METHOD AND APPARATUS FOR MANUFACTURING PLASMA BASED PLASTICS AND BIOPLASTICS PRODUCED THEREFROM

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Field of Classification Search .............. 435/2, 372
See application file for complete search history.

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ABSTRACT
A method of making a bioplastic, and a bioplastic produced thereby, by using human plasma in which human plasma is clotted, either dried through its gel phase or dried and powdered, and processed into a bioplastic with the addition of at least one plasticizer followed by forming and heating to form a final bioplastic construct.

26 Claims, 4 Drawing Sheets


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Genipin powder % in PRP/Glycerol (65/35, w/w)

- 0.25
- 0.5
- 1.0

Genipin in ETOH % in PRP/Glycerol (65/35, w/w)

- 0.25
- 0.5
- 1.0

FIG. 2
1. METHOD AND APPARATUS FOR
MANUFACTURING PLASMA BASED
PLASTICS AND BIOPLASTICS PRODUCED
THEREFROM

CROSS REFERENCE TO RELATED
APPLICATIONS

This application is based on U.S. Provisional Patent
Applications No. 60/852,368, filed Oct. 17, 2006; and No. 60/961,
580, filed Jul. 23, 2007, on which priority of this patent
application is based, and which are hereby incorporated by
reference in their entireties.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention pertains to bioplastics for patient implantation
or application, made at least in part from patient tissue or
fluids such as plasma.

2. Description of Related Art

Fibrin-based plastics were invented in the 1940s as part of
a U.S. Defense-sponsored research program to develop medical
strategies for wounded military personnel. For example, fibrin-based
plastics were developed out of the human blood program led by Edwin Cohn at Harvard University. John
Ferry, then at Woods Hole, led the group that was involved in
developing fibrin elastomers. As a result of this work, elastomeric
forms of fibrin were developed and used successfully in
neurosurgical applications, burn treatments, and
peripheral nerve regeneration. See, for example, Ferry, J.D. et
Invest. 23:597-600 (1944); Cronkite et al., JAMA. 124:976-8
(1944); and Ferry J.D. et al., Am. Chem. Soc J. 69:400-409
(1947). Hard fibrin plastics were fabricated into implants and
were finding clinical success as early as the 1940s. See, for
example, U.S. Pat. No. 1,786,488, No. 2,385,802, No. 2,385,
3,523,807, No. 4,548,736, and No. 6,074,663, all incorporated
herein by reference. Research sponsored by the Hungarian
government led to the development of similar products in the
1950s through the early 1970s. Forms of hard plastic fibrin was demonstrated to have clinical efficacy in orthopedic
applications of bone resurfacing. See, for example, Zinner,
Gerendos, M., Ther. Hung., 7:8-16 (1959); and Gerendos, M.,
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Despite the efficacy of fibrin products, concerns about
disease transmission from purified human fibrinogen from
pooled plasma remained. However, during the late 1970s and
thereafter, fibrin was developed as a tissue glue and sealant,
and although this application required purified human fibrinogen, new techniques had been developed to ensure the
safety of blood products. Consequently, fibrin-based glues
and sealants have been used in clinical practice for over
twenty years in Europe (and since 1998 in the United States)
with no disease transmission concerns. Recently, the
development of recombinant human fibrinogen and thrombin
and purified salmon fibrinogen and thrombin have helped further
to address concerns over both safety and market availability.
See, for example, Butler S.P. et al., Transgenic Res. 13:457-
450 (2004); Prunkard D. et al., Nat. Biotechnol. 4:867-871
(1997); U.S. Pat. Nos. 5,527,692; 5,502,034; 5,476,777;
6,037,457; 6,083,902; and U.S. Pat. No. 6,740,736. Autologous
sealants and glues are also available (see for example U.S. Pat. No. 6,979,307).

Despite such advances in the field, interest in the use of
protein bioplastics in plastic foams, such as fibrin elastomers,
has significantly declined over time. Silicone rubber sheets,
which were introduced in the 1960s and 1970s, have replaced
fibrin elastomeric sheets in the clinic, despite inherent
problems with their permanence. There are also limitations with
current synthetic bioresorbable plastics, such as polyurethane,
polyactic acid (PLA), polylactic-co-glycolic acid (PLGA), polyglycolic acid (PGA) and polyacrolactone. These polymers degrade in the body by hydrolysis, via bulk
degradation, or through surface erosion, all of which operate
independently of the surrounding biological environment.
The inability of these polymers to degrade in response to
cellular invasion and to promote directly the ingrowth of host
tissues remains a profound limitation of bioresorbable
implants.

In contrast, protein bioplastics can degrade in response to
cellular proteolytic processes so that degradation occurs in
concert with the growth and healing of host tissues. Also,
many synthetic materials do not inherently bind growth factors
of interest for therapeutic delivery options, whereas fibrin
binds to growth factors directly and indirectly through
molecular interactions with growth factors, including those
which may bind the cell. However, fibrin materials—
including certain of the present inventors’ own fibrin-based
plastics based on purified fibrinogen/thrombin from pooled
human or animal plasma—have certain constraints or limitations
such as not inherently containing endogenous growth
factors. Moreover, fibrin materials of the prior art are very
expensive especially when prepared from human sources and
with the required large amounts of starting material necessary
to give desired yields. Commonly used synthetic materials,
such as bioresorbable polymers, can be associated with
inflammatory interactions, whereas these interactions would
be less pronounced if one were to use protein-based plastics.

