary to prevent brittle fracture and maintain peak load and stiffness values comparable to fresh bone since the behavior of fresh, wet bone is considered ideal. The results of the rehydration study demonstrated that with rehydration of two hours and longer, the grafts did not exhibit brittle failure. All grafts rehydrated at thirty minutes, however, exhibited brittle failure and demonstrated a wide range of values for peak load and stiffness. The average peak load and stiffness did not change after two hours of

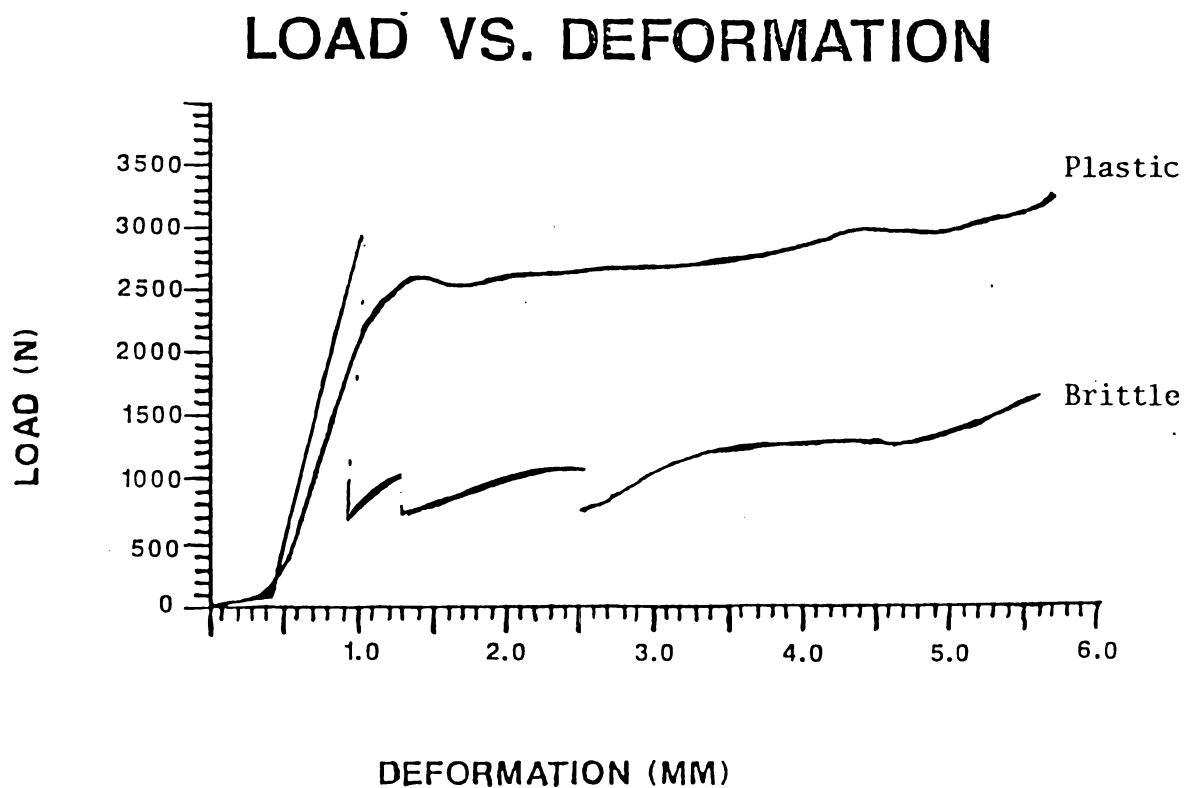


Figure 8: Characteristic Load vs. Deformation curves for plastic and brittle failure.

rehydration. The fresh grafts exhibited higher average load and stiffness values and less variation in values when compared with any rehydration time for freeze-dried bone (Figures 9 and 10).

Irradiation dosage and rehydration time or water content were compared in terms of failure mode, stiffness, and peak load changed. The stiffness of the irradiated grafts increased with each two Megarad dosage when compared with non-irradiated grafts. Brittle fracture was observed in 38% of the two Megarad samples and 27% of the four Megarad samples (Table 1) and grafts that failed with brittle fracture mode were of higher than average density. When grafts at the two Megarad level of irradiation were rehydrated for four hours or irradiated while frozen with their original water content, brittle fracture was not observed. The frozen irradiated samples did, however, have a higher average stiffness than the freeze-dried rehydrated bone at the two Megarad level (Table 2).

b. Statistical Results

Student t tests were used to analyze the data statistically. For the rehydration study, each rehydration time between two and eighteen hours were compared to fresh bone and to all other rehydration times (Tables 3 and 4). No significant difference was found, at the 95% confidence level, in peak load or stiffness between different rehydration times or between rehydrated bone and fresh bone. Fresh bone, however, exhibited a smaller standard deviation for the mean than did any of the freeze-dried bone.

