(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13971 A1

(51) International Patent Classification[7]: A61L 27/36

(21) International Application Number: PCT/US00/23342

(22) International Filing Date: 24 August 2000 (24.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/382,331 25 August 1999 (25.08.1999) US

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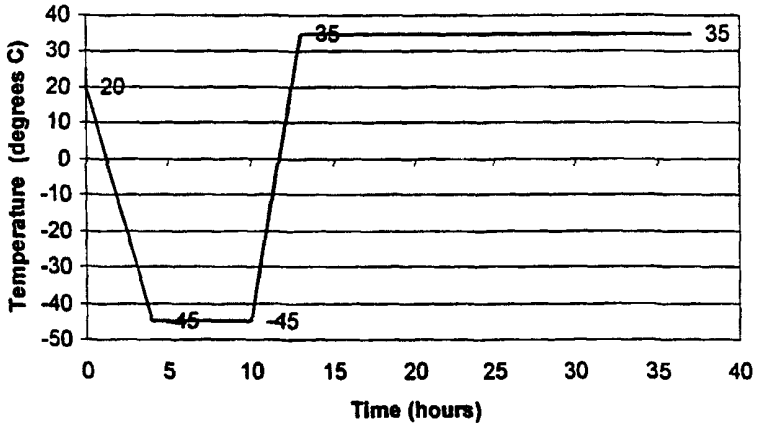
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF TREATING BONE FOR IMPLANTATION AND RESULTING BONE



(57) Abstract: Provided herein is a method for dehydrating and, optionally treating with a mechanical-strength conserving agent, a monolithic bone intended for implantation. The method serves to conserve at least one of the biomechanical properties of the bone during the dehydration of the bone and its subsequent packaging. Also provided is a monolithic bone for implantation and a method of using the bone for the repair of damaged bone.

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METHOD OF TREATING BONE FOR IMPLANTATION AND RESULTING BONE

FIELD OF THE INVENTION

Provided herein is a method for dehydrating and, optionally treating with a mechanical-strength conserving agent, a monolithic bone intended for implantation. The method serves to conserve at least one of the biomechanical properties of the bone during the dehydration of the bone and its subsequent packaging. Also provided is a monolithic bone for implantation and a method of using the bone for the repair of damaged bone.

BACKGROUND OF THE INVENTION

Bone grafting is widely used to treat fractures, non-unions and to induce arthrodeses. Autogenous cancellous bone, which is taken from one site in the graftee and implanted in another site of the graftee, is considered by many to be the most effective bone graft. Autogeneous cancellous bone provides the scaffolding to support distribution of the bone healing response. Autogeneous cancellous bone also provides the connective tissue progenitor cells which form new cartilage or bone. However, the harvest of autogenous bone results in significant cost and morbidity, including scars, blood loss, pain, prolonged operative and rehabilitation time and risk of infection. Furthermore, in some clinical settings, the volume of the graft required by the graft site can exceed the volume of the available autograft. Accordingly, alternatives to autografts have been developed in an attempt to reduce the morbidity and cost of bone grafting procedures.

The use of preserved bone intended for implantation to replace diseased or missing parts is common. The successful application of such bone is predicated on sound knowledge of its biologic properties and its capacity to withstand the stresses to which it will be subjected. When mineralized bone is used in grafts, it is primarily because of its inherent strength, i.e., its load bearing ability at the recipient site. The biomechanical properties of bone grafts upon implantation are determined by many factors, including the specific site from which the bone is taken; the age, sex, and physical characteristics of the donor; and the method chosen to prepare, preserve, and store the bone prior to implantation. A more detailed explanation of the alteration of the biomechanical properties of bone by the methods chosen for its preservation and storage may be found in Pelker et al., *Clin. Orthop. Rel. Res.*,174:54-57(1983). However, the needs for processing (e.g., to preserve the graft for later use and to remove immunogenic cellular materials) can conflict with the need to conserve the mechanical strength of the bone.

During the preparation of bone intended for implantation, the porous matrix is typically contacted with one or more treatment fluids to variously clean, defat, sterilize, virally inactivate, disinfect, and/or demineralize the bone or to impregnate the bone with one or more pharmacological agents (antibiotics, bone growth factors, etc.) so the bone can act as a drug delivery system. See U.S. Patent No. 5,846,484 for a detailed explanation of the treatment of bone intended for implantation. Some treatment processes, such as irradiation and lyophilization, can work against conservation of the mechanical strength of bone and can lessen the bone's weight bearing properties. Processing requirements can also create dimensional changes in the allograft bone. Such changes of dimension can create damage within the tissue,

and may also make it difficult for a machined piece to mechanically engage with surgical instruments, other allografts, or the prepared surgical site. Treatment processes also can have a deleterious effect on such important mechanical properties as toughness. Implants demonstrating improved toughness are important as the insertion of some allografts can be quite energetic, e.g., the hammering in of cortical rings used in spinal fusion surgery.

It is generally accepted that freezing monolithic bone to temperatures as cold as -70° C. prior to its packaging and storage results in little if any alteration in its physical properties. However, freezing bone as a preservation technique is costly and can be logistically difficult, e.g., shipping and storage. Lyophilization (freeze-drying, i.e., freezing, then sublimation of moisture) is commonly performed on bone to permit its shelf storage for up to several years without spoilage. Lyophilization removes excess moisture from the bone and reduces its antigenicity. According to the American Association of Tissue Banks ("A.A.T.B"), lyophilized whole bone containing no more than 6% moisture can be stored at ambient temperatures for up to five years after processing. However, adverse changes in the biomechanical properties of the bone have been found to result from the lyophilization procedure. Lyophilization can result in damage to the bone due to dimensional changes that occur during the freezing and dehydrating operations. The adverse mechanical changes appear to be associated with damage occurring in the bone matrix, specifically, ultrastructural cracks along the collagen fibers. These effects appear to be magnified when lyophilization and gamma irradiation are used together. Studies using rat bones to model the effects of lyophilization upon the compressive properties of cancellous bone (compression strength of tail vertebrae) and the bending and

torsional properties of the long bones indicate that compressive strength can be reduced by up to 30% with little or no change in stiffness, bending strength can be reduced by as much as 40%, and torsional strength can be reduced by up to 60%. These changes, resulting in bone that is brittle, have been found to occur even after the bone has been rehydrated. A more detailed explanation of the effects of lyophilization on mineralized bone can be found in Kang et al., *Yonsei Med J* 36:332-335(1995), and Pelker et al., *J. Orthop. Res.* 1:405-411(1984). Thus, a problem exists in providing a bone intended for implantation that is both tough and convenient to store and maintain.

Because freezing and thawing bone is minimally damaging to the bone, whereas lyophilization results in reduction in the mechanical strength of the bone, it is the inventors' belief that toughness is maintained and/or enhanced in the bone by dehydrating the bone using methods that remove the water associated with the bone while diminishing the dimensional changes associated with lyophilization. It is also believed that, when used, a mechanical strength-conserving agent is not acting as a cryopreservative (i.e., minimizing crystal growth during freezing) but rather in some new, not entirely understood, manner to diminish the dimensional changes associated with lyophilization. The dehydration of tissue, for example, by treatment with an anhydrous polar organic solvent such as ethanol, is known. See, for example, U.S. Pat. No. 5,862,806 to Cheung. However, it has not been previously appreciated that applying this technique to bone results in an implant having improved biomechanical properties, e.g., toughness. Thus, it is desirable to provide a method for dehydrating monolithic bone intended for implantation prior to its packaging and storage that will better conserve the biomechanical properties of the bone, i.e., its toughness and/or

dimensions as compared to lyophilized bone, from the time the bone is harvested through the packaging and storage operations and to time of implantation.

It has been determined by the inventors herein that the diminishment of dimensional changes is related to the extent to which the mechanical strength-conserving agent, when used, has penetrated the bone before or during the dehydration process. Similarly, an improvement in the toughness of the implant has been seen to be related to the extent to which the mechanical strength-conserving agent, when used, has penetrated the bone before or during the dehydration process, providing further evidence of the advantage of one aspect of the invention herein.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a method for dehydrating monolithic bone intended for implantation in order to conserve the biomechanical properties of the bone during its dehydration process and subsequent packaging and storage and to substantially maintain such biomechanical properties throughout its rehydration and subsequent implantation.

It is a further object of the invention to provide a method of dehydrating monolithic bone that reduces the dimensional change associated with the lyophilization of bone.

It is a further object of the invention to provide a method of dehydrating monolithic bone that improves the toughness of the bone graft.

It is a further object of the invention to provide a method of dehydrating monolithic bone with minimal negative impact to the biological properties of the bone graft.

It is a further object of the invention to provide a treatment that acts as an antimicrobial/preservative agent.

It is a further object of the invention to provide a method for packaging dehydrated monolithic bone so that the bone may be stored at ambient temperatures for an extended period of time, e.g., up to five years or longer without excessive loss of its toughness.

It is a further object of the invention to provide a method for the rehydration of dehydrated monolithic bone such that the toughness of the bone at the time of its implantation is optimized.

It is a further object of the invention to provide a method that minimizes the tendency for a partially rehydrated graft to fracture due to the insertion forces applied by the surgeon.

It is a further object of the invention to provide a dehydrated monolithic bone implant optionally containing one or more medically/surgically useful substances, e.g., an osteogenic material such as bone morphogenic proteins (BMPs) and platelet derived growth factor (PDGF).