Fibrin glue—bovine-derived fibrinogen is potentially less
expensive—although similar to its purified human
counterpart in not containing human growth factors—yet disease transmission and immuno-sensitization with repeated use are
potential major drawbacks due to its xenogenic source.

Analogously to plastic implants, allogenic bone grafts also
have several limitations, including high variability of graft
quality from donor to donor. This variability arises from
several factors including amount of active endogenous
growth factors in each donated graft, and there are no practical means for quality assessment and/or quality control of
allogenic bone graft materials with respect to these growth
factors.

To date, methods and compositions previously developed for
bioplastics, including but not limited to fibrin, elastin and
e., are not sufficiently adaptable for modern clinical use. For
example, the original manufacturing methods developed for
certain protein-based bioplastics required high temperatures
(i.e., as high as 155° C.). Such high temperature processing
precludes the use of exogenously added drugs and proteins as
well as destroys any inherent biological activity. In addition,
methods for making these materials porous have not been
reported or developed previously. Prior to the present invention,
one had solved the problem of manufacturing bio-
plastics while avoiding the disadvantages of known
processing techniques, such as high temperatures and pressures and/or difficulty in retaining desirable physical characteristics of
the plastics. More importantly of all, perhaps, no one has
heretofore addressed how effectively to reduce or eliminate
the issues of disease transmission and immune response of bioplastics derived from animal or human pooled donor sources.

Therefore, certain needs remained prior to the present invention. Methods of incorporating heat-sensitive materials such as biological response modifiers, including but not limited to growth factors and extracellular matrix molecules, and drugs into elastomeric and/or pliant and/or hard materials were needed. Compositions having the ability to respond to the local cellular milieu were also needed, with or without spatial patterns in the overall construct or sheet to provided such responses where desired. Prior efforts to crosslink fibrin-based bioplastics were either post-fabrication methods, which generally created unwanted effects such as swelling, or used toxic crosslinking agents such as formaldehyde in addition to representing laborious processes. Fabrication methods were also needed that could be used to control properties of manufactured articles including for example the density, porosity, and mechanical properties of bioplastic materials. Methods of manufacturing biocompatible materials with anisotropic properties were needed, especially with regard to extrusion or directed strain and/or printing technologies to impart such anisotropic properties. For these and other reasons, more reliable, cost-effective substitute tissue scaffold materials including bioplastics have previously remained an illusive yet important clinical goal.

SUMMARY OF THE INVENTION

In order to meet these and other goals, the present invention is a method of making bioplastics using human plasma either in its whole form or from which one or more constituents have been removed, in which human plasma is clotted either before or after any removal of any desired constituents, dried through its gel phase or dried and powdered, and processed into a bioplastic with an added plasticizer. When elastomeric or elastomeric sheet materials are made containing the clotted and dried plasma of the present invention, the plasma or plasma fraction is “dried through the gel phase,” that is, dried until the plasma or plasma fraction(s) is/are drier than a colloidal plasma gel, and then further processed with a plasticizer (water and/or glycerol) into an elastic or elastomeric plastic in that form. (Because glycerol, glycerin and glycerine are equivalent, hereinafter the word “glycerol” will be used to indicate any or all of glycerol, glycerin and glycerine.) For rubbery-to-hard bioplastics which have little elasticity, quantities of powdered clotted plasma (whole or otherwise) are used to make a bioplastic, again together with a water and/or glycerol plasticizer or other biocompatible plasticizers known in the art. The clotted and dried plasma may alternatively be added to virtually any plastic base material that will cure at the desired temperatures, but generally speaking the clotted and dried plasma plus plasticizer (water and/or glycerol) is used to make a bioplastic material without other structural-plastic-making additives. In other words, except for constituting materials such as powders, additives, biologics or drugs or etc. which do not contribute significantly to the bioplastic itself, the present inventive materials are made predominantly of clotted and dried plasma plus plasticizer. When powdered clotted plasma is used as an ingredient in bioplastics, the powder is generally adjusted to a water content of 5-15% by weight, preferably 8-12% by weight, more preferably 8-10% by weight, prior to mixing the dough. Dough is defined as the combination of plasma powder, plasticizer and any other components that are mixed prior to plastification processing. By contrast, when the plasma or plasma fraction is dried through the gel phase, the water that is inherent from the original plasma clot represents about 10-25% by weight of the starting material. Also, at any time a stabilizer(s) may be added to the plasma constituents to protect during dehydration and rehydration. These stabilizers may include without limitation glycogen, sorbitol, mannitol, trehalose, maltitol, xylitol, isomaltitol, erythritol, amylose, amylopectin, inositol hexanehexulose, sulphated beta-cyclodextran, etc. or combinations thereof. In fact, the stabilizer may be any known nontoxic polysaccharide according to the general formula of C₆H₁₂O₆, where n is between 200 and 2500. For those bioplastics in which significant retention of biological activity of constituents is desired, the plasticizing temperature is between 55-65°C. If retention of biological activity is not necessary the clotted dried plasma containing composition may be plasticized at temperatures up to about 150°C, particularly to create harder and/or denser bioplastic materials. In any case, the clotted dried plasma containing admixtures of the present invention are plasticized at 9-25 kpsi (kilopounds per square inch), preferably at 9-15 kpsi and more preferably at least 10.7 kpsi or higher. The resulting plasma-based plastics (PBP's) of the present invention can thus be made with a range of biomechanical and degradation properties. PBP's can be used in a variety of clinical applications, including their use as substitute graft materials, drug delivery carriers, anti-adhesion and barrier membranes, and scaffolds for tissue engineering. PBP can also be used in cell culture as a non-animal source of endogenous or exogenous growth media.