The data for the irradiation was analyzed in a similar manner with the use of t tests. The peak load and stiffness of freeze-dried

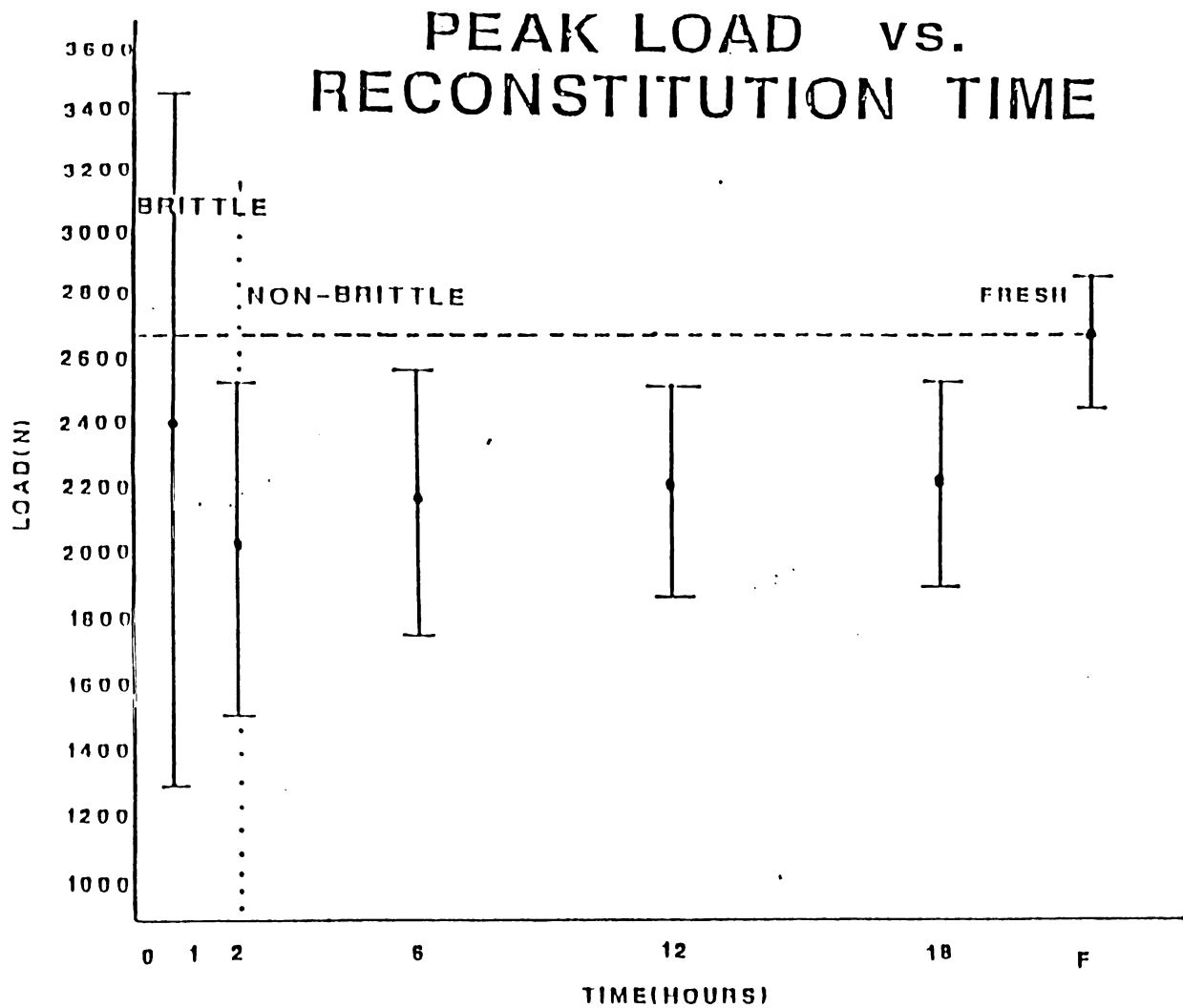


Figure 9: Peak Load vs. Rehydration time for rehydrated and fresh bone grafts.