It is a further object of the invention to provide a method that decreases the time necessary to rehydrate a lyophilized or otherwise dehydrated bone intended for implantation.

These and other objects not specifically set forth above will be apparent to those skilled in the art in view of the objects set forth above and the foregoing specification.

In keeping with these and related objectives of the invention, there is provided a method for dehydrating monolithic bone intended for implantation to conserve the

biomechanical properties of the bone during the dehydration process and subsequent packaging and to maintain such biomechanical properties during the storage of the bone preceding its implantation. The method comprises:

- dehydrating without lyophilizing the monolithic bone; and,
- packaging the dehydrated bone.

The method can further comprise prior to or during the step of dehydrating without lyophilizing, or, when utilized prior to lyophilizing:

- contacting the bone with a mechanical strength-conserving amount of at least one biocompatible mechanical strength-conserving agent, said agent being a liquid organic material or solution, mixture, or suspension thereof, which is capable of penetrating and remaining in the bone during the dehydration process, packaging and storage.

In another aspect, the invention includes the dehydrated bone obtained by the foregoing method(s) and use of obtained by the invention herein.

The expression "monolithic bone" as utilized herein refers to relatively large pieces of human or animal bone, i.e., pieces of bone, autograft, allograft or xenograft, that are of such size as to be capable of withstanding the sort of mechanical loads to which functioning bone is characteristically subjected. The monolithic bone of this invention is to be distinguished from particles, filaments, threads, etc. as disclosed in U. S. Patent Nos. 5,073,373, 5,314,476 and 5,507,813, which, due to their relatively small dimensions, are incapable of sustaining significant mechanical loads, either individually or in the aggregate. It is further to be understood that the expression "monolithic bone" refers to fully mineralized bone, i.e., bone with its full natural level of mineral content, and to such bone that has been demineralized to some minor

extent, i.e., to an extent which reduces the original strength of the bone by no more than about 50 percent. The monolithic bone can be provided as a single integral piece of bone or as a piece of bone permanently assembled from a number of smaller bone elements, e.g., as disclosed and claimed in U.S. Patent No. 5,899,939 the contents of which are incorporated herein by reference. Although monolithic bone can contain factors which are osteogenic, monolithic bone can also contain additional materials, e.g., as disclosed in U.S. Patent No. 5,290,558 the contents of which are incorporated herein by reference, which will remain with the bone after its rehydration and will be present at the time of implantation.

The expression "mechanical strength" as utilized herein is intended to mean any one of the principal biomechanical properties of bone, specifically including compression strength, flexural modulus, torsional modulus and yield strength, as well as the sum of these properties, that are characteristic of bone.

The expression "toughness" as utilized herein is intended to refer to any characteristic that qualitatively can be described as the way in which the bone fails, i.e., how the bone undergoes deformation prior to fracture. For example, bone that exhibits improvement in toughness would be more desirable than bone having less toughness. Quantitatively, "toughness" as utilized herein is a measure of the energy absorbed by the osteoimplant prior to breakage and is expressed in units of force times length, such as Newton-millimeters (N-mm).

The expressions, "toughness-enhancing", "conserving the toughness of the bone", "conserving the mechanical strength of the bone" and expressions of like import shall be understood herein to mean that the monolithic bone dehydrated in accordance with the invention shall demonstrate at least greater than about 5%

increase in toughness as compared to bone that has been lyophilized. That is, bone dehydrated in accordance with the invention herein will demonstrate improved ability to withstand the forces occurred during implantation as compared to bone that is lyophilized.

The expression "dimensional-conserving" and expressions of like import shall be understood herein to mean that the monolithic bone treated in accordance with the invention, shall demonstrate at least about 2% less decrease in length dimension as compared to bone that has been lyophilized. That is, bone dehydrated in accordance with the invention herein will demonstrate less shrinkage after dehydration than lyophilized bone.

The term "biocompatible" and expressions of like import shall be understood to mean the absence of an undesired stimulation of a severe, long-lived or escalating biological response to an implant and is distinguished from a mild, transient inflammation which accompanies implantation of essentially all foreign objects into a living organism and is also associated with the normal healing response. Materials useful to the invention herein shall be considered to be biocompatible if, at the time of implantation, they are present in a sufficiently small concentration such that the above-defined condition is achieved.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 represents a monolithic bone processed according to the invention herein.

Figure 2 is a representation of a ramp-shaped implant.

Figure 3 is a photograph of a freeze-dried monolithic bone after it was subjected to compression to fracture.

Figure 4 is a photograph of a frozen monolithic bone after it was subjected to compression to fracture.

Figure 5 is a graphical representation of a standard freeze-drying process.

Figure 6 is a graphical representation of an alternative freeze-drying process in which the tissue is subjected to some level of dehydration prior to freezing and sublimation of any remaining moisture.

Figure 7 is a graphical representation of the dimension change of a bone implant prepared as in Example 5.

Figure 8 is a graphical representation of the treatment effects on dimensional change.

Figure 9 is a graph of the average degree of fragmentation by donor.

Figure 10 is a graph of the average degree of fragmentation by treatment.

Figure 11 is a graph of the percentage dimensional change by treatment.

DETAILED DESCRIPTION OF THE INVENTION

Bone for implantation is obtained, e.g., aseptically in a morgue or an operating room from a cadaver donor or from a living donor's tissue obtained by surgical excision or amputation. The bone is cleansed, e.g., using 70% ethanol and washed with water for injection and sonification. This use of alcohol in the initial processing of bone for implantation is common to the industry and may have some dehydrating effect. However, the dehydrating effect, if any, is negligible and would not bring the bone within the less than 6% water content required by the A.A.T.B. prior to storage of bone intended for implantation. The bone may be treated with antibiotics such as polymyxin B sulfate, bacitracin, and/or gentamicin, and may contain trace amounts of residual antibiotics. Cleansing, cutting, sizing, shaping, container sterilization, filling,

lyophilization, and stoppering functions may be performed under conditions following industry standards for tissue handling. The bone employed in the invention is of monolithic proportions in contrast to "particles," "filaments," "threads," "strips," etc., as described in U.S. Patent Nos. 5,073,373, 5,314,476 and 5,507,813. Thus, the bone treated according to the method of the invention is generally a relatively large piece or segment of donor bone and is intended for implantation into a correspondingly relatively large defect or other implantation site. Typically, the bone herein will possess dimensions of length on the order of about 2 mm to about 500 mm and preferably at least about 5 mm to about 100 mm. Similarly, dimensions of width will be on the order of about 1 mm to about 600 mm and preferably at least about 1 mm to about 100 mm. Dimensions of thickness will be on the order of about 1 mm to about 30 mm and preferably at least about 1 mm to about 10 mm. Any one of several methods, including but not limited to, cutting, forming and machining can readily obtain such bone.

The expressions "dehydrating liquid", "volatile solvent" and other expressions of like import as utilized herein may be used interchangeably and are intended to refer to any suitable solvent or mixture of solvents having a vapor pressure at relevant temperature, i.e., the temperature at which dehydration takes place, such that the solvent is readily passed off by evaporation. It shall be understood that such terms when used interchangeably with respect to a specific liquid or mixture of liquids is intended to refer to the specific function, e.g., dehydrating liquid and/or volatile solvent, that is being performed at the time (depending on the specific conditions of its use) and may involve more than one function at the same time. That is, a suitable liquid may serve as both a dehydrating liquid and/or volatile solvent and such

function may occur simultaneously. Such dehydrating liquid(s) will be suitable even if ordinarily considered to be toxic so long as the amount of dehydrating liquid, if any, present at the time of implantation does not produce a toxic response. Examples of dehydrating liquids useful in the invention herein would include but not be limited to, polar organic solvents, e.g., alcohol, typically a low molecular weight alcohol such as methanol, ethanol, isopropanol, butanol, isobutanol, ethylbutanol, acetonitrile, pyridine, industrial methylated spirit, etc.; histological solution, e.g., Flex 100™; polar solvent, e.g., dimethylsulfoxide, small ketones, acetone; chloroform; methylene chloride and ethylene chloride; straight chain hydrocarbons, e.g., hexane, pentane and similar alkanes; low molecular weight alkenes; esters; ether, e.g., ethyl ether, tetrahydrofuran, dioxane, ethylene glycol monoethyl ether, crown ethers, etc.; aldehyde or solutions containing aldehydes, e.g., formaldehyde, formalin, etc., at low temperatures such that cross-linking does not proceed; super critical fluids, e.g., carbon dioxide or hydrogen sulfide at supercritical pressures, mixtures of any of the above liquids, etc. Such dehydrating liquids may be selected in accordance with the present virtue of their ability to extract water from the bone, while not reacting adversely with the bone. Such dehydrating liquids can preferably be present in a graded series of aqueous concentration, e.g., 60% ethanol, 70% ethanol, 95% ethanol, absolute ethanol, which serves to promote the removal of the water associated with the monolithic bone.

The dehydrating liquid should have a viscosity at 20° C. of no greater than about 1410 cps, preferably the viscosity is between about 2 and about 300 cps. The preferred dehydrating liquid is a series of graded dehydrating alcohols .

