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DELIVERY SYSTEMS FOR BONE MORPHOGENETIC PROTEIN (BMP)  
FOR REPAIR OF BATTLE INCURRED BONE INJURIES(U)  
CALIFORNIA UNIV LOS ANGELES M R URIST 01 NOV 87

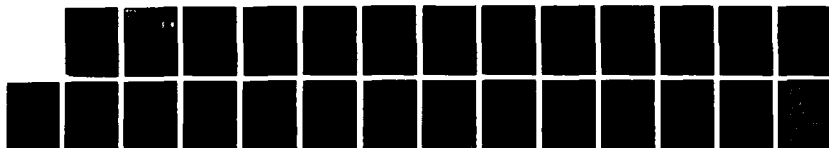
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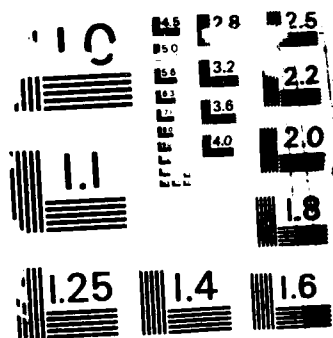
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DAND17-84-C-4845

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SECURITY CLASSIFICATION OF THIS PAGE

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**REPORT DOCUMENTATION PAGE**

Form Approved  
OMB No. 0704-0188

**AD-A191 564**

LE

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION/AVAILABILITY OF REPORT  
Approved for public release;  
distribution unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION  
University of California

6b. OFFICE SYMBOL  
(if applicable)

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)

Los Angeles, California 90024

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING/SPONSORING  
ORGANIZATION US Army Medical  
Research & Development Command

8b. OFFICE SYMBOL  
(if applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER  
DAMD17-84-C-4045

8c. ADDRESS (City, State, and ZIP Code)

Fort Detrick  
Frederick, Maryland 21701-5012

10. SOURCE OF FUNDING NUMBERS

PROGRAM  
ELEMENT NO.  
61102A

PROJECT  
NO. 3M1  
61102BS10

TASK  
NO.  
DA

WORK UNIT  
ACCESSION NO.  
371

11. TITLE (Include Security Classification)

Delivery Systems for Bone Morphogenetic Protein (BMP) for Repair of Battle Incurred  
Bone Injuries

PERSONAL AUTHOR(S)

Marshall R. Urist

13a. TYPE OF REPORT  
Final

13b. TIME COVERED  
FROM 7/16/84 TO 9/30/87

14. DATE OF REPORT (Year, Month, Day)  
1987 November 1

15. PAGE COUNT

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	01	
06	16	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Battle-incurred bone injuries; bone defects; fractures; bone  
regeneration; bone morphogenetic protein; non union; bone  
healing; osteogenesis; fracture compound comminuted.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The attached document is a summary of ten projects on bone  
regeneration under the influence of a bone morphogenetic protein (BMP),  
performed in collaboration with Lieutenant Colonel Jeffrey Hollinger and  
associates at D/C, W.R.A.I.R. in the three-year period from 1984 through  
1987 inclusive.

The ten projects have in common the objective of determining  
efficacy of BMP/NCP in augmentation of bone regeneration process in  
dogs. The tests were performed in standard bone defects of critical  
size for regeneration in adult dogs in skull trephine operations,  
segmental long bone loss, and spinal fusion. Implants of BMP were  
placed in heterotopic sites in a mouse muscle pouch for preliminary  
tests for verification of induced bone formation. Four delivery systems  
were tested: (1) a selection of insoluble non-collagenous bone matrix

DISTRIBUTION/AVAILABILITY OF ABSTRACT

☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION  
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL  
Mary Frances Bostian

22b. TELEPHONE (Include Area Code)  
301-663-7325

22c. OFFICE SYMBOL  
SGRD-RM1-S

cont'd.  
→ proteins, which form aggregates with BMP, (2) polylactic acid polymers, (3) biodegradable beta tricalcium phosphate, and (4) a polymethylmethacrylate. The report includes preliminary observations on implants of human BMP in human subjects under the guidelines of the Human Subject Protection Committee of the University of California, Los Angeles, School of Medicine, Center for the Health Sciences. Applied to this Annual/Final Report is a Summary of the Materials, Methods, and Results of the individual ten projects. The overall results demonstrate that the ideal delivery system for transmission of BMP for augmentation of bone regeneration in long-lived animals such as dogs and humans, must be sought by investigations with the aid of new and improved methods. These extended investigations should be facilitated by the development of a homogeneous recombinant BMP, which could become available in 1988-1989.