STIFFNESS vs. REHYDRATION TIME

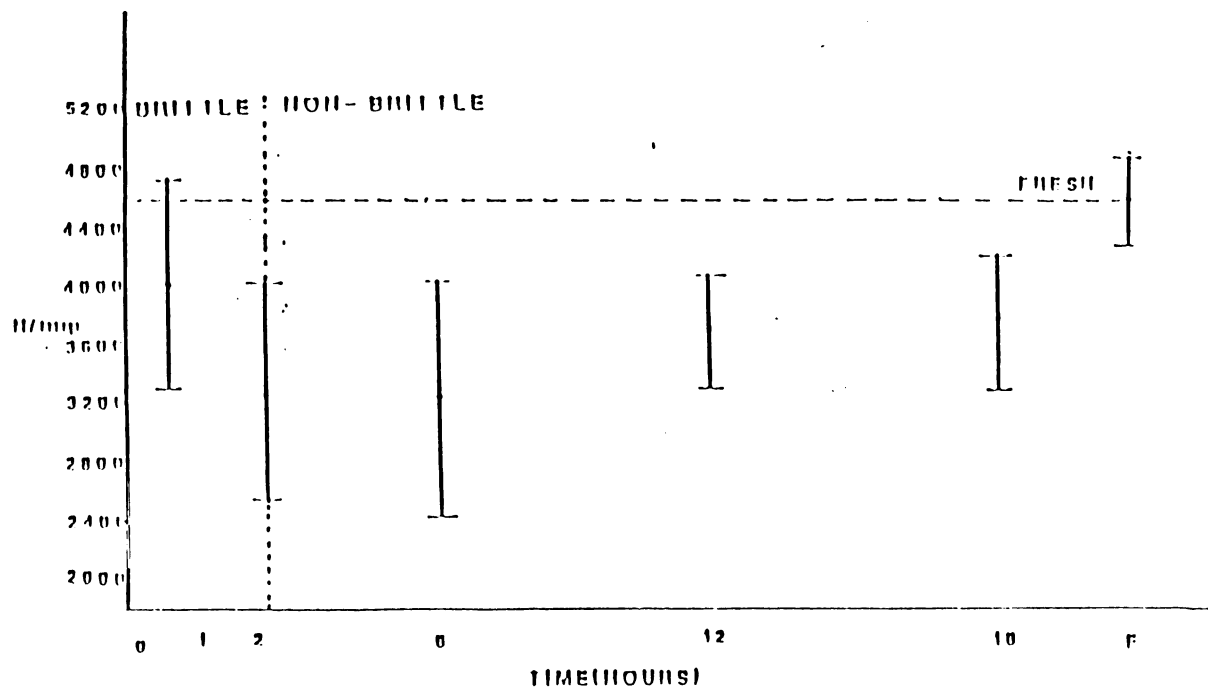


Figure 10: Stiffness vs. Rehydration time for rehydrated and fresh grafts.

Table 1

Peak Load, Stiffness, Percent Brittle Fracture and t Statistic for Cloward Plug Bone Grafts Rehydrated for Two Hours with Different Irradiation Dosages

	non-irradiated	2 Megarad	4 Megarad
Peak Load (N)	2029 $\pm$ 448	2108 $\pm$ 693	2399 $\pm$ 605
Stiffness (N/mm)	2601 $\pm$ 643	3299 $\pm$ 875	4437 $\pm$ 810
% Brittle Fracture	-----	38	27
t stat.	-----	2.500*	4.108*

Table 2

Peak Load, Stiffness, Percent Brittle Fracture and t Statistic for Cloward Plug Bone Grafts Irradiated at Two Megarads with Different Rehydration Time

	Frozen	2 hours	4 hours
Peak Load (N)	2339 $\pm$ 232	2108 $\pm$ 693	2027 $\pm$ 704
Stiffness (N/mm)	4294 $\pm$ 843	3292 $\pm$ 875	3846 $\pm$ 1353
% Brittle Fracture	-----	38	-----
t stat.	3.275*	2.500*	0.923

*t statistic for comparison between stiffnesses of irradiated groups compared with non-irradiated samples: $t > 2.131$ indicated statistical significance at the 95% confidence level.

Table 3

t Statistic* for Differences in Mean Peak Load Values for Freeze-Dried Grafts with Different Rehydration Times

	Fresh	2 hour	6 hour	12 hour	18 hour
Fresh	-----	0.245	0.379	1.591	1.693
2 hour	0.245	-----	0.333	0.727	0.727
6 hour	0.379	0.222	-----	1.839	1.235
12 hour	1.5911	0.727	1.839	-----	0.544
18 hour	1.693	0.727	1.235	0.544	-----

Table 4

t Statistic* for Differences in Mean Stiffness Values for Freeze-Dried Grafts with Different Rehydration Times

	Fresh	2 hour	6 hour	12 hour	18 hour
Fresh	-----	1.000	1.733	1.831	1.337
2 hour	1.000	-----	0.434	0.456	0.038
6 hour	1.733	0.434	-----	0.337	0.308
12 hour	1.831	0.456	0.337	-----	0.302
18 hour	1.377	0.038	0.308	0.302	-----

*t statistic for comparison between means: $t > 2.447$ indicates statistical significance at the 95% confidence level.

irradiated and freeze-dried non-irradiated grafts were compared to one another. No significant difference was observed between the peak load values of any of the groups at the 95% confidence level. Significant differences were found, however, between the average stiffness of irradiated bone compared to non-irradiated bone, the irradiated bone being of higher stiffness (Tables 1 and 2). After four hours of rehydration, the stiffness of the grafts irradiated at two Megarads was not significantly different than the stiffness of the non-irradiated grafts.