BRIEF DESCRIPTION OF THE DRAWING(S)

FIG. 1 are electron micrographs taken after osteoblastic precursor cells were cultured on PBP and then monitored for subsequent cell interactions using scanning electron microscopy.

FIG. 2 is a collection of photographs showing samples of the present bioplastic in which (top row) Genipin is added as a powder to the plasma bioplastic base without prior alcohol solubilization and (second row) Genipin is added to the plasma bioplastic base after solubilization in alcohol.

FIG. 3 is a set of micrographs that illustrate how smaller particle sizes enable more and better uniformity in mold fill and molded product.

FIG. 4 is a schematic flow diagram of one embodiment of the present method for making a bioplastic from plasma.

DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

The present invention is a method of making bioplastics using human plasma either in its whole form or from which one or more constituents have been removed, in which human plasma is clotted either before or after any removal of any desired constituents, dried through its gel phase or dried and powdered, and processed into a bioplastic with an added plasticizer. When elastomeric or elastomeric sheet materials are made containing the clotted and dried plasma of the present invention, the plasma or plasma fraction is “dried through the gel phase,” that is, dried until the plasma or plasma fraction(s) is/are drier than a colloidal plasma gel, and then further processed with a plasticizer (water and/or glycerol) into an elastic or elastomeric plastic in that form.
indicate any or all of glycerol, glycine and glycerine.) For rubbery-to-hard bioplastics which have little elasticity, quantities of powdered clotted plasma (whole or otherwise) are used to make a bioplastic, again together with a water and/or glycerol plasticizer. The clotted and dried plasma may alternatively be added to virtually any plastic base material that will cure at the desired temperatures, but generally speaking the clotted and dried plasma plus plasticizer (water and/or glycerol) is used to make a bioplastic material with other structural-plastic-making additives. In other words, except for constituting materials such as powders, additives, biologics or drugs or etc. which do not conduce significantly to the bioplastic itself, the present inventive materials are made predominantly of clotted and dried plasma plus plasticizer. When powdered clotted plasma is used as an ingredient in bioplastics, the powder is generally adjusted to a water content of 5-15% by weight, preferably 8-12% by weight, more preferably 8-10% by weight, prior to mixing the dough. By contrast, when the plasma or plasma fraction is dried through the gel phase, the water that is inherent from the original plasma clot represents about 10-25% by weight of the starting material. Also, at any time a stabilizer may be added to the plasma to protect it during dehydration and rehydration. These stabilizers may include without limitation glycerogen, sorbitol, mannitol, trehalose, maltitol, xylitol, isomaltitol, erythritol, amylose, amylopectin, inositol hexaphosphate, sulphated beta-cycloexextran, etc. or combinations thereof. In fact, the stabilizer may be any known nontoxic polysaccharide according to the general formula of Cn(H2O)m-1, where n is between 200 and 2500. For those bioplastics in which significant retention of biological activity of constituents is desired, the plasticizing temperature is between 55-65°C. If retention of biological activity is not necessary the clotted dried plasma containing composition may be plasticized at temperatures up to about 150°C, particularly to create harder and/or denser bioplastic materials. In any case, the clotted dried plasma containing admixtures of the present invention are plasticized at 9-25 kpsi (kilopounds per square inch), preferably at 9-15 kpsi and more preferably at least 10.7 kpsi or higher. The resulting plasma-based plastics (PPBs) of the present invention can thus be made with a range of biomechanical and degradation properties. PPBs can be used in a variety of clinical applications, including their use as substitute graft materials, drug delivery carriers, anti-adhesion and barrier membranes, and scaffolds for tissue engineering. PPBs can also be used in cell culture as a non-animal source of endogenous or exogenous growth media.

One embodiment of the present invention is a method of manufacturing autologous bioplastics by processing a patient's own donated blood plasma and products produced thereby. A typical method of making such an autologous PPB is as follows. Blood is collected prior to surgery. The blood is spun down to obtain platelet-rich plasma (PRP) and/or platelet poor plasma (PPP) and/or serum, or comparable methods such as whole blood collection or via apheresis are used to collect plasma from the patient without having to collect whole blood. The plasma is then clotted with calcium, thrombin or other known clotting agents, and the clotting when performed on platelet-rich plasma forms a platelet-rich plasma gel. To make rubbery-to-hard plastics, the platelet-rich plasma gel is first processed into a powder by drying it (this can include first removing any retained serum or not, although it is also possible to use only serum by drying it into a powder) and then ball milling or grinding or other powdering techniques. The drying step may or may not include lyophilization, but plasma dried "through the gel phase" for use in elastomers generally should not be lyophilized if possible (see below). Alternatively, a serum-free powder can be formed by first removing serum from the gel by spinning and then drying and comminuting the remaining plasma. In general, then, the present invention can use whole plasma or plasma from which one or more constituents has been removed as desired (even to the point of only serum's remaining).

