METHOD OF OBTAINING Viable SMALL TISSUE PARTICLES AND USE FOR TISSUE REPAIR

VERFAHREN ZUR GEWINNUNG VON LEBENSFÄHIGEN KLEINEN GEWEBETEILCHEN UND IHRE VERWENDUNG FÜR DIE GEWEBEREPARATUR

PROCÉDÉ DESTINÉ À OBTENIR DE PETITES PARTICULES TISSULAIRES VIABLES ET UTILISATION POUR LA RÉPARATION TISSULAIRE

Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE SI SK TR


Date of publication of application: 23.09.2009 Bulletin 2009/39

Proprietor: Zimmer Orthobiologics, Inc.
Austin, TX 78729 (US)

Inventors:
• YAO, Jian Q.
 Austin, TX 78759 (US)

• ZAPOROJAN, Victor
 Austin, TX 78727 (US)

Representative: den Hartog, Jeroen H.J. et al
Howrey LLP
Rembrandt Tower, 31st Floor
Amstelplein 1
1096 HA Amsterdam (NL)

References cited:
WO-A-2004/078032
WO-A-2006/002253

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
Description

TECHNICAL FIELD OF THE INVENTION

[0001] Preparation and use of tissue particles, sized from various sources, to repair tissue defects such as orthopedic tissue defects.

BACKGROUND

[0002] Articular cartilage is a thin, smooth, low friction, gliding surface composed of hyaline cartilage with resiliency to compressive forces. While only a few millimeters thick, it has excellent wear characteristics. Its mechanical and structural capacity depends on the integrity of its extracellular matrix, in which chondrocytes are sparsely distributed throughout structural macromolecules including collagen, proteoglycans, and noncollagenous proteins. Although chondrocyte cells produce the extracellular matrix, they compose less than 5% of the wet weight of cartilage.

[0003] The composition and highly complicated interaction of these components make regeneration and replacement techniques challenging. For example, the lack of a direct blood supply and few cells distributed widely among a dense extracellular matrix leads to a limited healing ability of damaged articular cartilage. This has led to a wide variety of treatment approaches for defects, for example, in the knee, with varying levels of success.

[0004] Procedures such as drilling, abrasion, microfracture, and debridement provide symptomatic pain relief and improved function. Collectively, these procedures may be referred to as subchondral bone marrow stimulation techniques where the bone underlying the cartilage, which has a rich blood supply, is caused to bleed. The goal of such procedures is to mobilize mesenchymal stem cells from the blood to differentiate into chondrocyte-like cells that synthesize repair tissue. Once the vascularized cancellous bone is disrupted, a fibrin clot forms and pluripotent cells migrate into the area. These cells eventually differentiate into chondrocyte-like cells that secrete type I, type II and other collagen types, as well as cartilage specific proteoglycans, after receiving appropriate mechanical and biological cues. The cells produce a fibroblastic repair tissue that on appearance and initial biopsy can have a hyaline-like quality, but over time, is demonstrated histologically as being predominantly fibrocartilaginous tissue. Fibrocartilage is a relatively disorganized lattice of collagen fibers, as opposed to the natural hyaline cartilage, and thus partially fills the defect with structurally weak tissue that also exhibits limited durability.

[0005] Other procedural options such as periosteal grafting, osteochondral autografts and allografts, and autogenous chondrocyte cell implantation have been used to repair cartilage defects for the purpose of reducing pain and restoring function. The success of these procedures generally diminishes over time, possibly due to formation of fibrocartilage, inadequate development of repair tissue, poor cell differentiation, and/or poor bonding to the surrounding articular cartilage borders. Intact full thickness grafts, such as osteochondral autografts and allografts, also may suffer from mismatched sizes, immunologic rejection, and poor adhesion of cartilage to bony surfaces. For autogenous chondrocyte cell implantation, two surgeries are required: chondrocytes are first obtained from an uninvolved area of cartilage and cultured for 14 to 21 days, then the cultured cells are injected into the defect exposed via an open incision and covered with a periosteal flap excised from the proximal medial tibia.