c. Analytical Results

A finite element model was constructed for the purpose of obtaining basic material properties of cancellous bone, Young's modulus and peak principle stress, from the experimental response curves. These basic properties then can be compared with properties of cancellous bone for any geometry or loading pattern reported in the literature. The model was constructed of 4000 elements, with 55 elements for the circular end of the cylinder and 800 rows along the long axis of the cylinder (Figure 11). The bottom nodes of the element denoted with X's were fixed and the top nodes denoted with arrows were displaced four millimeters corresponding to the experimental displacement of the grafts. Different values for Young's modulus were input into the model. Load vs. displacement curves were generated by the finite element model for the range of E values (Figure 12). The experimental curves were then compared with the theoretical curves generated by the model and E was predicated for different experimental stiffness or slope values. To demonstrate the dependence of Young's modulus on the mineral density of

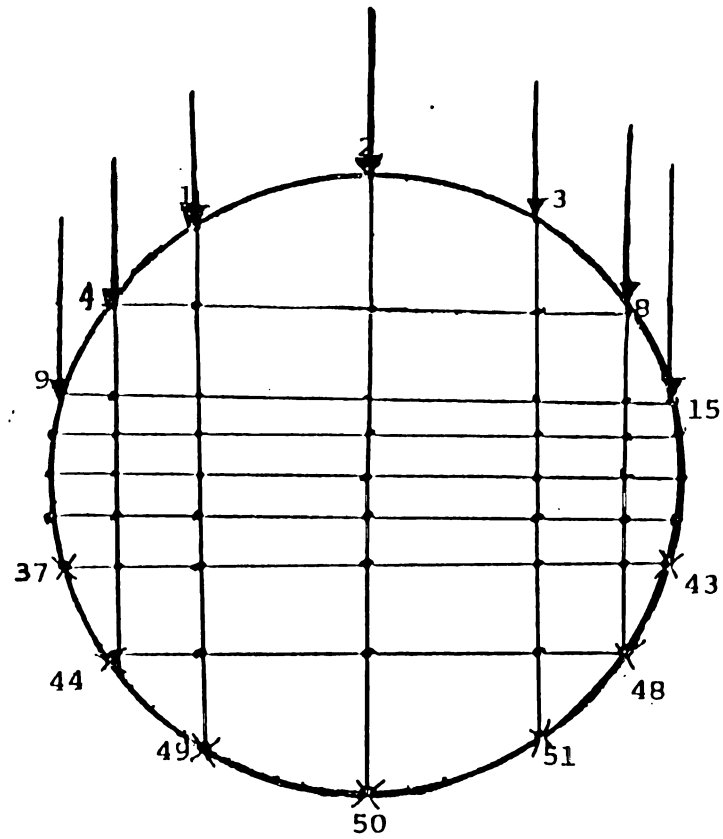


Figure 11: End View of the Finite Element Model of the Graft.

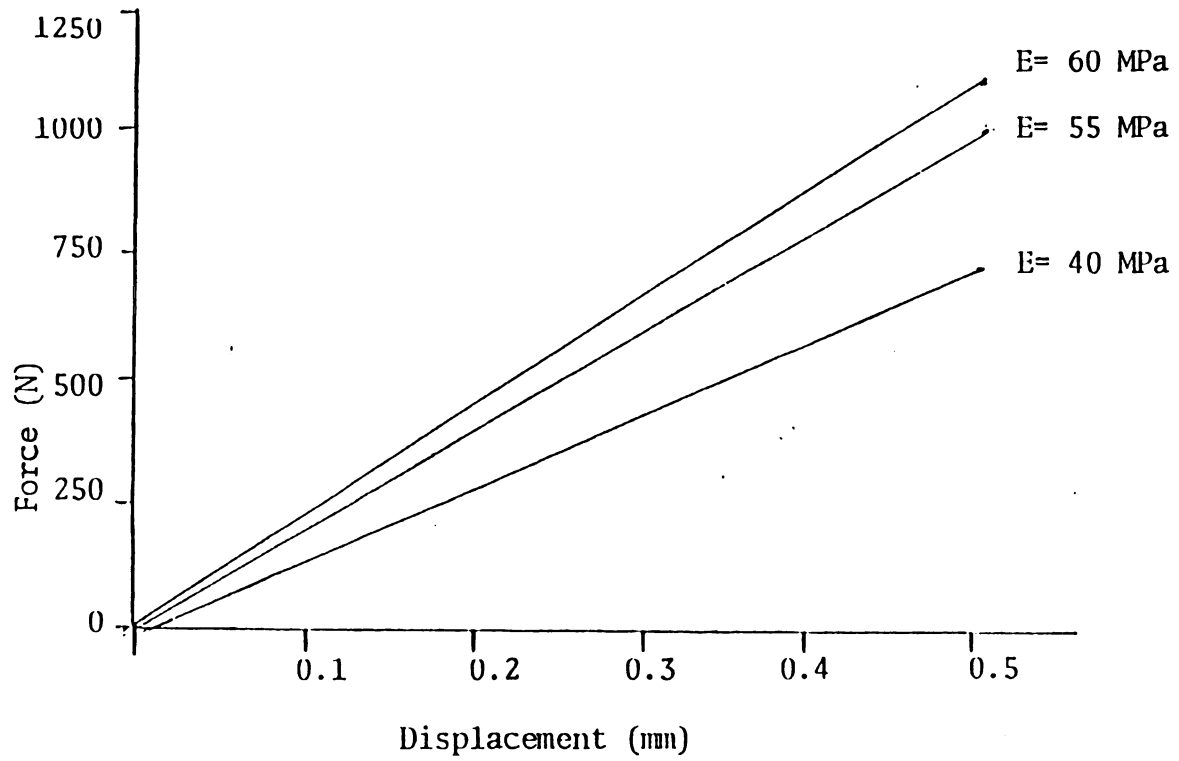


Figure 12: Plot of several Load vs. Deformation curves generated from the finite element model for a range of E values.