In an embodiment of the invention herein, prior to lyophilization or other dehydration methods, the prepared bone is contacted with a mechanical strength-conserving amount of a biocompatible mechanical strength-conserving agent. The biocompatible mechanical strength-conserving agent appropriate to the invention is a compound or solution that is liquid at the temperature at which it is contacted with the bone, more preferably from about 5°C. to about 65°C., and which penetrates the small pores of the bone remaining therein after lyophilization or otherwise being dehydrated. The conserving agent is biocompatible and nontoxic and does not substantially interfere with the normal healing of the graft. A suitable conserving agent will meet these criteria even if mixed with water or other volatile solvent and then subsequently the water or solvent is removed during lyophilization or other dehydration method(s) leaving the conserving agent behind, i.e., it has a eutectic point significantly below the freezing point of water and/or a vapor pressure less than that of the volatile solvent. Suggested classes of conserving agent would include polyhydroxy compound, polyhydroxy ester, fatty alcohol, fatty alcohol ester, fatty acid, fatty acid ester, liquid silicone, mixtures thereof, and the like.

Examples of suitable conserving agent include, but are not limited to:

(i) Polyhydroxy compound, for example, glycerol, 1,4-butylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, propylene glycol, dipropylene glycol; polysaccharides and their derivatives, e.g., hyaluronic acid; polyoxyethylene-polyoxypropylene copolymer, e.g., of the type known and commercially available under the trade names Pluronic and Emkalyx; polyoxyethylene-polyoxypropylene block copolymer, e.g., of the type known and commercially available under the trade name Poloxamer; alkylphenolhydroxypolyoxyethylene, e.g., of the type known and

commercially available under the trade name Triton, and the like. (ii) Polyhydroxy ester, for example, monoacetin, triacetin, poly(oxyalkylene) glycol ester, and the like. (iii) Fatty alcohol, for example primary alcohols, usually straight chain having from 6 to 13 carbon atoms, including caproic alcohol, caprylic alcohol, undecyl alcohol, lauryl alcohol, and tridecanol. (iv) Fatty alcohol ester, for example, ethyl hexyl palmitate, isodecyl neopentate, octadodecyl benzoate, diethyl hexyl maleate, and the like. (v) Fatty acid having from 6 to 11 carbon atoms, for example, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid and undecanoic acid.

(vi) Fatty acid ester, for example, polyoxyethylene-sorbitan-fatty acid esters; e.g., mono-and tri-lauryl, palmityl, stearyl, and oleyl esters; e.g., of the type available under the trade name Tween from Imperial Chemical Industries; polyoxyethylene fatty acid esters; e.g., polyoxyethylene stearic acid esters of the type known and commercially available under the trade name Myrj; propylene glycol mono- and di-fatty acid esters such as propylene glycol dicaprylate; propylene glycol dilaurate, propylene glycol hydroxy stearate, propylene glycol isostearate, propylene glycol laureate, propylene glycol ricinoleate, propylene glycol stearate, and propylene glycol caprylic-capric acid diester available under the trade name Miglyol; mono-, di-, and mono/di-glycerides, such as the esterification products of caprylic or caproic acid with glycerol; e.g., of the type known and commercially available under the trade name Imwitor; sorbitan fatty acid esters, e.g., of the type known and commercially available under the trade name Span, including sorbitan-monolauryl, -monopalmityl, -monostearyl,-tristearyl, -monooleyl and trioleylesters; monoglycerides, e.g., glycerol mono oleate, glycerol mono palmitate and glycerol monostearate, for example as known and commercially available under the trade names Myvatex,

Myvaplex and Myverol, and acetylated, e.g., mono-and di-acetylated monoglycerides, for example as known and commercially available under the trade name Myvacet; isobutyl tallowate, n-butylstearate, n-butyl oleate, and n-propyl oleate. (vii) Liquid silicone, for example, polyalkyl siloxanes such as polymethyl siloxane and poly(dimethyl siloxane) and polyalkyl arylsiloxane.

As stated above, the suitable biocompatible mechanical strength-conserving agent selected from the examples above preferably is capable of penetrating the small pores of the bone. Therefore, optionally, a solution of a conserving agent can be utilized. This solution can be aqueous or can be one utilizing a polar organic solvent or other volatile solvent such as those described herein above. Such volatile solvents or dehydrating liquids, when present prior to dehydration, will typically represent between about 20 to about 80, preferably about 30 to about 60 percent by volume of the biocompatible mechanical strength-conserving agent solution. Of course, it will be understood by those skilled in the art that any of the above liquids or mixtures thereof may be acting in a variety of functions such as a dehydrating liquid and/or volatile solvent and/or toughness enhancing agent and/or dimensional-conserving agent depending upon the specific conditions at the time of use. It is to be understood that such variety of function is intended to be within the scope of the invention herein.

The biocompatible mechanical strength-conserving agent, neat or solution, should have a viscosity at 20° C. of no greater than about 1410 cps, preferably the viscosity is between about 2 and about 300 cps. The preferred biocompatible mechanical strength-conserving agent is glycerol, more preferably a 50% aqueous or alcoholic solution of glycerol, most preferably a series of graded dehydrating alcohols and glycerol.

The bone is contacted with a mechanical strength-conserving amount of the mechanical strength-conserving agent and/or dehydrating liquid in a suitable container, e.g., a 120 ml or 500 ml bottle, optionally with mechanical stirring. Optionally, the conserving agent and/or dehydrating liquid can be applied by infusing, e.g., employing a pressurized system such as that described in U.S. Patent No. 5,846,484 the contents of which are incorporated herein by reference. Pretreatment of tissues using the process described in U.S. Pat. No. 5,846,484 can improve the speed at which the strength-conserving agent penetrates the tissue. Optionally, the conserving agent and/or dehydrating liquid can be contacted with the bone in the presence of a low pressure atmosphere such as that described in U.S. Patent No. 5,513,662 the contents of which are incorporated herein by reference or in the low pressure atmosphere provided by vacuum packaging the bone and strength-conserving agent utilizing a vacuum sealer. Optionally, the conserving agent and/or dehydrating liquid can be contacted with the bone in the presence of alternating vacuum and positive pressure such as that provided by the Hypercenter™ XP Enclosed Tissue Processor commercially available from Shandon Lipshaw USA or preferably a Sakura Tissue-TEK® VIP™ vacuum infiltration tissue processor commercially available from Sakura FineTek, USA. As one skilled in the art will readily appreciate, the optimal times and levels of alternating vacuum-positive pressure or varying positive pressure can be determined through routine experimentation. The tissue processor allows for the simultaneous contacting of the bone with a mechanical strength-conserving agent and/or dehydrating of the bone when a graded series of dehydrating agents is used as the volatile solvent for the strength-conserving agent. Such simultaneous

contacting/dehydrating may result in an implant having better mechanical properties than one that is lyophilized after being contacted with a strength-conserving agent.

To assist the mechanical strength-conserving agent and/or dehydrating liquid in penetrating the small pores of the bone, the bone and agent can be advantageously subjected to sonication. It has been determined that contacting the bone with strength-conserving agent in an ultrasonic bath improves the penetration of the agent into the tissue. Sonicating bone is well known in the art and is described in U.S. Patent 5,797,871 the contents of which are incorporated herein by reference. Of course, it will be understood by one skilled in the art, that the contacting of the bone with the strength-conserving agent and/or dehydrating liquid can be carried out by any combination of one or more of the foregoing.

After the bone has been in contact with the conserving agent and/or dehydrating liquid for a period of about 5 minutes to about 7 days, preferably at least about one hour, it can optionally be shaped prior to dehydration. Such shaping can be accomplished by cutting, forming, machining or other method of shaping bone. Thus, the bone can be rough cut, processed with strength-conserving agent, further shaped as desired, then subjected to further processing if necessary. Such shaping of bone intended for implantation is well known in the art and is described in U.S. Pat. No. 6025538 the contents of which are incorporated herein by reference.

After shaping or other optional processing steps, the bone intended for implantation may be further dehydrated following procedures well known in the art. For example, the bottle containing bone and conserving agent is initially frozen to -76°C. with the bone and conserving agent later being subjected to a vacuum of less than 100 millitorr while the temperature is maintained at or below -35°C. The end

point of the dehydration procedure is the determination of residual moisture of approximately 6% or less. Once the bone has been dehydrated, it is stored in sealed, vacuum-contained, bottles prior to its reconstitution and use although, of course, any suitable packaging means, e.g., polymeric tray packages (inner and outer sealed trays), are entirely suitable and are envisioned as being within the scope of the invention herein.

Other methods of dehydrating and/or dehydrating liquid removal would include but not be limited to: contacting the bone with a graded series of dehydrating liquids; contacting the bone with dehydrating liquid in the presence or absence of a desiccant, e.g., molecular sieve, anhydrous calcium chloride, anhydrous silica gel, etc.; subjecting the bone to microwave energy such as described in U.S. Pat. No. 4,656,047 the contents of which are incorporated by reference herein; subjecting the bone to heat at ambient or sub-atmospheric pressures, e.g., drying oven at temperatures from about 35°C. to about 85°C., preferably about 40°C. to about 50°C., or vacuum oven at temperatures from about 35°C. to about 85°C., preferably about 40°C. to about 50°C.; subjecting the bone to sub-atmospheric pressure in the presence or absence of a desiccant, e.g., closed container subjected to vacuum optionally containing a desiccant such as anhydrous calcium chloride, anhydrous silica gel or the like; subjecting the bone to ambient temperatures at ambient or sub-atmospheric pressures such as typically found in a laboratory bench-top or conventional fume hood; alternative lyophilization procedures such as starting the lyophilization cycle at a higher temperature to dehydrate the tissue then reducing the temperature and pressure to freeze the tissue and sublimate any remaining moisture as described in Balderson, et al., *The effects of freeze-drying on the mechanical properties of human*

cortical bone, 45th Annual Meeting of the Orthopaedic Research Society, : 785, (1999), the contents of which are incorporated by reference herein; or by a combination of one or more of the foregoing. It will be understood that all references to vacuum herein, unless otherwise specified, refer to vacuum pressures as are usually provide by standard sources of laboratory vacuum, e.g., vacuum pump, air-water venturi device, etc.