[0006] Various methods of promoting tissue growth and repair, and in particular cartilage repair, have been suggested and include the use of tissue particles derived from grinding non-demineralized, articular cartilage into pieces of about 60 μm to about 500 μm (Malinin U.S. Patent Application No. 20050196460); mincing tissue into particles using two parallel blades, resulting in particles of about 0.1 to about 3 mm³ in size and containing at least one viable cell (Binette et al. U.S. Patent Application No. 20040078090); pulverizing soft tissue into morsels of about 1 to about 100 μm that may then be combined with viable elements (cells) and/or bioactive molecules (Awad et al. U.S. Patent Application No. 20050288796); and, milling allograft cartilage, which is then lyophilized to create particles in the size of about 0.01 mm to about 1 mm that can be formulated into a paste (Gomes et al. U.S. Patent Application No. 20040219182). Various methods of tissue preparation have also been disclosed including a method of generating dermal tissue pieces of about 50 μm to about 1500 μm using a roller with multiple blades (Mishra et al. U.S. Patent Application No. 20040175690).

[0007] Cell and/or tissue viability for implants needs to be improved. For example, homogenizers used to generate tissue particles have resulted in about 5% of the cells remaining viable following homogenization. Enzymatic digestion, which is often used to generate cells for autogenous chondrocyte cell transplantation, results in poor cell viability following initial isolation.

[0008] Improved compositions and methods for repairing tissue defects and in particular, articular cartilage defects are desired.

SUMMARY OF THE INVENTION

[0009] One embodiment is a composition including isolated small tissue particles composed of cells and their associated extracellular molecules (e.g., proteins, polysaccharides, proteoglycans, etc.) known as the extracellular matrix (ECM). The tissue particles are sized such that in some embodiments, the particles have at least one dimension less than about 60 μm. In another embodiment, the particles have at least one dimension less than about 1 mm. In another embodiment, the particles are sized so that the volume is less than about 1
In some embodiments, at least about 50% of the cells in the tissue particles are viable. In other embodiments, at least about 80% of the cells in the tissue particles are viable. In some embodiments, the composition may also contain additives such as adhesives, solutions, and bioactive agents. Examples of adhesives include fibrin glue, Tisseal (Baxter BioScience, Deerfield IL), and Surgicel (Johnson & Johnson, New Brunswick NJ). Examples of bioactive agents include fibronogen, thrombin, bone morphogenic proteins (BMP), insulin-like growth factors (IGF), transforming growth factors (TGF) including the beta form (TGFβ), platelet-derived growth factor (PDGF), and bone marrow aspirate.

Another embodiment is a method for creating small tissue particles whereby a tissue sample is positioned on a cutting device containing at least two blades in parallel in one embodiment, and at least three blades in parallel in another embodiment. In embodiments containing at least three blades, spacing between the blades may be uniform or may vary. The space between the blades may define a dimension of the particle. In one embodiment, at least one blade is curved. In another embodiment, at least two blades are not parallel. In one embodiment, the sizing apparatus comprises three blades mounted in parallel and separated by spacers having a width of about 60 μm. By changing the relative spatial relationship between the tissue sample and the cutting apparatus, cuts can be made in the horizontal, vertical, and coronal planes. Because the angle between these planes can be varied, the resulting tissue particle can be sized to a variety of shapes, including cubes, triangles, quadrilaterals, and other polygons.

Another embodiment is the described compositions for ameliorating a tissue defect. In one embodiment, the defective tissue may be cartilage, bone, ligament, meniscus, tendon, muscle, nucleus pulposus, gingiva, annulus fibrosus, periosteum, perichondrium, fascia, and/or perineurium. In one embodiment, the defective tissue may be articular cartilage. In general, the use includes placing the isolated sized tissue particles into a tissue defect site. Retention of the tissue particles in the defect site is facilitated by the small particle size. In certain embodiments, retention of the tissue particles at the defect site may be enhanced by techniques such as microfracture and use of adhesives.