Prior to further processing, the plasma powder or dried plasma gel may be treated (washed) with ethanol or propanol to sterilize it and, if desired, to remove unwanted salts from the plasma by removing the wash-step alcohol. The sterilized dried plasma can then be further compositied with one or more of growth factors, drugs or other therapeutics, fillers, porogens, crosslinkers, plasticizers, and stabilizers, and then formed into a rubbery-to-hard plastic material according to methods described in PCT patent application PCT/US06/29754, incorporated herein by reference. Exogenous excipients or stabilizers including but not limited to sorbitol, mannitol and/or trehalose may be added to the plasma prior to processing to protect endogenous plasma proteins during lyophilization and/or subsequent milling. When it is necessary to minimize heat and shear damage to plasma proteins the powder formation technique may include without limitation jet milling, mechanical grinding/sieving, ball milling (as mentioned above) or other forms of particulate milling. The powder, with or without serum components, can be augmented with exogenous therapeutics (growth factors, drugs, analgesics, chemotherapeutics, antibiotics), fillers, porogens, crosslinkers and plasticizers, and then formed into rubbery to hard plastics. In addition, putty-like graft packing materials can be made by milling the plastics into pellets and mixing the pellets with self-hardening bone cements at the time of surgery.

To make elastic sheets, the clotted plasma is processed according to methods described in U.S. patent application Ser. No. 11/495,115, also incorporated herein by reference. It should be noted that platelet rich plasma has inherent antimicrobial properties, and therefore may not require exogenous factors to be added to produce an antimicrobial effect if such a property is desired. Alternatively, platelet-poor plasma is also useful in creating either autologous or allogenic plastic implants or other patient biomaterials.

As described above, the clotted and dried plasma generally retains a water content of 5-15%, more preferably 8-12%, and most preferably 8-10%. Different plasma-containing materials of the prior art have failed to create adequately cohesive polymer bases, and one reason may have been the absence of retained water to facilitate the plasticizing process.

Fabricated plastics can be milled or otherwise shaped by various approaches including but not limited to surface texturing, cutting and grinding. Surface textures can either be machined post-fabrication or can be molded into place. Alternatively, defined nano- and micro-textures can be imparted by molds used to form plastics, allowing direct molding of surface textures during bioplastic fabrication. Such textures may facilitate cell adhesion and/or physically direct cell behavior to the PPBs.

It is possible to practice the invention in an integrated system which can be, for example, installed in a blood bank. It should be noted that although an important embodiment of the present invention is the use of autologous plasma as a starting material for a patient's own bioplastic implant, pooled plasma or exogenous (allogenic) plasma may also be used to create shelf-stable implants and other materials that need not be custom manufactured patient-by-patient. In addition, the present bioplastics can be used as interfaces between tissues and prostheses to improve integration. Such a system can include a centrifuge, a dryer, a powder miller, disposable
molds in standard shapes, compression molds and a cooperating hot press, and a vacuum degasser. Custom molds, based on CT/MR imaging data, could also be made by using a compact CNC milling machine, on site, or by external vendors. Compression molds made out of disposable, high compression strength materials, for example polyetheretherketone (PEEK), eliminates the need for cleaning and sterilizing standard molds between usages. Of course, the system need not be present in a blood bank or hospital.

Referring now to FIG. 4, a schematic showing one embodiment of the preparation of a rubbery-to-hard inventive bioplastic from clotted, dried and powdered plasma, a patient donates blood (1) which is spun down (2) into separated PRP or PPP plasma and red blood cells, and optionally the red blood cells are reinfused into the patient (2a). The plasma is admixed with calcium, thrombin or another clotting agent to clot the plasma (3) and to create a gel comprised of plasma clot and serum (4). The gel is dried (5) and ground into a powder or otherwise comminuted (6). (For elastomeric materials the gel is dried through the gel phase but is not powdered – not shown.) The clotted dried plasma is then blended into a dough with glycerol and/or water as a plasticizer (7), together with adding any optional ingredients, excipients, biologicals, drugs or other ingredients which do not contribute substantially to the bioplastic itself, and/or a thermoplastic polymer additive which supplements the bioplastic matrix (7). The composited dough is packed into a compression mold (8) and plasticized at controlled, usually low, temperature, and under pressure (9), to make an embodiment of the present bioplastic (10). Alternatively, the same dough can be extruded instead of molded, according to means known in the art.

Forty-five (45) L of plasma, and possibly more, may be safely harvested by apheresis from a healthy individual every year. Taking only 25 L of plasma containing 10 grams of plasma-fibrin protein/L would yield 250 grams plasma-fibrin protein/year. Stated differently, a liter of platelet rich plasma yields 100 g solids. Considering that this yield can be mixed with various extenders, such as nanoparticulate calcium phosphate and plasticizers of various types, such as 1 part plasma to 3 parts extender(s), this would yield 1 kilogram of plastic per year per human donor. Alternatively, 100 g solids plus 66 g glycerol by weight will yield 166 g bioplastic, enough to constitute 132 cubic centimeters. The powdered plasma may be stored essentially indefinitely as a lyophilized powder or as a formed plastic under the appropriate conditions. Therefore, banking of materials becomes possible for private and/or military applications. Custom molds and compression molds, and/or extrusion, as described above may be included.

In the event of the use of pooled plasma, precautions are taken against diseases including but not limited to bloodborne pathogens. The pooled or non-autolesogous products are useful in the event of a traumatic event or emergency in which the patient has no opportunity to stockpile blood or plasma in advance of a surgery or procedure. Blood banks and hospitals therefore might well find it advantageous to manufacture and store such plastics, or their immediate components, and therefore salvage at least a portion of blood that has been collected but is nearing the end of its shelf life. For anticipated usage of these bioplastics, blood typing is unnecessary for most allo-genic applications. However, conventional blood typing procedures could be used to ensure maximum compatibility between the patient and the products made from these plastics and components.