The monolithic bone treated in accordance with the invention, i.e., lyophilizing or otherwise dehydrating such bone in the presence or absence of a mechanical strength-conserving agent or dehydrating without lyophilizing the bone, will exhibit a level of mechanical strength which is at least about 5%, preferably at least about 15%, and more preferably at least about 30% greater than that of a comparable specimen of monolithic bone which has been lyophilized in the absence of a mechanical strength-conserving agent. In addition, bone dehydrated according to the invention herein shows at least about 2 % less decrease in overall length dimension after dehydrating as compared to bone that has been lyophilized without being treated according to the invention herein. Further, bone treated according to the invention herein shows at least greater than about 5%, more preferably at least greater than about 19% improvement in overall toughness after dehydrating as compared to bone that has been lyophilized without being treated according to the invention herein. At this point, the bone can optionally be further shaped prior to packaging.

There are a variety of conditions by which dehydrated bone can be rehydrated prior to implantation. Soaking the dehydrated bone in rehydrating solution at normal atmospheric pressure can perform rehydration. Alternatively, the dehydrated bone can be rehydrated in a low atmospheric pressure environment, for example, the

rehydration solution can be introduced via hypodermic needle through the sealed rubber stopper. It is envisioned that implantation of the bone of the invention herein may at times proceed without prior rehydration. Such implantation is encompassed by the invention herein.

The rehydration solution can be any of a number of suitable agents such as sterile water, normal saline, physiologically buffered saline, dextrose solution, antibiotic solutions, and others of this sort. Optionally, it can contain one or more wetting agents such as any of the Pluronic™ agents or any of a variety of medically/surgically useful substances such as antiviral agents, particularly those effective against HIV and hepatitis; antimicrobials and/or antibiotics such as erythromycin, bacitracin, neomycin, penicillin B, tetracycline, viomycin, chloromycetin and streptomycin, cetazolin, ampicillin, azactam, tobramycin, clindamycin, gentamicin, etc.; amino acids, peptides, vitamins, inorganic elements, co-factors for protein synthesis; hormones; endocrine tissue or tissue fragments; synthesizers; enzymes such as collagenase, peptidases, oxidases, etc.; polymer cell scaffolds with parenchymal cells; angiogenic drugs and polymeric carriers containing such drugs; antigenic agents; cytoskeletal agents; bone morphogenic proteins (BMPs), transforming growth factor (TGF-beta), insulin-like growth factor (IGF-1), (IGF-2); platelet derived growth factor (PDGF), growth hormones such as somatotropin, etc.

The rehydrated monolithic bone prepared according to the invention herein is intended to be applied at a bone defect site, e.g., one resulting from injury, defect brought about during the course of surgery, infection, malignancy or developmental malformation. The bone, suitably sized and shaped as required, can be utilized as a graft or replacement in a wide variety of orthopedic, neurosurgical and oral and

maxillofacial surgical procedures such as the repair of simple and compound fractures and nonunions, external and internal fixations, joint reconstruction such as arthrodesis, general arthroplasty, cup arthroplasty of the hip, femoral and humeral head replacement, femoral head surface replacement and total joint replacement, repairs of the vertebral column including spinal fusion and internal fixation, tumor surgery, e.g., deficit filling, discectomy, laminectomy, excision of spinal cord tumors, anterior cervical and thoracic operations, repair of spinal injuries, scoliosis, lordosis and kyphosis method of dehydrating monolithic bones, intermaxillary fixation of fractures, mentoplasty, temporomandibular joint replacement, alveolar ridge augmentation and reconstruction, onlay bone grafts, implant placement and revision, sinus lifts, etc. Specific bones which can be repaired with the bone-derived implant herein include the ethmoid, frontal, nasal, occipital, parietal, temporal, mandible, maxilla, zygomatic, cervical vertebra, thoracic vertebra, lumbar vertebra, sacrum, rib, sternum, clavicle, scapula, humerus, radius, ulna, carpal bones, metacarpal bones, phalanges, ilium, ischium, pubis, femur, tibia, fibula, patella, calcaneus, tarsal and metatarsal bones.

The invention will be more fully understood by way of the following examples that are intended to illustrate but not limit methods in accordance with the present invention.

Example 1

Human diaphyseal segments from the humerus were treated for 72 hours in an ultrasonic bath containing 50% (v/v) aqueous glycerol. An M-4 threaded hole was drilled and tapped into the cortex of the diaphyseal bone. The specimens were then frozen at – 40°C. following this first phase of treatment. Specimens were then treated

by one of two processes in a Virtis Unitop 600L lyophilization unit. The first used a standard freeze-then-dry (FD) process that sublimates the water off from the frozen specimen. The time-temperature relationship for this process is outlined in figure 5. The second process uses a dry-then-freeze (DF) process that evaporates off much of the liquid prior to freezing and sublimation of the remaining liquid. The time-temperature relationship for this process is outlined in figure 6. It has been reported by Balderson, et al., *The effects of freeze-drying on the mechanical properties of human cortical bone*, 45th Annual Meeting of the Orthopaedic Research Society, : 785, (1999), that bone specimens using such a lyophilization procedure as the second process tend to have superior toughness and other mechanical properties.

Example 2

Human cortical bone specimens from the diaphysis of a long bone are manufactured into the shape of a threaded cylindrical dowel. Specimens are then treated in a stirred 60% propylene glycol/40% ethanol solution, while in an oven at 35° Celsius for at least 24 hours. At the end of 24-48 hours, the specimen is removed from the solution, and is then placed in a vacuum oven at 30° Celsius and standard laboratory vacuum. The specimen remains in the oven for a period of time necessary to evaporate off the remaining solvent, to remove the remaining water from the tissue, and to allow adherent treatment solution to penetrate. This time is determined by standard assays of solvent content and of moisture content. The samples are then packaged for surgical use.

Example 3

Human cortical bone specimens, prepared by cutting on a band saw into strut allografts, are placed into the retort chamber of an automated tissue-processing

machine, such as the Sakura Tissue-Tek® VIP™ tissue processor (Sakura FineTek, USA, Torrance, CA). Solutions of the following compositions (Table 1) are automatically pumped into the retort chamber for at least 4 hours per solution. This sequence of alcohol/glycerol solutions will concurrently dehydrate the tissue, while at the same time replacing the moisture with glycerol. Solutions are applied using alternating pressure (0.35 kg/cm^2 – 90 seconds) followed by ambient pressure (30 seconds), then vacuum (50 cm Hg – 90 seconds), then ambient pressure (30 seconds) in a cycle to assist and to speed fluid penetration into the tissues. Following the last solution, specimens are taken out of the retort, and placed into a bell jar with a vacuum attachment. The remaining volatile alcohol solvent is evaporated off into a hood, until only trace alcohol remains.

Table 1

Solution	Time (hr)	Temperature (°C)	Pressure/Vacuum
70% ethanol/30% water	4	40	ON
95% ethanol/5% water	4	40	ON
95% ethanol/5% glycerol	4	40	ON
70% ethanol/30% glycerol	4	40	ON
50% ethanol/50% glycerol	4	40	ON

Example 4

Human cortical bone specimens, prepared by cutting on a bandsaw into diaphyseal cross-sections, are placed into a closed container with a 50% ethanol/50% glycerol solution. The specimen is stirred continuously for 24-48 hours, then the container is opened under a hood to allow the volatile ethanol solvent to evaporate at ambient pressure. Specimens are then removed from the container, and placed into a

Virtis Unitop 600L lyophilization unit, using a standard lyophilization procedure, to complete the dehydrating/solvent removal steps.

Example 5

Five ramp shaped graft pieces (described in figure 2) were prepared from human bone tissue that had been treated for viral inactivation using processes described in US Patent No. 5,846,484. Specimens were individually placed in Kapak™ bags, and partially filled with 50% (v/v) aqueous glycerol solution. The bags were then sealed in a vacuum sealing device so that the air was removed prior to sealing and the implants were each surrounded by the glycerol solution at a reduced pressure. Specimens were allowed to equilibrate in the solution overnight, and were then removed from the Kapak™ bags. Specimens were then freeze-dried in a Virtis Unitop™ 600L lyophilization unit using standard methods. Dimensions were measured prior to treatment, and following treatments and with rehydration in physiological saline after 1.5 hours. Figure 7 shows the percent difference from fresh cut dimensions. Specimens were also visually checked for warpage or deformation of the ridges. None was found. Specimens were also checked to determine whether they would accept a mating screw into the threaded hole. All specimens did accept a threaded screw.

Example 6

Samples of the monolithic bone of figure 1 were obtained utilizing the same donor material. Initial measurements of 6 sites were determined. The samples were then processed to dehydrate in a VIP® tissue processor according to the conditions given in Table 2 below.

Table 2

Step		Concentration	Time (hr)	Temperature (°C)
1	Ethanol	70%	4:00	45
2	Ethanol	70%	4:00	45
3	Ethanol	95%	4:00	45
4	Ethanol	95%	4:00	45
5	Flex 100	100%	4:00	45
6	Flex 100	100%	4:00	45

After processing, the specimens were removed from the VIP® processor and placed in a closed container under continuous vacuum for about 4 hours to outgas any remaining solvent.