Another embodiment is a use of the inventive small tissue particles under cell culture conditions and, for example, as part of in vitro experimentation and/or to propagate cells in culture.

The method and composition will be further appreciated with reference to the following figures and description.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1A** is a photograph of particles with only live cell staining.

**FIG. 1B** is a photograph of particles with only dead cell staining.

**FIG. 2** shows an apparatus for sizing tissue particles.

**FIG. 3A** shows the percentage of viable cells following one embodiment of the method.

**FIG. 3B** shows the percentage of viable cells following enzymatic digestion versus an embodiment of the invention.

**FIG. 4** shows a schematic representation of the three particle planes defined by the x-axis, y-axis, and z-axis.

**FIG. 5A** is a photograph that shows a surface of a porcine knee joint that has been subjected to one embodiment of the invention.

**FIG. 5B** is a photograph that shows a surface of a porcine knee joint that has been subjected to another embodiment of the invention.

**FIG. 5C** is a drawing of the photograph of **FIG. 5A**.

**FIG. 5D** is a drawing of the photograph of **FIG. 5B**.

**DETAILED DESCRIPTION**

In one embodiment, a composition comprising a plurality of isolated tissue particles is disclosed. The particles comprise cells and their associated extracellular molecules, (e.g. proteins, polysaccharides, proteoglycans, etc.), which collectively are termed a matrix. In another embodiment, the tissue particles are comprised of cells wherein at least about 50% of the cells are viable. In another embodiment, the tissue particles are comprised of cells wherein at least about 60% of the cells are viable. In another embodiment, the tissue particles are comprised of cells wherein at least about 70% of the cells are viable. In another embodiment, the tissue particles are comprised of cells wherein at least about 80% of the cells are viable. Cell viability indicates that the cell is alive and able to perform one or more intrinsic biological functions (e.g., cellular signaling, maintenance of cellular homeostasis, etc.), and also may include cells that are dormant or arrested in a stage of the cell cycle. Cells that are not viable are cells that are dead. The absolute number of viable cells may vary depending on, for example, the tissue type used to make the particles and/or particle size. The presence of viable cells in the tissue particle composition facilitates use of the composition in ameliorating tissue defects, as described more fully below. For example, viable cells provide stimulators and/or cues for tissue remodeling, growth, and/or repair. Methods to measure cell viability are known to one skilled in the art and include facilitated dyes and biochemical assays. For example, cell viability in the tissue particles was measured using the LIVE/DEAD® viability assay (Invitrogen, Eugene OR) where the calcein dye is re-
tained in live cells and emits a green fluorescence and the ethidium homodimer is able to enter cells with dam-
age membrane and emits red fluorescence when inter-
acting with nucleic acids. FIG. 1A shows dead cells that emit red fluorescence in tissue particles in one embod-
iment of the invention. FIG. 1B shows living cells that emit green fluorescence in tissue particles in one embod-
iment of the invention. The results in FIGs. 1A and 1B showed satisfactory uptake of the dyes in to the small tissue par-
ticles.

[0016] Along with cells, the tissue particles also contain extracellular molecules, often referred to as the extracellu-
lar matrix (ECM). The ECM surrounds and supports cells within mammalian tissues, and is composed of three 
major classes of biomolecules: (i) structural proteins such as collagen and elastin; (ii) specialized proteins such as 
fibrillin, fibronectin, and laminin; and (iii) proteoglycans. Proteoglycans are composed of a protein core that is 
attached to long chains of repeating disaccharide units termed glycosaminoglycans (GAGs) and form complex 
high molecular weight components of the ECM. The ECM has many roles including cellular organization, guidance 
of cell migration and growth, and structure; the promi-
nence of these roles can vary depending on the tissue. For example, the ECM plays an important role in force 
transmission and tissue structure maintenance especially in cartilage, tendons, ligaments, bone, and muscle. The 
precise composition of the ECM in the particles depends on factors such as the tissue from which the particles 
are obtained and any treatments or modifications thereof. Thus, the composition of the ECM will vary depen-
ding on the endogenous composition for that tissue type. Along with variations in ECM composition based 
on tissue type, the ECM may also be modified. As one 
example, the particles may be treated with enzymes 
that hydrolyze protein and/or glycans, such as trypsin 
and hyaluronidase, to increase the accessibility of the par-
ticles. Also, the particles may be treated with enzymes 
acting with nucleic acids. FIG. 1A shows dead cells that 
emits red fluorescence when interacting with nucleic acids. FIG. 1B shows living cells that emit green fluo-
rescence in tissue particles in one embodiment of the invention. The results in FIGs. 1A and 1B showed sat-
sactory uptake of the dyes in to the small tissue par-
ticles.