the grafts, Young's modulus was plotted vs. mineral density of the bone (Figure 13). A series of stress plots was generated for different values of E (Figures 14, 15, 16). These figures show the stress isobars for each of the three principle stresses; the maximum stress intensity is indicated by red and decreases in magnitude for blue, green, and yellow. The maximum stress was found at nodes 37 and 43 in each row along the long axis, 0-800, corresponding to the fixed points at the interface between the grip and the bone graft. The principle stresses of the element were triaxial compression and the maximum principle stresses were plotted vs. mineral density of the grafts (Figure 17). By comparing the maximum stress found in the grafts at a given density with the experimental loads incurred, some conclusions could possibly be drawn concerning where the graft would be expected to fail.

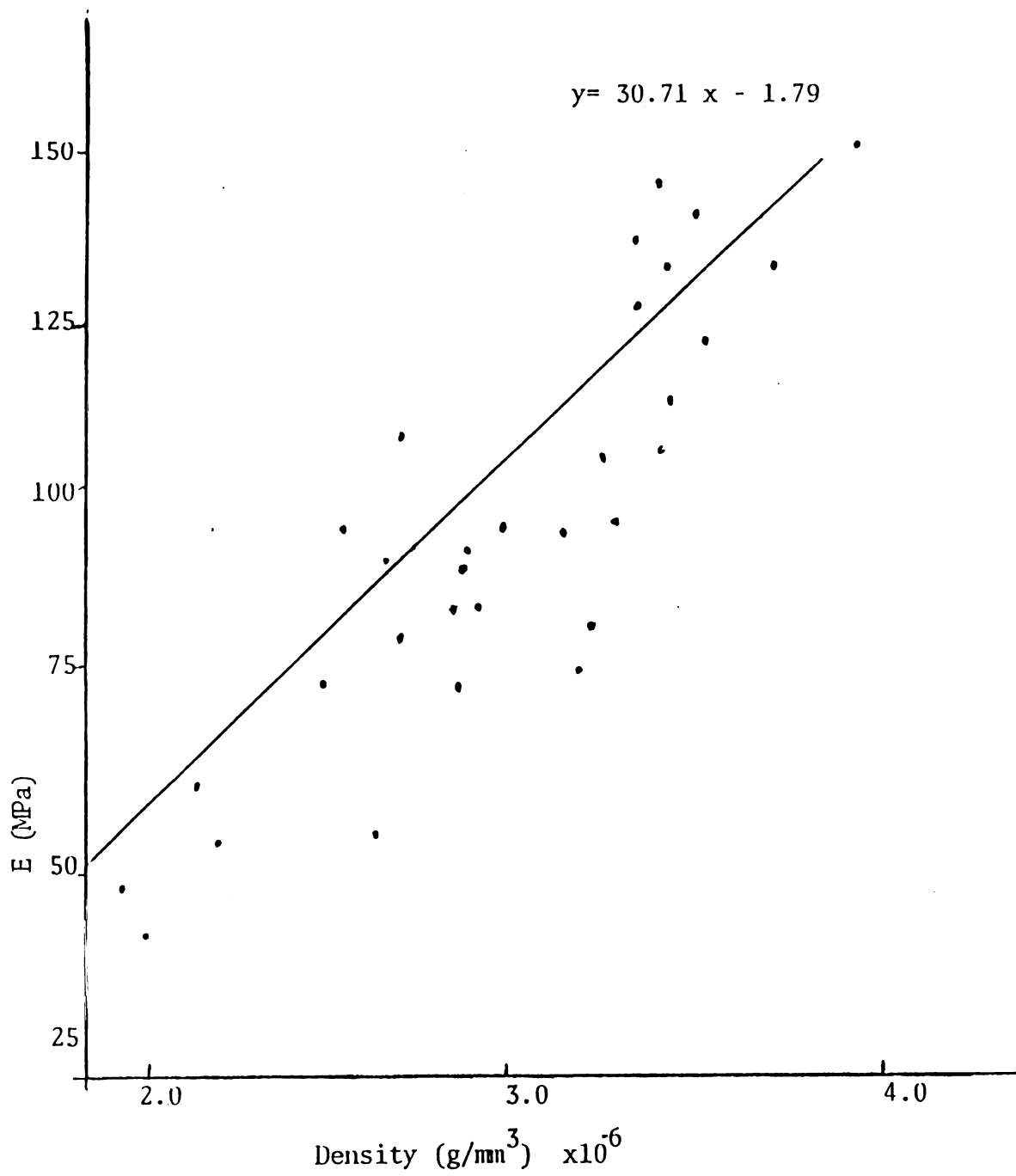


Figure 13: A Plot of Young's Modulus vs. Mineral Density.