Another specimen was treated using the process of Table 2. At the end of the process, the specimen was allowed to outgas for 4 hours 20 minutes in a vacuum desiccator. The specimen was weighed to 0.0001 grams.

In order to determine the level of dessication, the specimen was then placed in a lyophilization unit overnight to remove any remaining water from the specimen. After removal from the lyophilization unit, the specimen was again weighed. The difference indicated a water content of 3.45% water by weight for the alcohol-treated specimen.

After being rehydrated for about 5 minutes, the specimens were again measured to determine the existence of any dimensional changes. The percentage change in dimension was determined by calculating the difference between the pre-rehydration dimension and the initial dimension divided by the initial dimension and

multiplied by 100. The results of this measurement are given in Table 3 below. All values are in percentage units relative to the initial dimension.

Table 3

Alcohol only	Anterior Height	Posterior Height	Slot Width	Slot Height	Anterior/Posterior Diameter	Medial/Lateral Diameter
Specimen 1	-0.67	-0.46	-1.78	-1.03	-0.59	-0.28
Specimen 2	-1.13	-0.31	-0.54	-2.02	-0.84	-0.71
Avg	-0.90	-0.39	-1.16	-1.53	-0.71	-0.49
S.D.	0.33	1.11	0.88	0.70	0.18	0.31
Freeze-Dry						
Specimen 3	-5.40	-1.60	-2.12	-9.50	-3.70	-2.43
Specimen 4	-2.40	-1.45	-0.54	-7.11	-2.11	-2.41
Specimen 5	1.26	-0.65	-0.72	-5.53	-3.31	-0.79
Specimen 6	-1.97	-1.94	-1.79	-6.57	-2.36	-3.03
Avg	-2.13	-1.41	-1.29	-7.17	-2.87	-2.16
S.D.	2.72	0.55	0.78	1.68	0.76	0.96

Comparative Example 1

Bovine cortical bone specimens, 4mm x 4mm x 40mm (nominal) were prepared from the same bovine femur. Some specimens were soaked in a 50% aqueous solution of glycerol for three days prior to lyophilization. Other specimens were lyophilized without glycerol. After lyophilization, the specimens were tested in 3-point bending (30mm span, center loaded) in the MTS servo hydraulic testing system. Loading was conducted at a rate of 25mm/min under displacement control. Specimens were loaded to failure. Data were collected on maximal load, failure load and energy absorbed to break (a measure of how brittle the material is). Factors were compared by the Wilcoxon non-parametric test.

The results are given in Table 4 below.

Samples:
Glycerol/Dry n=4 No glycerol/dry n=3
Glycerol/Saline n=2 No glycerol/Saline n=3

Table 4

	Break Load (kN)	Energy to break (N-m)
Glycerol/Dry	0.277 ± 0.022	0.037 ± 0.0025
Glycerol/Saline	0.205 ± 0.037	0.037 ± 0.0021
No glycerol/Dry	0.234 ± 0.064	0.028 ± 0.0072
No glycerol/Saline	0.152 ± 0.016	0.028 ± 0.0135

Many of the specimens that were exposed to saline showed a number of fine, internal longitudinal cracks that were visible macroscopically. Both glycerol-treated and non-treated specimens displayed this morphology. For all specimens, peak load was equivalent to break load. Glycerol application was a significant factor in determining breaking load ($p=0.05$), and marginally significant in the energy to breakage ($p=0.08$). Saline hydration was significant to the breaking load ($p=0.03$) but not other parameters.

Glycerol application prior to lyophilization reduces brittleness in the bone samples. Freeze-drying, composed of a freezing step and a subsequent water-removal step, is damaging to bone and has been shown to negatively affect mechanical properties. Yet, the bone literature teaches that freezing itself is not detrimental to bone to any significant degree. Thus, it is believed that the damage protection offered by the strength-conserving agent does not act by eliminating damage in the freezing, but rather by eliminating damage due to dimensional changes during the dehydrating aspects of freeze-drying. Although the mechanism of the invention is not entirely

understood, the inventors believe that this improvement is achieved by maintaining the liquid environment of the bone to reduce damage during lyophilization. Strength was improved by an average of 34% and energy absorption prior to fracture was improved by up to 32%.

Comparative Example 2

Human bone was treated for viral inactivation using the process described in U.S. Patent No. 5,846,484. From these bones diaphyseal segments, 2 cm in length, were cut on a band saw. Other specimens were not treated by this 5,846,484 process, but were rather cleaned of adherent soft tissues and processed using standard techniques. To determine penetration of a treatment solution, penetration was affected by either of two treatment processes: Specimens suspended in a stirred solution and specimens suspended in an ultrasonic bath. The treatment solution used was 50% (v/v) aqueous glycerol, and also contained 0.5%(w/v) methylene blue dye to allow assessment of penetration. Specimens of each group were removed at 1 hour, 4 hours, 11 hours, 24 hours, and 48 hours. Sectioning the bone transversely to the middle (1cm) point, and qualitatively describing penetration assessed penetration of the conserving agent. The table below, Table 5, summarizes penetration into Haversian Canals (HC) and Matrix (M) regions of the middle (1cm) section.

Table 5

	1 hr		4 hr		11hr		24 hr		48 hr	
	HC	M	HC	M	HC	M	HC	M	HC	M
5,846,484 pretreated ULTRASONIC	X	P	X	P	X	P	X	X	X	X
5,846,484 pretreated STIRRED	O	O	X	P	X	X	X	X	X	X
ULTRASONIC	O	O	X	P	X	P	X	P	X	X
STIRRED	O	O	O	O	P	P	P	P	X	P

Key: X= Mostly Penetrated; P=Partly Penetrated; O=Minimally Penetrated

Pre-treatment of tissues using the process described in patent 5,846,484 improved the speed at which the tissue was penetrated by the treatment solution. Further, effecting penetration by ultrasonic bath also substantially reduced the penetration time for the solution.

Comparative Example 3

Human bone was treated for viral inactivation using processes described in U.S. Patent 5,846,484. From these treated bones, human diaphyseal bone segments were shaped into a ramped structure (figure 2) using standard techniques. One specimen from each of four donors (16 total specimens) received each of four treatments: A.) treated for 3 days in an ultrasonic bath containing 50% (v/v) aqueous glycerol solution, then freeze-dried using standard methods; B.) treated for 3 days in a container containing 50% (v/v) aqueous glycerol solution stirred continuously (Stir), then freeze-dried using standard methods; C.) freeze-dried only; D.) frozen only (-70°C.). Dimensional measurements were taken after initial manufacture, and then again after all treatments and following 1 hour rehydration in physiological saline. The threaded hole was also tested using a mating screw prior to the process and at the end of the process. Results in Figure 8 show the difference between final and initial measurements for the overall length (OL) and overall width (OW). Each of the treated groups showed substantially less dimensional change than the freeze-dried only group, though differences in all groups were greater than that of the specimens that were frozen and still contained water at the time of rehydration. The threaded hole (shown on the left side of figure 2) was also tested at each stage, and found to accept the screw for the treated specimens (Stir and Ultrasonic) and the frozen specimens at each stage. For the freeze-dried specimens, two of four specimens failed

to accept the screw following the freeze-drying step, though one of these did accept a screw after rehydration.

Comparative Example 4

Donor specimens prepared as in Comparative Example 3 where subjected to mechanical testing utilizing a MTS 858 Bionix™ compressive testing instrument at a loading rate of 25 mm/min for single-cycle compression to 2mm displacement testing to determine the toughness of each treatment group. The results are contained in Table 6 below.

Table 6

	A	B	C	D
Toughness (N-m)	17.2	14.7	12.3	12.5
Improvement in toughness as compared to lyophilized only (%)	39.8	19.5	0	-1.6

The results demonstrate that toughness is improved when bone is treated in accordance with one embodiment of the invention herein.

Comparative Example 5

Samples of the monolithic bone of figure 1 were obtained from three different donors. The bone was then either processed according to the procedure of Example 6 with five minutes or two hours of hydration time, lyophilized under industry standard conditions or frozen. The samples were then subjected to compression to breakage using a uniaxial servo-hydraulic test machine (MTS model 858 Bionix) under displacement control to apply a constant compressive displacement to each test articles at a rate of 25 mm/min. Before the start of each test, a compressive preload was applied to each specimen (under load control) of 250N and machine displacement

was zeroed at this point. This was done to provide a consistent zero displacement starting point for each test. Tests terminated when a total compressive displacement of 2.5mm had been imparted to the test article. In all cases, failure (a peak in load, followed by a sharp drop in load) was observed before 2.5 mm of compressive displacement was reached. Test articles were loaded using stainless steel inserts which conformed to the upper and lower ridged surfaces of the test article, with the exception of two small mounting holes on both the lower and upper insert. The system 100 kN load cell in the 100 kN range was used to collect load information. Displacement information was measured from the test systems displacement transducer (LVDT). Peak loads and energy to break values were extracted from the load vs. displacement curves using Testworks version 3.6 test software.

Measurements of the breaking energy values was determined as an indicator of toughness. The results are contained in Table 7 below. All units are in N-mm.