[0017] The size of the tissue particles of the inventive 
composition may vary depending on such factors as the 
type of source tissue used, the age of the tissue, and the 
intended subsequent use of the composition. In one em-
bodyment, tissue particles are sized such that at least one 
dimension of the particle is less than 1 mm. In another 
embodiment, the tissue particles are sized such that at 
least one dimension is less than 60 μm. In another em-
bodyment, the tissue particles are sized such that the par-
ticle is substantially cubical with each side about 60 μm 
or less. In another embodiment, the tissue is derived from a 
juvenile source and the tissue particles have a volume 
less than 1 mm³. In another embodiment, the tissue par-
ticles have a volume of about 2 x 10⁻⁴ mm³. The tissue 
particles may be any shape, including but not limited to 
cubes and elongated strips. Sizing refers to cutting of the 
tissue sample into the desired size and/or shape, and is 
further described below.

[0018] The tissue particles may be derived from a va-
riety of tissue types and tissue sources. The tissue may 
be autogenic, allogenic, or xenogenic with respect to the 
recipient of the inventive composition, as explained be-
low. Any tissue is potentially suitable for use and tissue 
types may include cartilage, bone, ligament, meniscus, 
tendon, muscle, nucleus pulposus, gingival, annulus fi-
brusos, periosteum, perichondrium, fascia, and/or peri-
neurium. In one embodiment, the tissue is articular 
cartilage. In another embodiment, the articular cartilage 
is hyaline cartilage and/or fibrocartilage.

[0019] In one embodiment, the tissue is engineered 
tissue. Engineering of the tissue refers to altering the 
physiology of the tissue such that it possesses traits that 
it would normally not have, magnifying and/or muting 
the existing tissue traits, and/or growing tissue in vitro. En-
gineered tissue may include tissue derived from a trans-
genic donor. Transgenic donor refers to tissue sources, 
such as animals, in which exogenous genetic material 
has been incorporated into the genome of the source. 
The incorporated genetic material may provide for the 
expression of a non-endogenous gene or may alter the 
expression levels of an endogenous gene. In another 
embodiment, the donor tissue may be genetically altered 
following removal from the donor. Examples of altera-
tions of the tissue following excision and prior to or con-
comitant with culturing include alterations brought about 
by introduction of genetic material and/or bioactive 
agents. In the case of genetic manipulation, the tissue 
may be treated with genetic vectors using various meth-
ods of genetic introduction, e.g. viral- and lipid-mediated, 
as known in the art, to bring about alterations in endog-
avenous or exogenous gene expression.Bioactive agents, 
such as growth factors, may be incubated with the cul-
tured tissue to bring about alterations in tissue physiolo-
gy. Engineered tissue may also refer to tissue that has 
been propagated or grown in vitro. Tissue grown in vitro 
refers to the creation and/or propagation of tissue outside 
an animal host. For example, in vitro grown tissue may 
result from tissue culture manipulations where cells are, 
for example, stimulated to form a tissue in an incubating 
vessel. Methods for producing in vitro tissue are known 
to one skilled in the art.