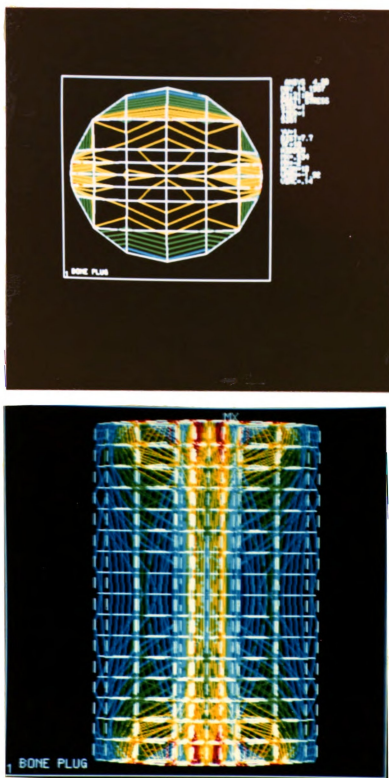


Figure 14: End and Axial View of the Stress Plots Generated by the Finite Element Model for Sigma 1.

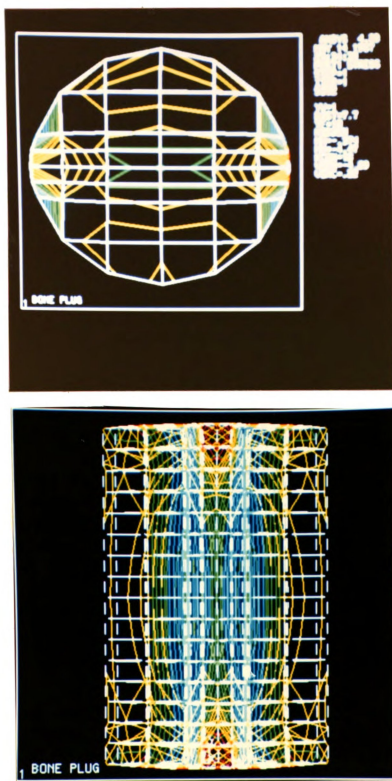


Figure 15: End and Axial View of the Stress Plots Generated by the Finite Element Model for Sigma 2.

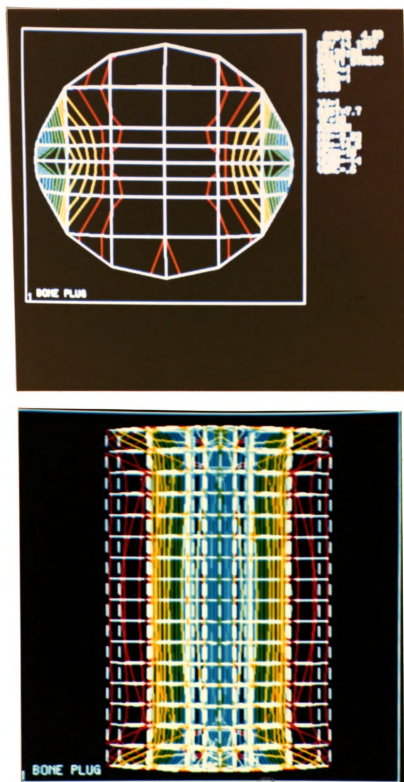


Figure 16: End and Axial View of the Stress Plots Generated by the Finite Element Model for Sigma 3.