Uses and applications of bioplastics formed with the present clotted and powdered plasma fractions include, with-out limitation: bone grafts, including packing materials; tissue engineered scaffolds (particularly to coordinate with a patients’ own stem cells as stem cell culture becomes a commercial reality); fixation devices; surgical guides; scaffolds for tendon repair; prosthetic/tissue interfaces; sutures; nerve guides; wound protection; and protection of dura.

The method as described in association with FIG. 4 emphasizes the preparation of a dough and then the molding or extrusion of the resulting bioplastic dough. Fabrication can also be by powder molding according to the following alternative method. Molds are filled with powdered materials, including the powdered plasma, and subsequently infiltrated with plasticizer such as glycerol under positive pressure. Similarly, negative pressure may be applied to the bottom of the powder bed as glycerol or other plasticizer, is applied over the top, or a combination of both, by vacuum casting. The resulting powdered structure can be compacted by compression molding according to PCT/US06/29754.

As an alternative to molding, powdered materials can be selectively deposited, voxel-by-voxel and layer-by-layer into a mold cavity to form either homogeneous or heterogeneous 3D structures. Then, glycerol, or other plasticizer, can be infused into the structure under positive pressure, or by applying a negative pressure to the bottom of the powder bed as glycerol is applied over the top, or a combination of both. The resultant powdered structure may be compacted by compression molding according to PCT/US06/29754.

Elastomeric sheets may, without limitation, include layered, rolled or tube structures and may include machined sheets which may include the processing step of punching out or otherwise forming holes, possibly of defined geometries or patterns, to facilitate host tissue interstitial communication throughout the construct. Topical applications of sheet materials include, without limitation, skin substitutes following burn and chronic non-healing wounds/sores; surgical soft tissue defect fillers; post skin and breast cancer resection; plastic surgery related applications to help minimize scarring; and dental applications, including guided tissue regeneration. Interior (rather than topical) applications include duraplasty, peripheral nerve guides, adhesion prevention in various applications such as gastrointestinal and cardiovascular surgery, hernia repair, degradable thermal insulators for cryosurgery, renal applications, anastomoses, tendon/ligament repair, heart valves and patches, bursa repair to prevent adhesions, and drug delivery of growth factors, analgesics, chemotherapeutics, antibiotics and other drugs via implanted reservoirs or impregnated plastics with or without pores. Solid forms of the present materials (with solid ranging from rubbery plastic to very hard plastic) may be used for any of the above-mentioned applications or also in fillers or shaped grafts for craniofacial, dental, orthopaedic, neurosurgical and plastic surgical applications; or in “granular” filler, tubes and other shapes to fill defects due to trauma, cancer resection, spinal fusion, cranial defect, diseased or degraded joints such as due to arthritis or osteonecrosis; or in resorbable implants for arthroplasty, prosthetic-to-prosthetic interfaces; degradable screws, plates and other fixation devices; cartilage and meniscus graft applications; to provide fillers for cartilage defects; to create intervertebral disks to use as replacements for failed or failing disks; and to create bone resurfacing molds. Solid forms may also be used in tissue engineering applications, with capability also to deliver cells and/or growth factors for a wide range of tissue types. Such autogenic plasma-based plastic scaffolds may also be formed so as to incorporate autogenic adult stem cells. With the ever increasing banking of cord stem cells, the structures described herein could meet the demand for scaffolds capable
of delivering stem cells for other than hematopoietic stem cell applications. Microbars can be used for attaching graft materials, including corneal grafts, cartilage grafts, for blood vessel and other tubular structure Anastomoses. Finally, for cell culture applications PBP wafers can be constructed and placed in cell culture dishes, or porous spheres can be suspended in cell culture. Armed with the disclosure herein and the inventive feature of making bioplastic from autologous or allogenic plasma or plasma fraction(s) with at least one plasticizer, the remainder of the features and characteristics of the material can be readily controlled by those skilled in the art.

Incidentally, the market potential is significant. Bone grafts, including autografts, allografts and synthetics are far from ideal, yet these are currently the second most implanted of all biomaterials (blood products are first). Autologous plastics could economically address many of the problems associated with the current options. Beyond bone grafts, there are many other important applications, such as nerve guides, prosthetics/tissue interfaces, tendon repair, and wound protection bandages. A potential business model is an integrated plastics manufacturing system for hospitals that can be placed in or adjacent existing blood banks, including (as recited above) a centrifuge, a dryer, a powder miller, disposable molds in standard shapes, compression molds and a cooperating hot press, and a vacuum degasser. Another business model is batch manufacturing in any location.

The following examples are illustrative. In general, initial experiments were performed using rabbit and human plasma testing such variables as dried plasma particle size, percent plasticizer (such as glycerol), plasma powder/plasticizer equilibration time, and processing temperature and pressure. Furthermore, ammonium acetate porogen and genipen crosslinking validation experiments have been performed. In general, as overall conclusions, when plasma powder/plasticizer ratio is 55/45 and is held constant, and mixing equilibration time for dough mixing is varied, the resulting relative hardness of the bioplastic decreases as the dough incubation time increases. However, when plasticizer concentration is varied, while holding dough mixing and processing temperature and pressure constant, such an approach results in a decrease in relative hardness of the bioplastic as the relative plasticizer concentration increases.