Keywords: osteogenesis; wounds and injuries; healing; biomaterials

military medicine

Accession For	
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DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
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Availability Codes	
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A-1	



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DELIVERY SYSTEMS FOR BONE MORPHOGENETIC PROTEIN (BMP)  
FOR REPAIR OF BATTLE INCURRED BONE INJURIES

FINAL REPORT

NOVEMBER 1, 1987

MARSHALL R. URIST

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4045

University of California, Los Angeles  
Los Angeles, California 90024

Approved for public release; distribution unlimited

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Department of the Army position unless so designated by other  
authorized documents.

FINAL REPORT OF DAMD17-84-C-4045 PROJECT NO. 3M1 6102BS10

INTRODUCTION

Summarized in this report, the 10 projects performed under this Contract demonstrate the extraordinary potential of BMP research for improvement of treatment of battle-incurred extensive bone injuries.

Bone differs from other tissue not only in physiochemical structure but also in its extraordinary capacity for growth, continuous internal remodeling, and regeneration throughout postfetal life, even in long-lived higher vertebrates. How much of this capacity can be accounted for by proliferation of predifferentiated osteoprogenitor cells and how much can be attributed to induced differentiation of mesenchymal-type cells have been challenging questions for a long time. A basic assumption is that regeneration occurs by a combination of the two processes. The process of induced cell differentiation has been observed from measurements of the quantities of bone formed in response to implants of either bone matrix or purified bone morphogenetic protein (BMP) in extraskeletal and intraskeletal sites. The osteoprogenitor cell proliferation process has been well known for more than a century and is measured in reactions of periosteum and endosteum to injury, diet, vitamins, and hormones. Bone-derived growth factors stimulate osteoprogenitor cells to proliferate in serum-free tissue culture media. The mechanisms of action of BMP and growth factors are primarily local, but secondary systemic immunologic reactions could have either permissive or depressive effects.

The most important and indispensable substitutes for experiments in human beings are adult mongrel dogs, monkeys, and sheep. Experimental

observations on local conditions and biochemical factors regulating bone regeneration are recorded in literature dating back over a century. The largest part of present knowledge was obtained by experiments on rabbits, guinea pigs, rats, and mice. Relatively little information has been obtained from research on dogs, a species comparable to man with respect to regulatory mechanisms, time relations, and surgical problems. Such information that is available on bone repair in dogs is chiefly descriptive. Present research was performed on dogs because, as observed by Coulson, the metabolic activity index (MAI) of the dog is 1.58, remarkably close to that of human beings (1.0); in comparison, the MAI is 5.15 for the rat and 15.60 for the mouse.

Young growing individuals repair cranial trephine defects more rapidly than adults. Normally, infants can regenerate the entire vault of the cranium, but in mature individuals, a defect with a diameter of more than 1 cm will heal only with the aid of a transplant of autogeneic bone. In the adult, the incorporation of the transplant is incomplete often enough to warrant a search of substitutes for autogeneic bone. The newest substitute is bone morphogenetic protein (BMP), recently isolated from bovine and human bone. BMP is the hypothetical paracrine agent augmenting the process of bone regeneration by induction of the morphogenetic phase of bone development.

#### 1. CRANIOTOMY DEFECTS IN DOGS.

In the adult dog, a 14-mm skull trephine defect regenerates only incompletely in the lifetime of the individual. Only about half of the defect is repaired; the regenerated part develops by extension of growth from the bony rim. Correlated roentgenographic and histomorphometric