[0020] The developmental or maturation stage of the 
tissue used in the invention may also vary. For example, 
the tissue particles may be derived from embryonic, fetal, 
neonatal, juvenile, or adult tissue. In an embodiment 
where juvenile tissue is used, juvenile is defined as being 
less than 12 years old in the case of humans. Further, 
the tissue may be acutely isolated or cultured prior to 
sizing into particles. In the case of cultured tissue sam-
ples, the tissue is maintained in an environment that pre-
serves the viability of the cells in the tissue. However, it 
is also understood by one skilled in the art that some cell 
death may occur as a result of in vitro tissue culturing. 
The tissue, either in preparation of culturing or following 
acute isolation, may be sized into smaller pieces that
either facilitate subsequent sizing, e.g. results in a size that is easier to manipulate in the subsequent creation of tissue particles, or promotes cell viability in tissue culture, e.g. increases the surface area of the tissue and thus oxygen and nutrient accessibility to the cells. In another embodiment, the tissue is sized to the desired particle size prior to culture.

[0021] The composition may also include additional components. In another embodiment, the tissue particles of the composition are maintained or suspended in a solution. The solution may be a buffer that maintains the solution pH in a desired range. For example, the buffer may maintain the tissue particles in a solution in the range from about pH 6.8 to about pH 7.5. In other embodiments, the buffer may maintain the pH in the range of about pH 5 to about pH 7. The buffer, and the resulting buffering pH range chosen, depend on factors known to one skilled in the art including the tissue type and the effects of certain pH on that tissue type.

[0022] In another embodiment, the composition include bioactive agents. The bioactive agents may be either residual from culturing of the tissue sample as described above or may be added to the tissue particles at another time. Examples of bioactive agents include but are not limited to growth factors, hormones, and nutrients.

[0023] The inventive composition may also comprise an adhesive that aids in the attachment of the tissue particles to the site of tissue defect. The adhesive may be a naturally occurring bioadhesive such as fibrin. Thrombin converts soluble plasma fibrinogen into molecules of fibrin that polymerize and form a fibrin clot. Fibrin may encapsulate and/or enmesh the tissue particles at the sites of tissue defect. It should also be noted, however, that due to the small size of the inventive tissue particles, the particles are intrinsically adhesive to the site of tissue defect. In another embodiment, the tissue particles may be treated such that they become positively charged. The tissue particle may be charged by a variety of treatments including exposing the particles to an ionic detergent or a magnetic field, resulting in the creation of an overall positive charge on the particles. The overall positive charge of the particle facilitates adhesion of the particle to the predominantly negatively charged tissue defect. Increased adhesion of the particles to the tissue defect site may reduce the time required for tissue defect repair.

[0024] In one embodiment, a method of preparing a composition comprising tissue sized into particles is disclosed. The tissue sample is initially cut into smaller pieces to facilitate subsequent sizing into tissue particles, e.g., using surgical tools known to one skilled in the art, such as a scalpel. In one embodiment, the tissue is initially cut into pieces of about 5 mm to about 11 mm. In another embodiment, the tissue, which may have been cultured, has already been subjected to the initial cutting process and is of the approximate size for subsequent sizing. As shown in the schematic of FIG. 2 (not to scale), once the tissue is of the appropriate initial size, it is mounted on a jaw 12 of an axial cylinder. By extending the axial cylinder along axis A, the tissue 10 contacts the blades 14 mounted in opposition of the jaw. In one embodiment, the tissue is contacted with at least two blades mounted in parallel on a substantially flat surface. In another embodiment, the blades 14 are not parallel to each other and may also include blades that are not straight, e.g., curved. The configuration of blades 14 will also include spacers 16 between the blades, the width of which will correspond to the desired dimension between parallel cuts. In certain embodiments, the spacers 16 between the blades 14 will be the same size and in other embodiments, the spacers 16 may be of different sizes. The blades will be sufficiently sharp so that damage and/or loss of the tissue will be minimized.