Table 7

	Frozen	Freeze-Dried (5 minute rehydration)	Alcohol (5 min rehydration)	Alcohol (2 hour rehydration)
Donor 1	39731	24638	31587	41004
Donor 2	29546	24425	18078	27142
Donor 3	25531	22038	24122	19222
Donor 4	24534	13924	25926	N/A
Average	29835	21256	24928	29122
Standard Deviation	6943	5028	5565	11025
Percentage Relative to Frozen	100%	71.2%	83.6%	97.6%
Percentage relative to lyophilized (freeze-dried) bone	140.4%	100%	117.3%	137.0%

After being subjected to compression to fracture, the monolithic bone was visually evaluated to determine the relative degree of fragmentation of each treatment method. This relative degree of fragmentation is also an indicator of toughness. The degree of fragmentation of the monolithic bone was scored on a relative scale from 1 to 10 wherein 1 represented no fragmentation and 10 represented highly fragmented. The results are contained in Table 8 below.

Table 8

	Frozen	Freeze-Dried (5 minute rehydration)	Alcohol (5 min rehydration)	Alcohol (2 hour rehydration)
Donor 1	2	6	2	2
Donor 2	2	7	4	2
Donor 3	2	4	2	2
Donor 4	2	7	4	N/A
Average	2	6	3	2

From this above data, two separate measures of energy absorption indicate that freeze-drying decreases toughness. Alcohol treatment also decreases toughness, but by less than for a comparable freeze-dried graft (29% reduction for FD vs. 16% reduction for alcohol). The may be very significant at the time of graft insertion, since many of the grafts that would receive this treatment will receive mechanical blows (hammering, etc.) to place them in the surgical site. At later time points, after rehydration for 2 hours (whether in a container in the operating room, or rehydrating in the patient's surgical site), the graft behaves similarly to the frozen tissue in its toughness characteristics.

It will be understood that various modifications can be made to the embodiments and examples disclosed herein. Accordingly the above description should not be construed as limiting but merely as exemplifications of preferred embodiments. Those skilled in the art will envision various modifications that are within the scope and spirit of the claims appended hereto.

WHAT IS CLAIMED IS:

1. A method for treating a monolithic bone intended for implantation to conserve at least one of the biomechanical properties of the bone during dehydration, subsequent packaging and storage of the bone, the method comprising:

contacting the monolithic bone with at least one dehydrating liquid and/or volatile solvent;

dehydrating the monolithic bone;

removing the dehydrating liquid and/or volatile solvent; and,

packaging the dehydrated bone.

2. The method of Claim 1 further comprising:

contacting the bone with a mechanical strength-conserving amount of at least one biocompatible mechanical strength-conserving agent before and/or during the step of dehydrating, said agent being a liquid organic material which is capable of penetrating and remaining in the bone during its dehydration, packaging and storage;

3. The method of Claim 1 or 2 further comprising:

contacting the dehydrated bone just prior to implantation with a rehydrating amount of at least one rehydration solution to rehydrate the bone.

4. The method of any one of the above claims wherein the dehydrating liquid and/or volatile solvent is selected form the group consisting of methanol, ethanol, isopropanol, butanol, isobutanol, ethylbutanol, acetonitrile, pyridine,

industrial methylated spirit, graded series of dehydrating agents, histological solution, Flex 100™, dimethylsulfoxide, small ketones, acetone, chloroform, methylene chloride, ethylene chloride, straight chain hydrocarbons of less than 12 carbons, hexane, pentane, low molecular weight alkenes, esters, ethers, ethyl ether, tetrahydrofuran, dioxane, ethylene glycol monoethyl ether, crown ethers, aldehyde, solutions containing aldehydes, formaldehyde, formalin, super critical fluids, liquid carbon dioxide, liquid hydrogen sulfide, and mixtures of two or more of the above liquids.

5. The method of any one or more of Claims 1to 3 further comprising infusing under constant or alternating positive pressure the dehydrating liquid and/or volatile solvent and/or mechanical strength-conserving agent.

6. The method of any one or more of Claims 1to 3 further comprising sonicating the bone and dehydrating liquid an/or volatile solvent and/or mechanical strength-conserving agent.

7. The method of any one or more of Claims 1 to 3 further comprising contacting the bone and the dehydrating liquid and/or volatile solvent and/or mechanical strength-conserving agent in the presence of a low pressure atmosphere.

8. The method of any one or more of Claims 1 to 3 further comprising contacting the bone and the dehydrating liquid and/or volatile solvent and/or mechanical strength-conserving agent in the presence of alternating vacuum and positive pressure.

9. The method of Claim 3 wherein the rehydration solution is selected from the group consisting of normal saline, physiologically buffered saline, dextrose

solution, wetting agents, medically/surgically useful substance(s) and antibiotic solutions.

10. The method of Claim 9 wherein the medically/surgically useful substance(s) is at least one member of the group consisting of bone morphogenic proteins (BMPs), transforming growth factor (TGF-beta), insulin-like growth factor (IGF-1), (IGF-2) and platelet derived growth factor (PDGF).

11. The method of Claim 2 wherein the strength-conserving agent is selected from the group consisting of polyhydroxy compound, polyhydroxy ester, fatty alcohol, fatty alcohol ester, fatty acid, fatty acid ester, liquid silicone and mixtures thereof.

12. The method of Claim 11 wherein the polyhydroxy compound is selected from the group consisting of glycerol, 1,4-butylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, propylene glycol, dipropylene glycol; polyoxyethylene-polyoxypropylene copolymer, polyoxyethylene-polyoxypropylene block copolymer, and alkylphenolhydroxypolyoxyethylene.

13. The method of Claim 2 wherein the mechanical strength-conserving agent is glycerol.

14. The method of Claim 2 wherein the mechanical strength-conserving agent is an aqueous solution of glycerol.

15. The method of Claim 2 wherein the mechanical strength-conserving agent is an alcoholic solution of glycerol.

16. The method of Claim 11 wherein the polyhydroxy ester is selected from the group consisting of monoacetin, triacetin and poly(oxyalkylene) glycol ester.

17. The method of Claim 11 wherein the fatty alcohol is selected from the group consisting of caproic alcohol, caprylic alcohol, capric alcohol, undecyl alcohol, lauryl alcohol and tridecanol.

18. The method of Claim 11 wherein the fatty alcohol ester is selected from the group consisting of ethyl hexyl palmitate, isodecyl neopentate, octadodecyl benzoate and diethyl hexyl maleate.

19. The method of Claim 11 wherein the fatty acid is selected from the group consisting of hexanoic acid, heptanaoic acid, octanoic acid, decanoic acid and undecanoic acid.

20. The method of Claim 11 wherein the fatty acid ester is selected from the group consisting polyoxyethylene-sorbitan-fatty acid ester, polyoxyethylene fatty acid esters, mono-, di-, and mono/di-glycerides, sorbitan fatty acid esters, n-butyl stearate, n-butyl oleate and n-propyl oleate.

21. The method of Claim 11 wherein the liquid silicone is selected from the group consisting of polyalkylsiloxane and polyalkylarylsiloxane.

22. The method of Claim 21 wherein the polalkylsiloxane is selected from the group consisting of polymethylsiloxane and poly(dimethylsiloxane).

23. The method of Claim 1 wherein the step of dehydrating is carried out by contacting the bone with a graded series of dehydrating liquids; or, by subjecting the bone to microwave energy; or, by subjecting the bone to heat at

ambient or sub-atmospheric pressures; or, subjecting the bone to sub-atmospheric pressure in the presence or absence of a desiccant; or, by a combination of one or more of the foregoing.

24. The method of Claim 1 or 2 wherein the step of dehydrating the bone and/or volatile solvent removal is carried out by subjecting the bone to microwave energy; or, by subjecting the bone to ambient temperatures at ambient or sub-atmospheric pressures; or, by subjecting the bone to heat at ambient or sub-atmospheric pressures; or subjecting the bone to sub-atmospheric pressure in the presence or absence of a desiccant; or, by a combination of one or more of the foregoing.

25. Monolithic bone obtained by any one or more of Claims 1 to 24.

26. The bone of Claim 25 wherein the toughness is at least about 5% greater, preferably at least about 10% greater and most preferably at least about 15% greater than that of a comparable specimen of monolithic bone which has been lyophilized.

27. The bone of Claim 25 wherein the strength is at least about 10% greater, preferably at least about 20% greater and most preferably at least about 30% greater than that of a comparable specimen of monolithic bone which has been lyophilized.

28. Use of the bone of Claim 25 for repair of at least one bone selected from the group consisting of: ethmoid, frontal, nasal, occipital, parietal, temporal, mandible, maxilla, zygomatic, cervical vertebra, thoracic vertebra, lumbar vertebra, sacrum, rib, sternum, clavicle, scapula, humerus, radius, ulna, carpal bones,

metacarpal bones, phalanges, ilium, ischium, pubis, femur, tibia, fibula, patella, calcaneus, tarsal and metatarsal bones.

29. A dehydrated monolithic bone implant containing at least one biocompatible mechanical strength-conserving agent, said agent being a liquid organic material which is capable of penetrating and remaining in the bone during its lyophilization, packaging and storage.

30. The monolithic bone of Claim 29 wherein the strength-conserving agent is selected from the group consisting of polyhydroxy compound, polyhydroxy ester, fatty alcohol, fatty alcohol ester, fatty acid, fatty acid ester, liquid silicone and mixtures thereof.