methods demonstrate that new bone develops by proliferation of preexisting osteoprogenitor cells lining the diploe and perivascular cells of the bone marrow stroma. An autogeneic bone graft, including bone marrow, provides a supplementary framework and generally completes the repair process. Transplants of bone marrow alone fail to repair the defect. Implants of bovine bone morphogenetic protein (bBMP) and a carrier consisting of matrix  $\gamma$ -carboxyglutamic acid rich protein (without any additional bone or bone marrow) induce repair almost as completely as an autograft. BMP-induced bone regeneration is incomplete in the thin lateral temporal marrow-deficient part of the cranium. Implants of BMP plus bone marrow induce complete repair, suggesting that calvarial bone regeneration is bone marrow stroma-dependent for a supply of target cells. The target for BMP in cranial bone regeneration is the perivascular connective tissue cells (pericyte) of the host bed marrow stroma and endosteum. The molecular mechanism of differentiation of pericytes into osteoprogenitor cells is not known, but the process is irreversible, heritable, and presents a solvable problem.

## 2. BONE REPAIR INDUCED BY BONE MORPHOGENETIC PROTEIN IN ULNAR DEFECTS IN DOGS.

In dogs, resection of a length of the ulna equal to twice the diameter of the mid-shaft leaves a defect which consistently fails to unite. In response to an implant of 100 mg of bovine bone morphogenetic protein (bBMP), the defect becomes filled by callus consisting of fibrocartilage, cartilage and woven bone within four weeks. The cartilage is resorbed and replaced by new bone in four to eight weeks. Woven bone is then resorbed, colonized by bone marrow cells and remodeled into lamellar bone. Union of the defect is produced by 12

weeks. Control defects filled with autogeneic cortical bone chips unite after the same period.

In regeneration induced by bone morphogenetic protein (BMP) and in repair enhanced by bone graft, union depends upon the proliferation of cells within and around the bone ends. Our working hypothesis is that BMP induces the differentiation of perivascular connective tissue cells into chondroblasts and osteoprogenitor cells and thereby augments the process of bone regeneration from the cells already present in the endosteum and periosteum.

### 3. CALCIUM PHOSPHATE DELIVERY SYSTEM FOR BMP WAS TESTED IN MICE BEFORE EXPENSIVE EXPERIMENTS WERE PERFORMED IN DOGS.

An aggregate of biodegradable B-tricalcium phosphate and bone morphogenetic protein (BMP/TCP) induces the differentiation of cartilage within eight days, cartilage and woven bone within 12 days, and lamellar bone including bone marrow, within 21 days. The yield of new bone from a 1-mg dose was more than 12 times greater from the TCP/BMP than from the BMP alone. Whether TCP acts as a slow-release delivery system, potentiates the activity of BMP, or serves to distribute BMP in a favorable three-dimensional pattern requires further investigation.

### 4. BMP-BETA TRICALCIUM PHOSPHATE COMPOSITE DELIVERY SYSTEM IN DOGS.

Beta tricalcium phosphate (TCP) was employed as a nonimmunogenic biodegradable delivery system for bovine bone morphogenetic protein (bBMP). A bBMP/TCP composite was implanted in adult dogs with skull trephine defects of a critical size of 1.4 cm that would otherwise remain unhealed in the lifetime of the individual. bBMP/TCP implants induced 91%-100% incorporation by deposits of new bone. In comparison,

control implants of TCP impregnated with bovine serum albumin (BSA/TCP) induced 0%-8% incorporation, or only marginal host bed reactive bone formation. The retention of unabsorbed TCP in the host bone four months after implantation suggests that further research should be encouraged to obtain a formulation of sintered calcium phosphate that could be resorbed more rapidly and in the process more completely replaced by bone.

#### 5. BMP POLYLACTIC POLYGLYCOLLIC ACID POLYMER COMPOSITE IN SPINAL FUSIONS IN DOGS.

To investigate the action of BMP on the growth of host bed derived bone in experimental spinal fusions, posterior intervertebral spinal fusions of the lower thoracic spine were performed in 13 mature mongrel dogs. The procedures were limited to single intervertebral levels. Three levels in each dog were employed for controls for BMP levels. The spinal columns were examined by radiohistomorphometric methods at three weeks, six weeks, 12 weeks, and six months, and showed the BMP levels to have two to three times more new bone than control levels. At the BMP level, an increase in the amount of bone was observed in the interval from three to 12 weeks, in contrast to a decrease seen at control levels. Fusion was present in 5/7 of the BMP levels compared with 0/7, 1/7, and 2/7 of control levels. The BMP level exhibited an increased number and volume of areas de novo cartilage and woven bone formation at all time intervals of dogs compared to all control levels. The PLA/PGA polymer was incompletely resorbed and partially retained in the fusion site. These preliminary observations suggest that BMP may serve as a useful adjunct in spinal fusions, but research is required to find a more rapidly degradable delivery system. The objective of BMP research

is to augment the host bed capacity for bone generation and regeneration for patients with missing bone substance from battle-incurred wounds.