When making elastomeric sheet materials from the present collected, clotted and dried plasma (dried through the gel phase as to one or more plasma fractions, but not powdered), it is possible to make elastomeric sheets based on plasma from either platelet rich or platelet poor plasma clotted and dried down through the gel phase. When making elastomeric plastic sheet materials from plasma in this way, it should be noted that lyophilization may not be used as the drying method for platelet rich plasma unless one adds additional thrombin to the plasma during or after clotting of the plasma.

Also, when processing plasma for use in elastomeric sheet type materials, it is useful to use existing "gel dryers" known in the art to dry the plasma through the gel phase.

As with fibrin or other protein-based plastics we have tested, plasma powder based plastics can be readily crosslinked with a crosslinker such as, without limitation, Genipin ((Methyl)1R,2R,6S)-2-hydroxy-9-(hydroxymethyl)-3-oxacycloc[4.3.0]nono-4,8-diene-5-carboxylate). Genipin can be added as a powder up to about 2% by weight of the powder weight, prior to dough mixing and plasticizing. Preferably, Genipin powder (known in the art) is solubilized in alcohol, such as ethanol, methanol, glycerol, isopropanol, propylene glycol, or any of the di-, tri- or tetra-polyethylene glycols and is incorporated into the plasma-based bioplastic dough in the amount of about 2% Genipin by weight of the dough (net of the alcohol or polyol carrier). Interestingly, an alcohol is used to sterilize the plasma powder. Genipin or other crosslinking agent may be added to the ethanol during sterilization, and can be retained in the bioplastic dough while the alcohol fraction is removed. It should also be understood that Genipin is preferably admixed into the bioplastic dough in solution—or and it can be infused into a plasma gel in solution—but it may also be incorporated as a dry powder with any of the present bioplastic ingredients at any step of processing. Other crosslinkers, both water- and alcohol-soluble, known in the art may be substituted. By example, proanthocyanidin may be used. Similarly, the introduction of particular ammonium acetate crystals, pre-sized to 150-250 microns, during the dough mixing phase, and following sublimation (drying under vacuum) post processing, resulted in a controlled porous plastic with a pore size of 150-250 microns. Other porogens known in the art may be substituted.

Sterilization of PBPs can be performed throughout processing, ranging from screening of plasma based on established donor collection protocols, by techniques known and developing for bacterial and viral minimization, alcohol, gamma- or other sterilization techniques of plasma powder and/or final post-packaging that represents minimal loss of biological activity, such as gamma radiation and ethylene oxide gas.

The following examples are illustrative.

EXAMPLE 1

As an example of initial biocompatibility of plasma-based plastics, plasma-based constituents (plasma powder/glycerol) 55/45 were vibrated to 300 micron thickness samples and sterilized via incubation in 70% ethanol for ten minutes. MG-63 human osteoblastic cells were seeded upon samples and incubated for three days. Cell containing samples were processed for scanning electron microscopy (SEM). Cells exhibited ready binding, proliferation and migration upon the bioplastic surface. Furthermore, cell proteolytic remodeling of the plastic was readily apparent and extensive cellular processes are interacting directly with the bioplastic, with proteolytic degradation creating a porous material from a smooth surface.

EXAMPLE 2

Rabbit plasma bioplastic samples were prepared and placed in cell free serum containing cell culture medium and held at 37°C for up to 60 days. Samples were weighed and measured for surface area at indicated times. The bioplastic was found to swell about 50% upon addition to media but thereafter to remain constant in size throughout the duration of sampling. This indicates that the present bioplastic will not spontaneously degrade consistent with cell proteolytic degradation.

EXAMPLE 3

Human plasma powder was sized into ≤38 micron and ≤150 micron distributions. Using similar processing conditions to those described in the first sentence of Example 1, processed slurries were thermomechanically molded into micron peg molds. The smaller particle size of ≤38 microns resulted in finer structural features compared to particle sizes of ≤150. It is believed that the present particle size ranges for the human plasma powder are preferably 38-500 microns, more preferably 50-200 microns and most preferably 75-150 microns.
Retained biological activity in plasma based plastics (PBBs). Due to processing parameters we are able to retain biological activity within PBBs. This biological activity results primarily, but not exclusively, as a function of growth factors and extracellular matrix (ECM) molecules contributed by platelets and to a somewhat lesser extent the plasma itself. Our principle processing parameter that clearly establishes the ability to create bioplastics with substantially preserved biological activity of biological constituents is the "low" temperature processing during plastification. Historically, both US and Hungarian art was based on "high" temperature processing well above 100°C. Knowledge of tissue repair promoting growth factors and ECM molecules contained in blood was not discovered until several decades later. Furthermore sterilization was based upon steam sterilization which completely denatured any biological activity with the purified blood proteins used historically, resulting in essentially a complete denaturation of any biological components. We teach the art of low temperature processing of <65°C when it is the purpose of the resulting plastic to retain growth factor activity. The critical temperature range is 55°C to 65°C with the preferred temperature being 60°C. Below 55°C the plastification reaction can be incomplete while increasing temperature to above 65°C results in significant loss of biological activity. However, if the purpose of the PBBs is mechanical not biological, higher temperature of up to 140°C and even in some cases 150°C, can result in superior mechanical characteristics.

There are two important aspects to temperature, namely, magnitude and duration. Table 1 illustrates the importance of temperature duration. As pressing time at 60°C increases from 7.5 to 30 minutes there is a significant loss in biological activity in the resulting PBBs. Biological activity was determined by taking known quantities of PBB samples, pulverizing to powder under liquid nitrogen, extracting soluble growth factors from the powder, and determining the ability of powder extracts to stimulate osteoblastic precursor cell proliferation in vitro.