[0025] The method may be conducted in the absence of exogenously added digestive enzymes. Although digestive enzymes promote cell dissociation, they also may decrease the percent of viable cells resulting from the treatment. In FIG. 3, the viability of cells following one embodiment of the inventive method, as determined by LIVE/DEAD® viability assay, was about 85% (FIG. 3A) while viability of cells following enzymatic treatment with collagenase resulted in about 25% viable cells (FIG. 3B). Without being held to a single theory, it is believed that the digestive enzyme damages the cell membrane components, contributing to the death of the cell.

[0026] The first contact between the blades and the tissue results in parallel cuts in, for example, the x-axis plane of the tissue sample, as shown in FIG. 4. In different embodiments, the tissue may be pushed against the blades or alternatively the blades may be pushed against the tissue. In another embodiment, the blade and tissue sample are both moved towards each other. In certain embodiments, the blades may not be parallel and therefore, would not result in parallel cuts. However, for simplicity, the inventive method will be described in terms of parallel blades making parallel cuts but in all cases, the blades and resulting cuts may not be parallel, and also may be non-straight, e.g., curved. Following the first contact between the blades and the tissue, the sample and/or blades can then be translationally moved along axis B, e.g. without rotation, so that further cuts can be made in parallel with the previous cuts and still in the same plane. In one embodiment, the blades are translationally moved with precision of about 1 μm using a digital micrometer 18, as shown in FIG. 2. The blades and/or tissue sample is then rotated relative to one another in rotation C about axis A, defining a second orientation and the blades and tissue are again caused to contact, making cuts in, for example, the y-axis plane, as shown in FIG. 4. In this second orientation, the blades and/or tissue can again be translationally moved so that a series of parallel cuts can be made. In one embodiment, the second contact between the blades and tissue results in substantially perpendicular cuts in the tissue wherein the angle be-
tissue particle is sized such that at least one dimension is less than 1 mm. In another embodiment, the resultant tissue particle is sized such that at least one dimension is less than 1 mm. In another embodiment, the blades contact the tissue and the tissue is derived from a juvenile, non-dermal source, the resultant tissue particle is sized such that at least one dimension is less than 1 mm. In another embodiment, the resultant tissue particle size is less than 1 mm. The method produces tissue particles of the desired dimensions and also maintained a high percentage, e.g. above about 85%, viable cells (See FIG. 3A).

As described above, the tissue source used by the inventive method to generate tissue particles may be used from any source and with any type of tissue including autogenic, allogenic, xenogenic, cultured, and engineered tissue and of any maturation stage.

In one embodiment, the composition for ameliorating a damaged tissue in a mammal is used for ameliorating a damaged tissue. In one embodiment, the composition for ameliorating a damaged tissue in a mammal is used for ameliorating a damaged tissue in a mammal. The tissue particle composition is introduced into or in proximity to a damaged tissue under conditions sufficient to ameliorate the damaged tissue. The composition may include a plurality of tissue particles sized from tissue. In addition, the particles may include both cells and extracellular molecules organized in a matrix, as described above. In one embodiment, the damaged tissue may be articular cartilage. In the case of damaged particular cartilage, the cartilage lesion may be debried back to a stable base cartilage and loose or fibrillated cartilage may be resected, In one embodiment, in the case of articular cartilage, the subchondral base is microfractured until bleeding occurs from the subchondral bone. Microfracture entails creating a series of small fractures in the bone of about 3 mm to about 4 mm in depth using an awl. Alternatively, a drill may be used to create holes in the subchondral bone, with care to not cause heat necrosis in the site. The composition is applied into the area of and/or proximate the defect.