#### 6. CRANIOTOMY DEFECTS IN SHEEP.

An aggregate of partially purified bovine bone morphogenetic protein (bBMP) and bone matrix insoluble noncollagenous proteins (iNCP) weighing a total of 100 mg of lyophilized BMP/iNCP, was implanted using ultra thin gelatin capsuled in skull trephine defects in adult sheep. One hundred milligram samples of freeze-dried bovine serum albumin (BSA) were similarly implanted in 18-20 mm trephine skull defects and also in posterior cervical muscle pouches for heterotopic controls. In two out of five sheep, the trephines were repaired with bone as early as four weeks after the operation. Eight to 12 weeks after surgery, repair was complete in the other three sheep. In the control contralateral trephines, one-third to one-half of the defect was incompletely repaired. Neither the BMP nor the BSA control implants induced bone formation in the muscle. While the BMP/iNCP prepared from bovine bone consistently induced regeneration in skull trephine defects, only fibrous tissue and no extraskeletal bone was induced to form in cervical muscle pouches in sheep.

#### 7. CRANIOTOMY DEFECTS IN MONKEYS.

Large cranial defects do not always heal spontaneously, especially in humans; often they have to be obturated with metallic or acrylic fillers. Bilateral cranial trephine defects, measuring 14-20 mm in diameter, were created in three Rhesus monkeys, providing a typical primate model system for investigations in comparative physiology of bone regeneration. Each skull had one control and one experimental

trephine defect. Control defects were implanted with bovine serum albumin (BSA). Experimental defects were implanted with 100-200 mg of a partially purified fraction of bovine bone morphogenetic protein (bBMP) in two monkeys. In one monkey, the BMP was incorporated in a 1:1 poly(lactic) poly(glycollic) acid copolymer, high-viscosity formula. The animals were killed at eight weeks, ten weeks, and 16 weeks after implantation. The tissue responses were analyzed by computed histomorphometry and routine histologic examination. At each time interval, the BMP implanted defects produced more complete regeneration than the control implants. The morphogenetic response occurred in the following sequences: mesenchymal cell proliferation, chondrogenesis, and increased bone formation. BMP-induced osteogenesis may initiate the regenerative process. The copolymer releases BMP but may constitute a barrier to the end stages of replacement by new bone and would be more useful in a low-viscosity rapidly biodegradable form.

#### 8. BMP DELIVERY SYSTEM OF BONE MATRIX NON COLLAGENOUS PROTEINS (BMP/NCP) IN A BONE TUMOR DEFECT.

A 29-year-old woman with an enchondroma that was expanding and eroding the palmar cortex of the middle phalanx was successfully treated by curettage and implantation of bone morphogenetic protein. The metaphysis and cortex were repaired by lamellar bone within two months. The medulla was completely filled with trabecular bone by nine months. The full range of motion and normal functions of finger and hand joints were restored, and there was no recurrence or abnormalities at follow-up visits 2.5 years after the operation.

#### 9. BMP/NCP IN A METAPHYSEAL DEFECT.

The response of protodifferentiated and differentiated bone cells to bovine bone morphogenetic protein (bBMP) was observed in implants in the adult rabbit distal femoral metaphysis. Bovine serum albumin and denatured bBMP were implanted in the contralateral femur of controls. The changes of the bone marrow reflected the reaction of protodifferentiated cells. The changes in preexisting trabecular bone tissue reflected the reaction of differentiated cells to bBMP. <sup>45</sup>Ca radioisotope quantitative methods demonstrated that the bone morphogenetic response was superimposed upon the reaction to the injury of surgical implantation. By the end of the fourth week, roentgenograms and histologic sections showed larger deposits of intrametaphyseal cartilage and bone in bBMP than in control implanted femurs. By the end of the eighth week, bone formation was associated with remodeling of the entire distal femur and expansion of the external diameter of the metaphysis. These observations indicate the need for investigation of perisinusoid and perivascular cells of periosteum, endosteum, and marrow stroma.