### TABLE 1

<table>
<thead>
<tr>
<th>Pressing Time¹</th>
<th>Biological Activity (% above control)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Control³</td>
<td>158³</td>
</tr>
<tr>
<td>PBB: 7.5 min</td>
<td>285</td>
</tr>
<tr>
<td>PBB: 15 min</td>
<td>150</td>
</tr>
<tr>
<td>PBB: 30 min</td>
<td>48</td>
</tr>
</tbody>
</table>

¹PBB pressed at 60°C at 10.7 kpsi for indicated times
²% above non-serum, cell culture media control
³10% FBS in cell culture media
⁴Values represent the mean of triplicate determinations

### EXAMPLE 5

Interestingly, pressing temperature appears to have little or no impact on biological activity. An example experiment is presented in Table 2. Therefore, temperature not pressure is the limiting factor in retention of growth factor biological activity in PBBs.

### TABLE 2

<table>
<thead>
<tr>
<th>Pressing Pressure</th>
<th>Biological Activity (% above control)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Control⁵</td>
<td>148⁵</td>
</tr>
<tr>
<td>PBB: 60°C, 10.7 kpsi</td>
<td>96</td>
</tr>
<tr>
<td>PBB: 60°C, 14.7 kpsi</td>
<td>120</td>
</tr>
<tr>
<td>PBB: 55°C, 10.4 kpsi</td>
<td>142</td>
</tr>
<tr>
<td>PBB: 55°C, 14.7 kpsi</td>
<td>148</td>
</tr>
</tbody>
</table>

⁵PBB pressed at indicated temperature and pressure for 15 min
⁶% above non-serum, cell culture media control
⁷10% FBS in cell culture media
⁸Values represent the mean of triplicate determinations

### EXAMPLE 6

Another example of retained biological activity as well as biocompatibility is shown in FIG. I. Osteoblastic precursor cells were cultured on PBBs and then monitored for subsequent cell interactions using scanning electron microscopy. Increasing magnification from FIG. 1A-1D illustrate positive cell-PBB interaction with active remodeling of the PBB substrate.

### EXAMPLE 7

Genipin modification of PBBs. Historically, prior art has either not utilized chemical crosslinking to modify protein-based biomedical plastics or has used toxic crosslinking agents such as formaldehyde. With the use of formaldehyde, purified fibrin-based plastics could be maintained in vivo for time frames approaching one year. However, formaldehyde remains a toxic substance and crosslinking procedures occurred post-plastification. Post-plastification processing is rather complex procedure requiring extensive, multiple sequences to minimize undue swelling of the plastic due to temporal differences in water transport and the formaldehyde crosslinking reaction. The present approach is to utilize Genipin, a natural plant-based chemical crosslinker 100ks of times less toxic than formaldehyde, and as we teach here the Genipin can be added prior to plastification. Because transport of Genipin is not an issue, crosslinking occurs during plastification, stabilizing the PBBs and minimizing any swelling when placed in biological fluids. In addition, we disclose here not only of the timing of the addition of the Genipin, but also the form in which it is added to the preplasticized dough. FIG. 2 demonstrates that desolving Genipin crystals in ethanol prior to addition to the bioplastic dough results in a more homogenous distribution of crosslinking (the second line of bioplastic samples is demonstrably more homogenous than the top line). Note that when Genipin is delivered in crystalline form, it first dissolves locally within the forming PBB, resulting in “islands” that eventually create a non-homogenous distribution of crosslinking in PBBs. When Genipin crystals or powder are solubilized in ethanol prior to adding to the bioplastic dough phase, a homogenous color change occurs throughout the PBBs creating a more monolithic product.

As we have shown previously, Genipin treated plastics promote residence time in biological environments. Also, within the context of delivering growth factors and other biological components, although there is a slight loss in biological activity, substantial biological activity remains in Genipin treated PBBs (Table 3). Biological assessments were conducted as with Table 1 and 2. There is no difference
between the forms of Genipin added to the bioplastic dough, either crystalline or dissolved in ethanol.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect of genipin on biological activity of PBP</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PBP Sample</td>
</tr>
<tr>
<td>Serum Control</td>
</tr>
<tr>
<td>PBP: No Genipin</td>
</tr>
<tr>
<td>PBP: 2% Genipin (powder)</td>
</tr>
<tr>
<td>PBP: 2% Genipin (ETOH)</td>
</tr>
<tr>
<td>PBP: ETOH</td>
</tr>
</tbody>
</table>

1PBP pressed at 60 C, 10.7 kpsi for 15 min
2% above serum, cell culture media control
3% FBS in cell culture media
4Values represent the mean ± SEM of triplicate determinations

As expected, the inclusion of genipin in PBP has a significant influence on PBP mechanical properties, with the inclusion of genipin increasing the Young’s modulus by 4.9 fold (Table 4).

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanical properties of PBP</strong></td>
</tr>
<tr>
<td>% Genipin</td>
</tr>
<tr>
<td>Powder</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Powder + Water</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>ETOH</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

PBP were 65/35 PRP/glycerol (w/w) pressed at 60 C, 10.7 kpsi for 15 min

EXAMPLE 8

Lyophilized plasma particle size on PBP characteristics. Another aspect to PBP formulation where we teach new art is particle size of source biological powder for bioplastic dough preparation. Historically, purified protein starting materials were screened to remove large particles, but there is nothing in the prior art concerning the importance of initial powder particle size and the resulting PBP characteristics. As the particle size becomes smaller this denotes a faster equilibration time of “wetting” powder with added plasticizer during the dough preparation. Smaller particle size enables more uniform mold fill as demonstrated in FIG. 3. These properties are desirable during micromolding or for micromachining preparation of PBP; whereas, larger particle size will enable better macromolecular interlock between particles during plasticification, resulting in PBP's with greater mechanical properties.