In one embodiment, the composition for ameliorating damaged tissue also including adhering the inventive composition to the damaged tissue. It is conducted in conjunction with microfracture, adhesive properties of bleeding bone secure the tissue particles in place, as shown in FIGS. 5A and 5C. Specifically, the resulting blood clot from the bleeding bone serves as a biological glue that maintains the particles on the surface near the defect. Also, due to the small size of the tissue particles, the particles naturally remain in the defect, as shown in FIGS. 5B and 5D, possibly as a result of surface tension. The inventive composition may also include adhesives such as fibrin, hyaluronic acid, fibrin glue, fibrin clot, collagen gel, alginate gel, gelatin-resorcin-formalin adhesive, mussel-based adhesive, dihydroxyphenylalanine (DOPA) based adhesive, chitosan, transglutaminase, poly(amino acid)-based adhesive, cellulose-based adhesive, polysaccharide-based adhesive, synthetic acrylate-based adhesives, platelet rich plasma (PRP), platelet poor plasma (PPP), clot of PRP, clot of PPP, MATRIGEL® (BD Biosciences, San Jose CA), monostearoyl glycerol co-succinate (MGSA), monostearoyl glycerol co-succinate/polyethylene glycol (MGSA/PEG) copolymers, laminin, elastin, proteoglycans, and combinations thereof.

In one embodiment, the tissue particles of the inventive composition may have been treated such that the particles exhibit a net charge, as described above, that facilitates electrostatic adhesion to the tissue defect. Other techniques known to one skilled in the art, such as flaps, may also be used to keep the particles In the defect site.

In one embodiment, the composition for ameliorating damage tissue may be applied using a minimally invasive procedure, e.g. arthroscopy. The use of a minimally invasive procedure allows a smaller incision, resulting in less pain, a shorter in-patient stay, and a faster recovery time than traditional more invasive procedures. The use of a minimally invasive procedure such as arthroscopy may also aid in diminishing potential post-operative complications such as soft tissue fibrosis.

In one embodiment, the damaged tissue may be orthopedic tissue such as cartilage, bone, ligament, meniscus, tendon, and/or other muscle. In another embodiment, the damaged tissue may be nuclear pulposus, gingival, annulus fibrosus, peristeum, perichondrium, fascia, and/or perineurium. In one embodiment, the damaged tissue and the tissue that is used as the source for the Inventive composition are the same tissue type, e.g. articular cartilage. In another embodiment, the damaged tissue and the tissue that is used as the source for the inventive composition are different tissue types, Including autogenic, allogenic, xenogenic, cultured, engineered tissue, and of any maturation stage.

One embodiment discloses a biocompatible implantable composition comprising a plurality of biological tissue particles sized from tissue derived from viable ju-
venile cartilage, wherein the particles are comprised of chondrocytes having at least about 80% viability and extracellular proteins, each particle less than 60 μm, and the composition is capable of implantation in a mammal.

[0035] In one embodiment, particulate cartilage compositions are created and used for cartilage regeneration by stimulating chondrogenesis. Articular cartilage may be obtained from the articular surfaces of joints, such as from distal femurs, proximal tibia, acetabulum, heads of femurs, and/or heads of radii, as well as from other sites where hyaline cartilage is present, e.g., auricular, nasal, temporomandibular joint, and costal margin. The cartilage may be removed, for example, with a scalpel blade, rongeur, or other surgical instrument. In one embodiment, cartilage is removed down to subchondral bone, without removing bone. The articular cartilage may include articular hyaline cartilage and/or fibrocartilage and may comprise allogeneic and/or xenogeneic cartilage.

[0036] The following example further illustrates embodiments of the invention.

EXAMPLE

[0037] Cartilage tissue particles were assessed for cell viability and evaluation in cartilage defect repair. All procedures were conducted in compliance with relevant regulations for the use of animal tissue. Porcine knee joints were obtained from a local abattoir. A knee joint was opened using a scalpel and the articular cartilage from the condyle load bearing area was exposed. Using a 7.5mm diameter coring reamer, an osteochondral plug was obtained.

[0038] The osteochondral plug was mounted on the jaw of the cutting device (FIG. 2) and a series of cuts were made using three multiple blades to obtain viable small tissue particles.