#### 10. OBJECTIVES AND SPECIFIC AIMS OF CLINICAL TRIALS OF BMP

Clinical experience is being documented on two groups of patients selected for preliminary clinical trials of implants of human bone morphogenetic protein (BMP) in an aggregate of associated bone matrix non-collagenous proteins BMP/NCP. One group of twelve patients illustrates the problem of intractable non-union of the femur. A second group consists of six cases of segmental tibia bone defects ranging from 3 to 17 cm in length. Many of the patients had been candidates for amputation.

This total series of eighteen patients was investigated with the objective of determining efficacy of BMP. The specific aim is to investigate the effects of BMP upon the process of regeneration of bone defects far beyond the critical dimensions for spontaneous repair in the lifetime of the individual. The bone defects were incurred in violent accidents of the character encountered in combat related fractures with missing substance.

Appended to this report is a sample copy of the informed consent procedures for patients treated under the guidelines of the Human Subject Protection Committee, University of California, Los Angeles, School of Medicine, Center for Health Sciences.

10A. REPAIR OF SEGMENTAL DEFECTS OF THE TIBIA WITH CANCELLOUS BONE AND BONE MARROW AUGMENTATION WITH HUMAN BONE MORPHOGENETIC PROTEIN (hBMP): A PRELIMINARY REPORT OF SIX PATIENTS.

Human bone morphogenetic protein (hBMP) is a bone cell differentiation inducing factor. Six patients with traumatic segmental tibial defects 3 to 17 cm developed solid union by implantation of hBMP, autogeneic cancellous grafts, and stabilization. There were no allergic, infectious, or surgical complications. If hBMP augmentation in biodegradable delivery systems can be established by a prospective, randomized, double blind investigation, the incidence of successful bone graft operations for treatment of large segmental defects would be measurably improved.

10B. BONE MORPHOGENETIC PROTEIN AUGMENTATION GRAFTING OF RESISTANT FEMORAL NON UNIONS.

Twelve patients with intractable nonunions of the femoral diaphyseal or metaphyseal-diaphyseal segments were successfully treated

by a combination of internal fixation and implants of human bone morphogenetic protein (hBMP). There was an average of 3.0 surgical procedures per patient attempting union prior to hBMP implantation. Union was obtained in 100% of patients, 11/12 with initial protocol and in one patient with a repeat stabilization and implantation of hBMP. Four patients received autogeneic cancellous bone graft and four patients received allogeneic bone grafts. The BMP implant was prepared in the form of an aggregate of hBMP and bone matrix water insoluble noncollagenous proteins (hBMP/iNCP). Fifty to 100 mg of hBMP/iNCP was either implanted in the fracture gap in ultra thin gelatin capsules, or incorporated in a strip of polylactic/polyglycollic acid copolymer (PLA/PGA) and placed as an onlay across the fracture gap. The average time to union was 4.7 months. Further clinical investigation should be performed on matched cases with and without BMP augmentation in order to distinguish hBMP effects from new or improved methods of fracture fixation and autogeneic cancellous bone grafts.

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Revised February 16, 1988

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Submitted for Publication:

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Revised: February 10, 1987

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CONSENT TO ACT AS A RESEARCH SUBJECT IN THE RESEARCH PROJECT: EVALUATION OF  
HUMAN BONE MORPHOGENETIC PROTEIN (hBMP)

I agree (my child/my ward agrees) to participate in the experimental study to determine whether hBMP will induce repair of my (his/her) by my (his/her) own bone. I have (my child/my ward has) a bone defect that may be cured by hBMP or by filling it with methalmethacrylate cement or by transplanting some of my (his/her) own bone from my (his/her) own hip. The purpose of the hBMP is to avoid the complications of removal of bone from my (my child's/ward's) own hip, or the pain, or disfigurement of an implant of cement which fills but does not heal my (his/her) bone defect. I understand that the circumstances for BMP treatment are failure of bones to heal after one or more conventional operations, or bone conditions for which there is no known cure. For example, patients with non-union of fractures, non-healing defects from bone tumors, old infections, congenital malformations that fail to heal are eligible for BMP treatment. I (my child/my ward) will be one of 50 patients to be treated with hBMP.