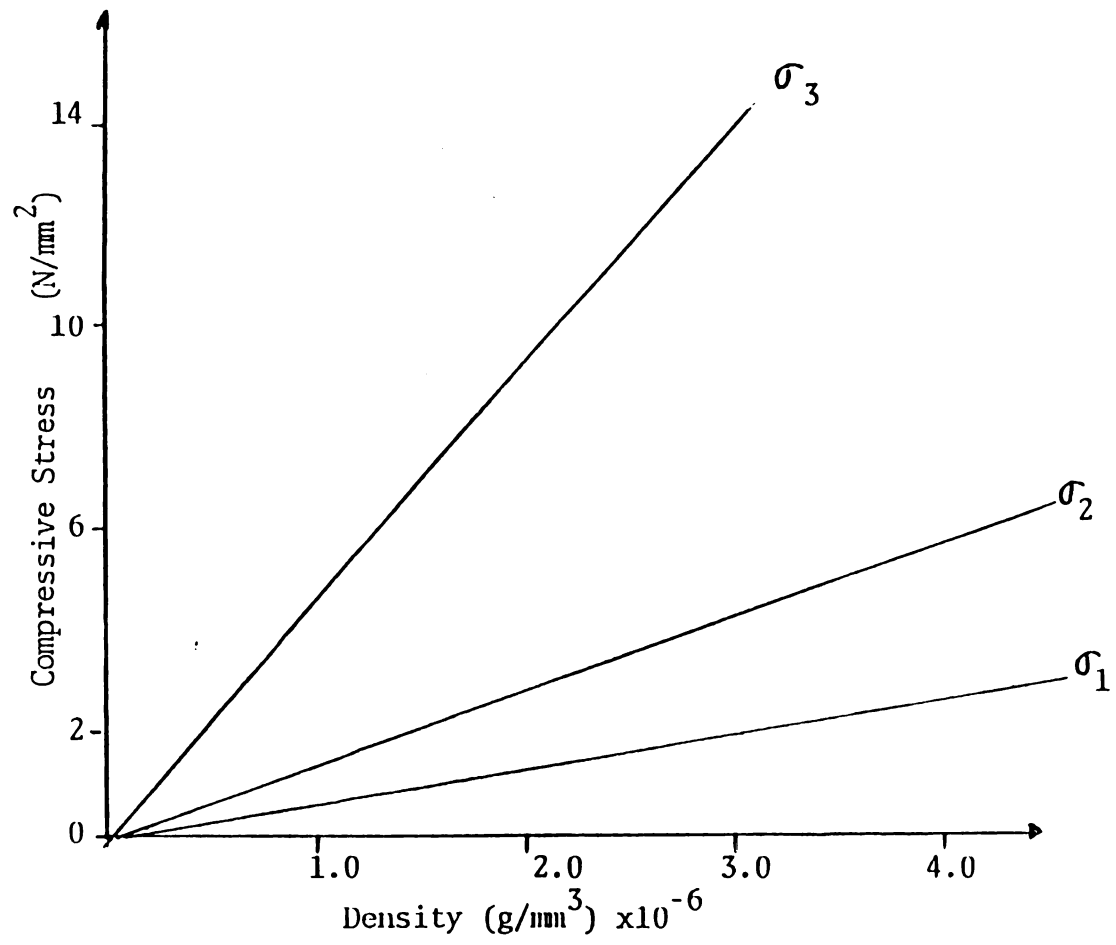


Figure 17: Plot of Peak Principle Compressive Stress vs. Mineral Density of the grafts.

V. Conclusion

The mechanical properties and behavior of cancellous bone is important to understand so that it may be used successfully as a transplant material and better understood in vivo. However, the literature demonstrates that there are many aspects of this research problem. Water content is an important aspect of all tissue and change in water content severely effects the behavior of tissue. Thus, freeze-drying, has a dramatic effect on the behavior of bone. Similarly, irradiation is known to cross-link tissue and cause free radical formation (35), thus the effects of irradiation secondary to the sterilization of bone should be studied. Finally, the constituents of fresh normally cancellous bone, specifically mineral density, should be examined to understand its effect on the material properties of the bone such as Young's Modulus and internal stresses.

The results of the rehydration study demonstrated that after 2 hours or more of rehydration, no grafts failed in a brittle mode and that there was no significant difference in peak load or slope. A parallel study done with NMR (42) showed the water content of freeze-dried bone was complete between 2 and 6 hours of rehydration in saline and after as long as 18 hours of rehydration never reached the percentage of water contained in fresh bone. These findings correspond to the Peak Load vs. Rehydration Time (Figure 9), if water is assumed to be a major constituent influencing the mechanical behavior of bone as suggested by many researchers including Carter (13) and Gibson (18). Once water has

been removed by the freeze-drying process, however, it may be that the original percent water cannot be replaced because a certain percentage is bound actively by the tissue (39). Since bone grafts from the femur or pelvis are transplanted into the spine and serve a non-physiological function of fusing the joint, further research should be done to determine what the desirable material properties of the graft are for the purpose of joint fusion.

A criterion was established for minimum acceptable load bearing characteristics by reviewing safety standards for impact injury to the head and neck region (28). Below 1500 N force, no fatal injury is caused in this region; therefore, if the graft can sustain 1500 N compression it would not be the "weak link" by this rough criterion. Figure 18 shows a plot of Density vs. Peak Load for the grafts used in the rehydration study. Most of the grafts failed at loads well above the 1500 N approximation; however, the group of donors for this study was males between ages 30 and 55. Further research should be performed with grafts from donors of different sexes and ages since all are presently used to prepare weight bearing grafts.