31. The monolithic bone of Claim 30 wherein the polyhydroxy compound is selected from the group consisting of glycerol, 1,4-butylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, propylene glycol, dipropylene glycol; polyoxyethylene-polyoxypropylene copolymer, polyoxyethylene-polyoxypropylene block copolymer, and alkylphenolhydroxypolyoxyethylene.

32. The monolithic bone of Claim 29 wherein the mechanical strength-conserving agent is glycerol.

33. The monolithic bone of Claim 29 wherein the mechanical strength-conserving agent is an aqueous solution of glycerol.

34. The monolithic bone of Claim 29 wherein the mechanical strength-conserving agent is an alcoholic solution of glycerol.

35. The monolithic bone of Claim 29 further comprising at least one medically/surgically useful substance.

36. The monolithic bone of Claim 35 wherein the medically/surgically useful substance is at least one member of the group consisting of bone morphogenic proteins (BMPs), transforming growth factor (TGF-beta), insulin-like growth factor (IGF-1), (IGF-2) and platelet derived growth factor (PDGF).

37. The bone of Claim 29 having at least about 2% less decrease in length dimension as compared to bone that has been lyophilized without being contacted with a mechanical strength-conserving amount of a mechanical strength-conserving agent.

38. The bone of Claim 29 wherein the toughness of the dehydrated bone is at least greater than about 19% as compared to bone that has been dehydrated without being contacted with a mechanical strength-conserving amount of a mechanical strength-conserving agent.

39. A rehydrated strength-conserved shaped bone implant prepared by:

contacting bone with a mechanical strength-conserving amount of at least one biocompatible mechanical strength-conserving agent, said agent being a liquid organic material which is capable of penetrating and remaining in the bone during its dehydration, packaging and storage;

dehydrating the bone containing the mechanical strength-conserving agent;

packaging the dehydrated bone; and,

rehydrating the bone prior to or during implantation.

40. The bone prepared by the method of Claim 39 wherein the step of dehydrating is carried out by lyophilization; or, by contacting the bone with a graded series of dehydrating liquids; or, by subjecting the bone to microwave energy; or, by subjecting the bone to heat at ambient or sub-atmospheric pressures; or, by subjecting the bone to sub-atmospheric pressure in the presence or absence of a desiccant; or, by a combination of one or more of the foregoing.

41. A rehydrated strength-conserved shaped bone implant prepared by:

contacting bone with a mechanical strength-conserving amount of at least one biocompatible liquid volatile solvent and/or dehydrating agent;

dehydrating the bone;

removing the liquid volatile solvent and/or dehydrating agent;

packaging the dehydrated bone; and,

rehydrating the bone prior to or during implantation.

42. The bone prepared by the method of Claim 41 wherein the step of volatile solvent and/or dehydrating agent removal is carried out by subjecting the bone to microwave energy; or, by subjecting the bone to ambient temperatures at ambient or sub-atmospheric pressures; or, by subjecting the bone to heat at ambient or sub-atmospheric pressures; or, by subjecting the bone to sub-atmospheric pressure in

the presence or absence of a desiccant; or, by a combination of one or more of the foregoing.

43. The shaped bone implant of Claim 42 wherein the shaped bone has been shaped before, during, or after its contacting with at least one biocompatible liquid volatile solvent and/or dehydrating agent.

44. The shaped bone implant of Claim 42 wherein the step of rehydrating is performed by contacting the bone with at least one rehydrating liquid selected from the group consisting of sterile water, normal saline, physiologically buffered saline, dextrose solution, antibiotic solutions, wetting agents, and medically/surgically useful substance(s).

45. Use of the bone of any one or more of Claims 42 to 44 for repair of at least one bone selected from the group consisting of: ethmoid, frontal, nasal, occipital, parietal, temporal, mandible, maxilla, zygomatic, cervical vertebra, thoracic vertebra, lumbar vertebra, sacrum, rib, sternum, clavicle, scapula, humerus, radius, ulna, carpal bones, metacarpal bones, phalanges, ilium, ischium, pubis, femur, tibia, fibula, patella, calcaneus, tarsal and metatarsal bones.

46. A method for treating monolithic bone intended for implantation to converse the mechanical strength of the bone during lyophilization and subsequent packaging and maintain such strength during the storage of the bone, the method comprising:

a) contacting the bone with a mechanical strength-conserving amount of at least one biocompatible mechanical strength-conserving agent, said agent being

a liquid organic material which is capable of penetrating and remaining in the bone during its lyophilization, packaging and storage;

b) lyophilizing the bone containing the mechanical strength-conserving agent; and

c) packaging the lyophilized bone.

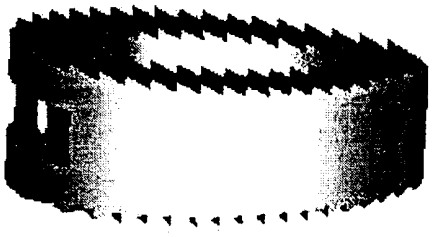
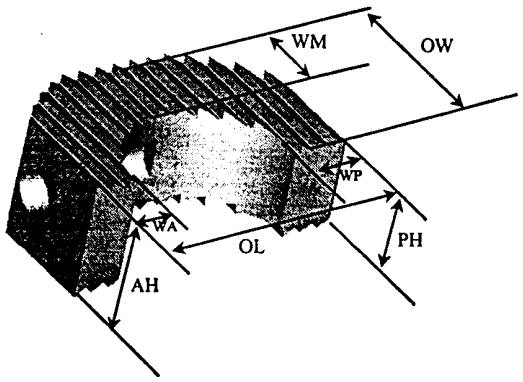


FIGURE 1



AH- anterior height
PH-posterior height
WA-anterior width
WP-posterior width
OL-overall length
OW-overall width
WM-medial width

FIGURE 2

FIGURE 3

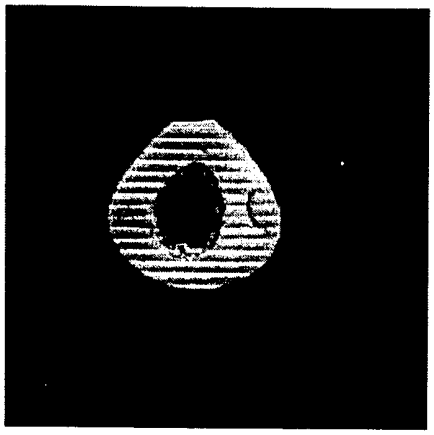


FIGURE 4

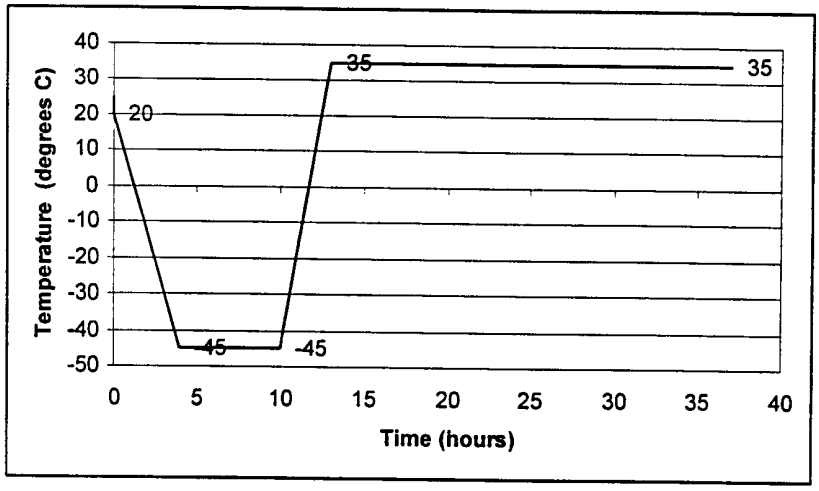

Temperature (degrees C)
40
30
20
10
0
-10
-20
-30
-40
-50
20
45
-45
35
35
0
5
10
15
20
25
30
35
40
Time (hours)

FIGURE 5

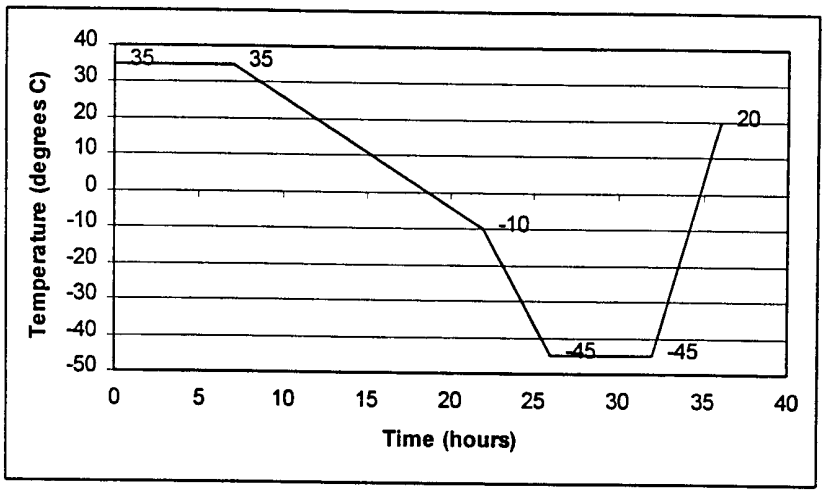

Temperature (degrees C)
40
30
20
10
0
-10
-20
-30
-40
-50
35
35
-10
-45
-45
20
0
5
10
15
20
25
30
35
40
Time (hours)