The potential risk is that my (my child's/my ward's) bone may fail to respond to hBMP and that I (my child/my ward) may need to have an implant of plastic cement or bone from my (his/her) own hip later. The potential benefits are that hBMP will stimulate my (his/her) own bone to grow.

I understand that as alternative treatment a plastic cement plug, a bone graft or stainless steel artificial skeletal parts are available but I (my child/my ward) choose(s) hBMP for treatment of my (his/her) condition which is known as \_\_\_\_\_. I am (my child/ward is) one of a few isolated cases selected on the basis of being likely to benefit from hBMP stimulation of new bone formation.

NSPC#86-12-592 • Date of Expiration: February 12, 1988

I have (my child/my ward has) the choice of having a plastic cement plug, or bone from my (his/her) own hip or leg, or bone donated by another individual, or the implant of hBMP. The hBMP is a purified protein that is the active ingredient of a bone graft. If the hBMP is used it will be placed in the bone defect in my (his/her) \_\_\_\_\_. The implantation of hBMP will be made at the same operation that treats my (his/her) \_\_\_\_\_, and does not require a separate operation unless, at the time of surgery, my (his/her) condition is found to require additional bone from another individual, or some bone removed from my (his/her) hip.

The part of the study that is experimental is the substitution of hBMP, a chemically purified crystalline protein for plastic cement bone graft, or stainless steel parts. The present experimental procedure consists of implantation of hBMP in an otherwise non-healing bone defect. The hBMP is obtained from human bone and is purified by a series of chemical fractionation processes which are known to remove all the non-BMP substances from the BMP. The transplantation antigens which are known to cause rejection of a bone graft are removed in the process of manufacturing hBMP.

HAZARDS. I (my child/my ward) understand(s) that the hazards of experimental hBMP concerns the possibility of toxicity and antigenicity. Toxicity means the quality of being poisonous. Toxic chemicals cause skin rash, nausea, allergic reactions, inflammation, tumors and other adverse reactions. None of these have been detected in animals of many different species treated with BMP. No toxicity has been found in experiments of 100 chickens, 200 mice, 2000 rats, 50 guinea pigs, 1000 rabbits, 6 dogs and 2 monkeys treated with BMP in the period from 1975 to 1982. These toxicity studies in animals have shown no observable organ damage and no allergic response. An allergic response means the rejection of the implanted BMP by local inflammation or a systemic reaction such as a skin rash, flushing of the skin, nausea, vomiting or fainting. None

of these reactions have been noted in animals thus far treated with BMP, nor in more than 10 human patients treated to date. Another hazard is the possibility of a wound infection from either the implant or the surgical operation on my (my child's/ward's) bone. Based on experiments on the animals listed above and more than 10 human patients successfully treated with hBMP, there is an 85% chance that hBMP will stimulate bone formation when implanted in a bone defect.

In 6 patients with 4 to 6 failed operations for non-healing leg bone fractures, all six have healed following implantation of hBMP. In one patient with a failure of treatment of a 2 1/2 year-old fracture of the femur, treated by electrical stimulation for 10 months, healing occurred within 6 months after implantation of hBMP. In another patient with a bone defect in the finger caused by excision of a bone tumor, the bone healed within three months after implantation of hBMP. In two patients with bone decay of the head of the femur (hip), decompression with implantation of hBMP has saved the patients from replacement of the joint with an artificial hip. No evidence of toxicity has been seen in these 10 patients in doses of 2.0 mg/kilo, possibly because hBMP is a normal constituent of the bone and blood. The effective dose is 0.1 mg/kilo in a phalangeal bone defect  $1.0^3$  cm in volume. These doses are 100 to 1000 times less than the safe doses in laboratory animals. The long term effect of hBMP in humans is not known.

