Interim Performance Report

Name and Address of the University: Board of Regents of the University of Wisconsin System

Title of Project: The Effect of Cementation and Autogenous Bone Grafting on Allograft Union and Incorporation

Grant Number: N00014-93-1-0745

Principle Investigator: Mark D. Markel, DVM, PhD

Covered Time Period: October 1, 1994, through January 31, 1995

Progress:

This study is designed to examine the reconstruction of bone after segmental bone loss. Project milestones may be divided as follows:

I. Phase I: *In vitro* evaluation of allograft/host bone constructs.

Status: Active. Cadaver bones have been collected and stored for subsequent testing with the custom designed nails.

II. Phase II: *In vivo* study - effects of cementation and cancellous graft.

A. Immunologic mismatching and base line values for dogs (radiographs, bone mineral density, and force plate analysis).

Status: Active. On schedule.

Accomplishments: (i) The original 32 dogs have been immunologically mismatched. We found 26% of the donor-recipient dog combinations evaluated were immunologically similar. While necessitating multiple combinations be evaluated, successful mismatches were obtained for all dogs in the study. (ii) Radiographic, bone mineral density, and force plate base line evaluation has been completed on all dogs.

B. Surgical procedures - segmental allografts plus treatments.

Status: Active. On schedule. Problematic.

Accomplishments: As indicated in previous progress reports, owing to the small size of available purpose-bred dogs, we had to custom design a small interlocking nail for the project. Dimensions of this nail were proportioned after larger, commercially available nails. The

initial nail designed was 4.0-mm diameter with 2.7-mm screw holes. Four screws were used in each nail to provide rotational stability and active loading of the nail-bone construct. A detailed summary of dogs and procedures performed is attached to this report. Briefly, the initial custom nail was used in 1 dog in August, 1994, which failed at 17 days after surgery.

The nail was then modified, changing the screw holes to 2.0-mm. This nail was mechanically tested and compared to the earlier design. The early nail bent and fractured at a load of 94 N. The new nail bent but did not fracture at a maximal load of 167 N. The new nail appeared to be almost twice as strong. This nail was placed in 9 dogs in September and early October. Eight of those dogs subsequently had fatigue failure of the nail and were euthanized from 17 to 31 days (mean of 22 days) after surgery. The surviving dog is currently out 129 days. She is larger than the others and had failure of 2 interlocking screws in each leg by 2 weeks, but the nail has remained intact.

The nail was modified again, increasing the size to 5.0-mm diameter and returning to 2.7-mm screws, as the remaining dogs were also of the larger size. This nail was placed in 22 dogs between November and early December. Fatigue failure has occurred in 8 of these dogs at 20 to 89 days (mean of 45 days). Fifteen dogs remain active in the study, ranging from 53 to 130 days post-surgery (mean of 83 days).

All implant failures appear to have been the result of cyclic fatigue of the nail. Dogs typically are moderately lame the first 1 to 3 days after surgery. They then begin to use their legs more and more. By 1 week, they typically are walking well. By 2 weeks, they trot around their runs and stand with their front paws on the fencing to great investigators that check on them. This activity leads to repetitive cyclic forces being transmitted through the nail-bone construct. Generally the dogs are very active up until the day they are found to be suddenly nonweight-bearing on one leg. When this happens, they are radiographed and evaluated for the cause of the lameness. If the nail is fractured and the bone displaced, they are euthanized to prevent suffering. In all cases of implant failure, the nail has fractured through a screw hole, involving either the second or third hole (the holes on either side of the allograft). The changes in nail diameter and screw hole size have been made to counter these cyclic forces.

At this time, the nail has been modified again, increasing the size to 6.0-mm diameter and maintaining 2.7-mm screw holes. The smallest commercial nail available is 6.0-mm diameter with 3.5-mm screw holes. No dogs have received the new nail thus far. We anticipate it will solve the cyclic fatigue problems we have encountered and that it can be applied to the beagle dogs with minimal reaming, allowing us to still answer the hypotheses of the study. However, as a result of the unexpected dog losses, we now find ourselves short of the necessary number of dogs to answer these questions. If we can obtain an additional 10 dogs, we will be able to maintain 6 dogs per group and retain statistical power for the study (power = 0.8 with n = 6, vs. power = 0.9 with n=8). Unfortunately, our approved budget does not provide for these additional animals. If the Navy would consider providing an additional $22,600 (a detailed budget is attached to this report), we can satisfactorily complete the study. Alternatively, if additional funds are not available, if the Navy would grant approval to shift money allocated for salary to the purchase and care of animals, the study can be completed. In either case, we expect the project will still be completed in the time frame originally approved (by May, 1996).

Despite these difficulties, we feel this is a worthwhile project and would like to complete it. The results will serve to advance the success of segmental allograft bone replacement. This project is the starting foundation to future projects we anticipate submitting to the Navy. Revascularization of large segmental allografts remains a problem area. The central portion of large grafts remains nonvascular, despite having the ends remodel and be incorporated into the host bone. The nonvascular region results in the bone being more prone to fracture. We would like to specifically address the revascularization of segmental allografts and the role that specific growth factors play in that process. Results of such studies will enhance the success of bone allografts and biosynthetic bone substitutes that are being developed.

C. Dog follow up - radiographs, bone mineral density, and force plate analysis.
Status: Active. On schedule.
Accomplishments: Follow up evaluations are being performed at 2, 4, 12, and 16 weeks after surgery. Dogs currently range from 8 to 18 weeks post-surgery.

D. Biomechanical testing and histologic analysis at 6 months post-surgery.
Status: Inactive. Mechanical testing will begin March, 1995, and finish October, 1995, if additional dogs are approved for the study. Histologic evaluation will be completed by February, 1996.

III. Publication and presentation of results.
Status: Inactive. With the difficulties encountered to date, we anticipate the study can still be completed by May, 1996. Results will be submitted for presentation at meetings and publication spring of 1996.