EXAMPLE 9

Addition of calcium phosphate particulates to PBP. Calcium phosphate particulates can be added during PBP dough preparation to create PBP with both organic and inorganic components. FIG. 4 shows the addition of up to 10% nano-particulate tricalcium phosphate (TCP) powder during dough formation with an increase in PBP opacity as TCP concentration increases. Alternatively, other clinically relevant forms of calcium phosphate, including but not limited to hydroxyapatite, can be substituted or mixed with TCP. The inclusion of such materials will alter mechanical properties, degradation, growth factor release rates, and provide additional osteoconductivity.

EXAMPLE 10

Uncrosslinked PRP-PBP is stable under in vitro conditions. PRP based PBP was placed under simulated in vivo conditions, 37° C. in serum containing media for 60 days. A slight swelling occurred within the first day, but there was no subsequent change throughout the incubation period.

Although the invention has been described with particularity above, with reference to specific materials and methods and results, the invention is only to be claimed insofar as is set forth in the accompanying claims.

The invention claimed is:

1. A method for making a plasma based plastic comprising: a) collecting a quantity of plasma, wherein said plasma is separated from whole blood; b) clotting said plasma; c) drying said clotted plasma, wherein said drying comprises lyophilization; d) contacting a quantity of the clotted dried plasma with at least one plasticizer to make a dough; and e) shaping and heating said dough to make a bioplastic article.

2. The method according to claim 1, wherein at least one plasticizer is added to said quantity of plasma before or after the step of clotting said plasma.

3. The method according to claim 1, wherein a crosslinking agent is added to said quantity of plasma before or after the step of clotting said plasma.

4. The method according to claim 1, wherein said quantity of plasma is a pooled quantity of plasma from a plurality of mammalian donors and wherein a crosslinking agent is added to said quantity of plasma before or after the step of clotting said plasma.

5. The method according to claim 1, wherein said quantity of plasma is a pooled quantity of plasma from a plurality of human donors.

6. The method according to claim 1, wherein said quantity of plasma is collected from a single human donor.

7. A method for making a plasma based plastic comprising: a) collecting a quantity of human plasma, wherein said plasma is separated from whole blood; b) clotting said plasma; c) drying said clotted plasma, wherein said drying comprises lyophilization; d) contacting a quantity of the clotted dried plasma with at least one plasticizer to make a dough; and e) shaping and heating said dough to make a bioplastic article.

8. The method according to claim 1 or 7, wherein said dough is heated at a temperature between 55-65° C.

9. The method according to claim 1 or 7, wherein said dough is heated at a temperature of no higher than 150° C.

10. The method according to claim 1 or 7, wherein the dough is shaped and heated at a pressure between 9-25 kilopounds per square inch.

11. The method according to claim 1 or 7, wherein the dough is shaped and heated at a pressure of at least 10.7 kilopounds per square inch or higher.

12. The method according to claim 1 or 7, wherein a porogen compound is added to the dough prior to shaping and heating.

13. The method according to claim 1 or 7, wherein said plasma is collected via apheresis.
14. The method according to claim 1 or 7, wherein said plasma is whole plasma.

15. The method according to claim 1 or 7, wherein said plasma is platelet-rich plasma or platelet-poor plasma.

16. The method according to claim 1 or 7, wherein said plasma is plasma from which one or more constituents has been removed.

17. The method according to claim 1 or 7 wherein the at least one plasticizer is selected from the group consisting of glycerol and water.

18. The method according to claim 1 or 7 wherein a stabilizer is added to said quantity of plasma and wherein said stabilizer is selected from the group consisting of glycerogen, sorbitol, mannitol, trehalose, maltitol, xylitol, isomaltitol, erythritol, amylase, amylopectin, inositol hexa sulphate, sulphated beta-cyclodextrin, and combinations thereof.

19. The method according to claim 1 or 7 wherein the dough is crosslinked by adding genipin as a powder to the clotted dried plasma in the amount of about 2% by weight of the dried plasma.

20. The method according to claim 19 wherein said genipin is solubilized in alcohol before adding said genipin to the dough.

21. The method according to claim 1 or 7 wherein the clotted dried plasma is adjusted to a percentage water by weight of 5-15%.

22. The method according to claim 1 or 7 wherein ammonium acetate is added to the dough prior to shaping and heating.

23. The method according to claim 1 or 7 wherein prior to shaping and heating, the dough is combined with a quantity of particulate ammonium acetate crystals, sized to 150-250 microns, and wherein during the shaping and heating the ammonium acetate crystals sublimate to result in a controlled porous plastic with a pore size of 150-250 microns.

24. The method according to claim 1 or 7 wherein said quantity of plasma is clotted, dried and comminuted to a particle size distribution of between 38-500 microns.

25. The method according to claim 1 or 7 wherein the dough is heated at a temperature between 100-140°C.

26. The method according to claim 1 or 7 wherein prior to shaping and heating, up to 10% nanoparticulate tricalcium phosphate is added to the dough.