Alternative procedures. Instead of using an implant of hBMP my (my child's/ward's) bone defect can be filled with plastic cement, or my (his/her) hip or leg may have a second operation for transfer of a bone graft into the bone defect in the \_\_\_\_\_. The plastic cement has been judged to be safe but it is not well tolerated because it does not bind to the bone but floats in the space between the bone and the soft parts and is a cause of pain and irritation. The bone that is transplanted from the patient's own hip is the best tolerated but requires a separate incision and a second

operation, which in turn can cause a painful scar, lump, infection, and/or blood clots. This is the reason it is desirable to test hBMP as a substitute for hip bone or leg bone transplants. I understand that I have (my child/my ward has) the option to have the transplant or plastic cement if the hBMP does not successfully induce repair of my (his/her) bone defect.

I have (my child/ward has) read the description of the experimental procedures to be used in this study as they are explained above, and I (my child/ward) give(s) my (his/her) consent to Marshall R. Urist, M.D. and his associate orthopedic and other surgical specialists to perform the operation named \_\_\_\_\_

I understand that as a consequence of my (my child/ward) signing, hBMP will be used.

Although I (my child/ward) now consent(s) to participate in this study by clinical follow-up visits, routine laboratory analyses, complete blood count, tests of kidney function and liver function, disability evaluations, and x-ray examinations for a period of 5 years, I (my child/ward) may elect to discontinue participation in this study at any time without prejudice to continued care or treatment. I (my child/ward) consent(s) to the blood tests that require 2 tablespoons of blood, and 24 hour samples of urine (not more than 3 times) for the above laboratory specimens when I am (he/she is) in the hospital. I (my child/ward) understand(s) that the potential hazards of venipuncture for blood collection in the above noted amounts are almost nil but could include pain, bleeding, light headedness, fainting, and rarely infection or blood clots.

I (my child/ward) agree(s) to have follow-up x-ray examinations at 2 month intervals for 6 months, and at one year intervals thereafter for 5 years. These x-rays are requested only at intervals that are not known to cause harmful side effects of radiation. Two to 5 years is the time necessary to determine all the

benefits of hBMP. I agree that I or my (my child's) insurance company will be responsible for all costs of the follow-up examinations. I understand that hBMP will be supplied at no cost to a third party carrier or to me (my child/my ward).

Information about hBMP will be available to me (my child/ward) at any time.

Any questions that may occur to me (him/her) will be answered at any time by Dr. Marshall R. Urist (Principal Investigator), 1000 Veteran Avenue, Room A3-34, Los Angeles, California 90024, (213) 825-6521, and/or his associates. Although the results of this study may be published, my (my child's/ward's) identity will not be disclosed unless (my child/my ward) I give(s) separate consent or unless required by law. I understand that if the investigational design or use of the information is to be changed, I will be so informed and my (his/her) consent reobtained. I do (my child/ward does) not expect a specific benefit from the use of hBMP other than to possibly help heal my (my child's/ward's) bone defect. It is my (his/her) understanding that the results of the investigation are personal and will be treated confidentially.

I understand that I (my child/ward has) have the right to refuse to participate in, or to withdraw from, this research at any time without prejudice to my (my child's/ward's) future medical care at UCLA.

I understand that circumstances may arise which might cause the investigator to terminate my (his/her) participation before completion of the study.

In signing this consent form, I acknowledge receipt of a copy of the form, as well as a copy of the "Subject's Bill of Rights."

I understand that if I am injured as a direct results of research procedures not done primarily for my own benefit, I will receive medical treatment at no cost. The University of California does not provide any other form of compensation for injury.

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I understand that if I have further questions, comments, or concerns about the study or the informed consent process, I may write or call the office of the Vice Chancellor-Research Programs, 3134 Murphy Hall, UCLA, Los Angeles, CA, 90024, (213) 825-8714.

\_\_\_\_\_  
Witnessed

\_\_\_\_\_  
Signed

\_\_\_\_\_  
Dated

\_\_\_\_\_  
Dated

\_\_\_\_\_  
Signature of Father or Guardian,  
patient is 18 years old or  
under.

\_\_\_\_\_  
Signature of Mother or Guardian, if if  
patient is 18 years old or under.

\_\_\_\_\_  
Signature of minor, if between  
12 and 18 years old.

\_\_\_\_\_  
Dated

HSPC #86-12-592

Expiration Date: February 12, 1988

UCLA HUMAN SUBJECT  
PROTECTION COMMITTEE

APPROVED FEB 18 1987

END

DATE

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