FIGURE 6

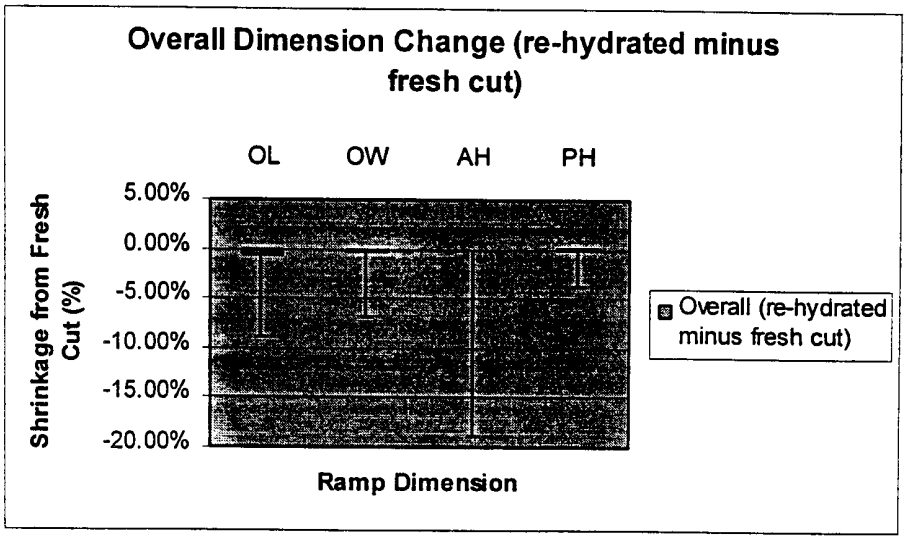

Overall Dimension Change (re-hydrated minus fresh cut)
OL
OW
AH
PH
5.00%
0.00%
-5.00%
-10.00%
-15.00%
-20.00%
Shrinkage from Fresh Cut (%)
Ramp Dimension
Overall (re-hydrated minus fresh cut)

FIGURE 7

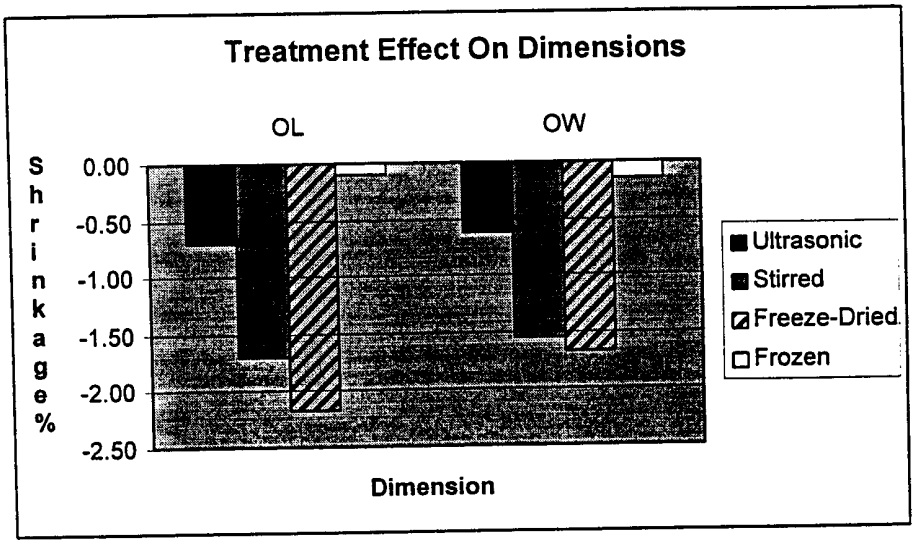

Treatment Effect On Dimensions
OL
OW
Shrinkage %
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
Dimension
Ultrasonic
Stirred
Freeze-Dried
Frozen

FIGURE 8

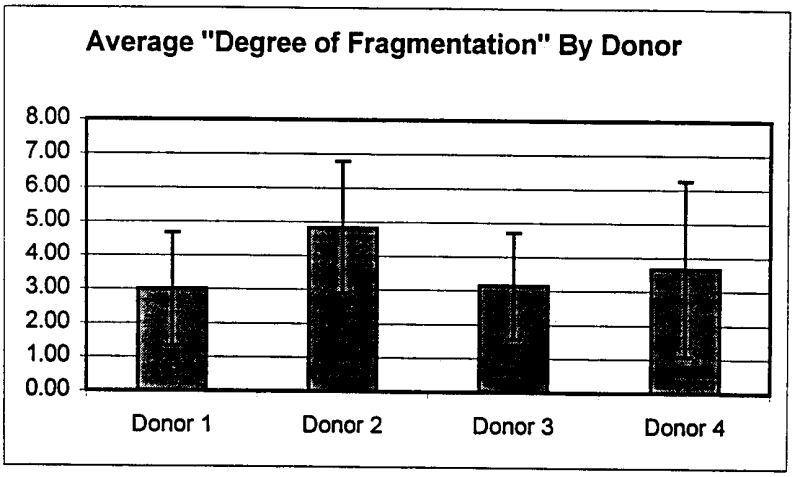
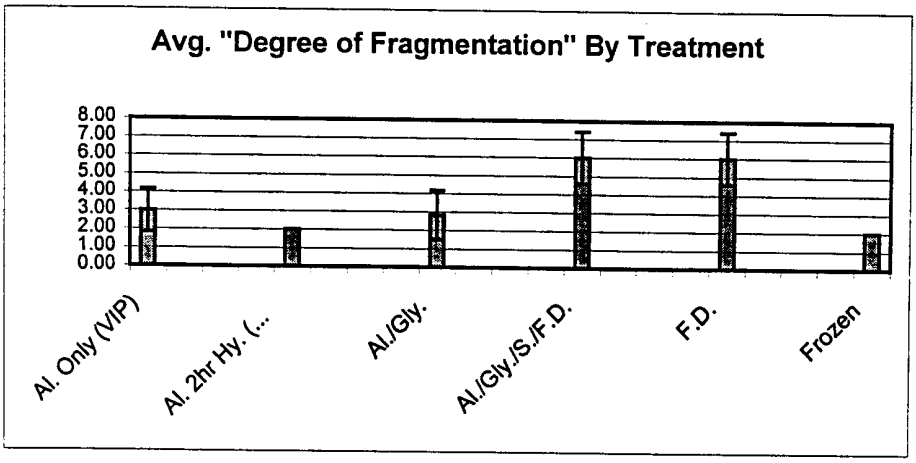

Average "Degree of Fragmentation" By Donor
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Donor 1
Donor 2
Donor 3
Donor 4

FIGURE 9



Scale: 10 = Highly Fragmented
1 = Intact (No Fragmentation)

Abbreviations: Al. = Alcohol
Hy. = Hydration
VIP = Vacuum Infiltration Processor
Gly. = Glycerol
S. = Sonicated
F.D. = Freeze-Dried

FIGURE 10

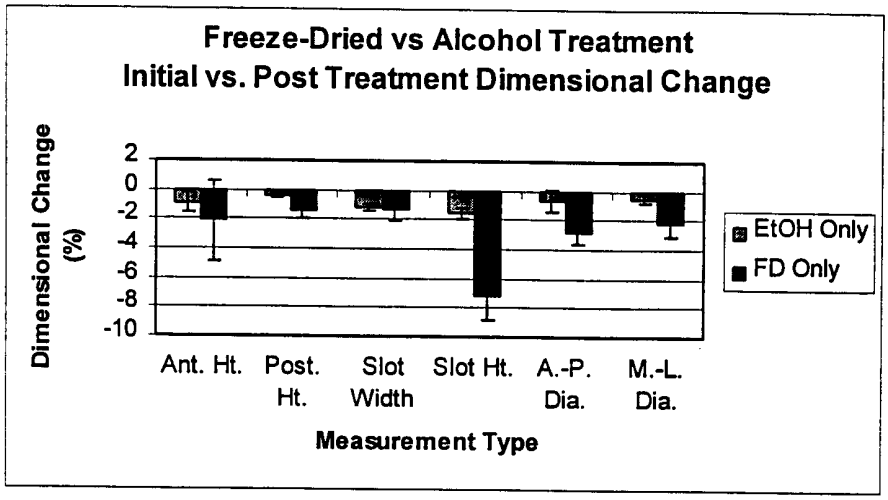

Freeze-Dried vs Alcohol Treatment
Initial vs. Post Treatment Dimensional Change
Dimensional Change (%)
2
0
-2
-4
-6
-8
-10
Ant. Ht.
Post. Ht.
Slot Width
Slot Ht.
A.-P. Dia.
M.-L. Dia.
Measurement Type
EtOH Only
FD Only

FIGURE 11

INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 00/23342

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 25941 A (OSTEOTECH) 24 July 1997 (1997-07-24) the whole document ---	1,25,29, 39,41,46
A	US 5 862 806 A (CHEUNG) 26 January 1999 (1999-01-26) cited in the application the whole document ---	1,25,29, 39,41,46
A	US 5 846 484 A (SCARBOROUGH) 8 December 1998 (1998-12-08) cited in the application the whole document ---	1,25,29, 39,41,46
A	US 5 264 214 A (RHEE) 23 November 1993 (1993-11-23) the whole document ---	1,25,29, 39,41,46
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2000	04/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Klein, C

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INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 00/23342

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US 5 899 939 A (BOYCE) 4 May 1999 (1999-05-04) cited in the application	
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