Results from the rehydration study were also used for comparison with the finite element model. The predictions for E from the model, when plotted vs. density (Figure 13), demonstrate a linear relationship ($y = 33.86x - 1.38$) between the two parameters and a correlation coefficient of 0.75 which is high for biological data (36). The graph of peak principle stress vs. density (Figure 17) was constructed from the stress plots of three points from the previous curve to illustrate what types of internal stresses the graft may be experiencing under the experimental loading conditions. Further work could be performed to

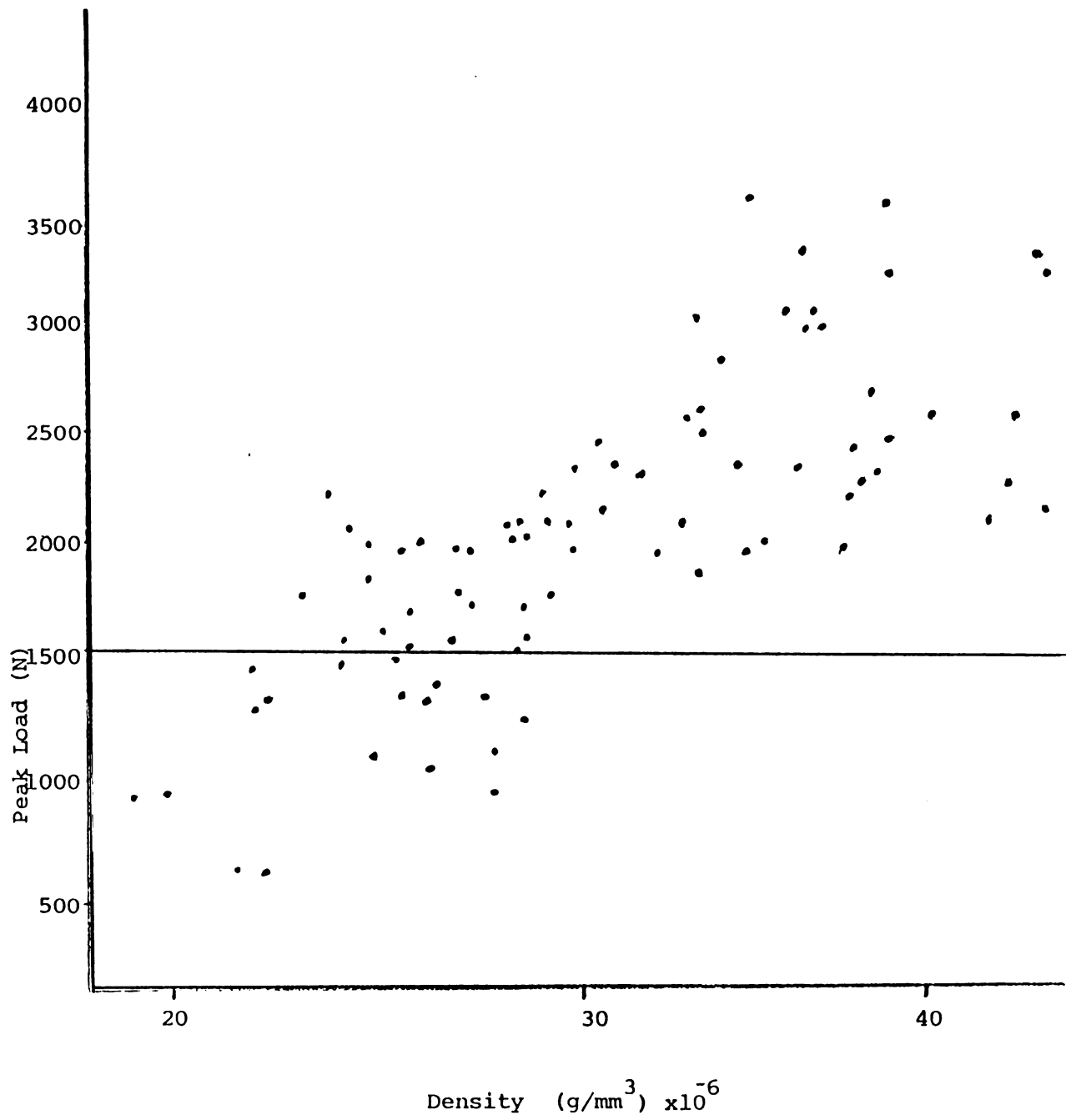


Figure 18: Plot of Peak Load (N) vs. Density (g/mm^3).

develop the model such that it could take into account the volume fraction or orientation of trabeculae.

Results of the irradiation study demonstrated a marked increase in the mean stiffness values of the grafts after irradiation. The data indicate that the higher mean stiffness demonstrated by the grafts irradiated at two Megarads and rehydrated for two hours is decreased upon rehydrating the grafts for four hours. This finding is consistent with the work presented by Bright (3) for cortical bone in dynamic loading. Interestingly, the grafts irradiated while frozen at -70 degrees also demonstrate an increase in stiffness while the original water content is maintained. Brittle fracture was not observed for the frozen irradiated samples even with the increase in stiffness. Brittle fracture only occurred in freeze-dried bone irradiated at two and four Megarads and rehydrated for two hours. The finding that frozen irradiated and four hour rehydrated irradiated grafts did not demonstrate brittle fracture suggests that the mechanism of water uptake is slower or that the freeze-dried irradiated bone has lower water content. Either of these ideas could be further researched to understand how gamma irradiation effects the hydroxyapatite crystals or collagen fibers of the bone.

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