[0039] Tissue particles were stained using LIVE/DEAD® stain to determine cell viability. The LIVE/DEAD® stain uses a membrane-permeant CALCEIN AM that is cleaved by endogenous esterases in the live cells to yield cytoplasmic green fluorescence, and the membrane-impermeant ethidium homodimer-1 labels nucleic acids of membrane-compromised cells, e.g., dead cells, with red fluorescence. Pictures of the stained slides were analyzed using NIH imaging software and the number of total and viable cells was calculated. For the enzymatic digestion method, cartilage was shaved off the articular surface and collected in a Petri dish. Tissue weight was recorded. The cartilage tissue blocks were digested in a 1:10 mass:volume ratio in 0.15% of collagenase type II for about 12-16 hours until no visible fragments remained. The cell-collagenase solution was filtered and washed with phosphate-buffered saline. Isolated cells were counted and viability was determined using LIVE/DEAD® staining. The number of viable cells was normalized to the tissue weight and represented as a percentage of the absolute number of cells in a given unit of tissue, as shown in FIG. 3A. Paired t-test statistical analysis was performed using Sigma Stat 2.0 software.

[0040] Results showed that tissue particles had a regular geometry. The majority of the particles were from 50 microns to 240 microns. LIVE/DEAD® staining experiments showed good tissue penetration of the dyes due to the size of the particles. Although a small percentage, about 10% to about 15%, of the tissue was lost during the cutting procedure, cell viability in the tissue particles was significantly higher than the percentage of viable cells obtained by digestion method (Compare FIG. 3A and 3B). Tissue particles seeded on the surface of the joint remained attached to the surface against gravity for an indefinite period of time as long as conditions were maintained (FIG. 5B) and the adhesion was increased when microfracture was simulated by compressing the subchondral bone and causing to bleed (FIG. 5A).

[0041] The above results showed that small tissue living particles were obtained from an autologous source. The results also showed that cell viability inside of the particle remained higher than 85%.

Claims

1. A composition comprising a plurality of isolated viable tissue particles, the particles comprising cells having at least 80% viability and cell-associated extracellular proteins, each particle sized to have at least one dimension less than 60 μm.

2. The composition of claim 1 wherein the extracellular proteins are a component of an extracellular matrix.

3. The composition of claim 2 wherein the extracellular proteins are selected from the group consisting of collagen, elastin, fibrillin, fibronectin, and laminin.

4. The composition of any preceding claim wherein the particles comprise tissue selected from the group consisting of cartilage, bone, ligament, meniscus, tendon, muscle, and combinations thereof.

5. The composition of any preceding claim wherein the particles comprises engineered tissue.

6. The composition of any preceding claim wherein the particles comprise juvenile tissue.

7. The composition of any preceding claim wherein the particles comprise articular cartilage.

8. The composition of any preceding claim wherein the articular cartilage comprises at least one of hyaline cartilage or fibrocartilage.

9. The composition of any preceding claim further comprising at least one of an adhesive, a buffer, or a...
10. The composition of claim 9 wherein the adhesive is selected from the group consisting of fibrin, hyaluronic acid, fibrin glue, fibrin clot, collagen gel, alginate gel, gelatin-resorcin-formalin adhesive, mussel-based adhesive, dihydroxyphenylalanine (DOPA) based adhesive, chitosan, transglutaminase, poly (amino acid)-based adhesive, cellulose-based adhesive, polysaccharide-based adhesive, synthetic acrylate-based adhesives, platelet rich plasma (PRP), platelet poor plasma (PPP), clot of PRP, clot of PPP, monostearoyl glycerol co-succinate (MGSA), monostearoyl glycerol co-succinate/polyethylene glycol (MGSA/PEG) copolymers, laminin, elastin, proteoglycans, and combinations thereof.

11. The composition of claim 9 wherein the bioactive agent is selected from the group consisting of growth factors hormones, nutrients, BMP, IGF, TGF, PDGF, and bone marrow aspirate.

12. The composition of any preceding claim further comprising thrombin.

13. The composition of any of the preceding claims for ameliorating damaged tissue in a mammal.

14. The composition for use according to claim 13, wherein the damaged tissue is selected from the group consisting of cartilage, bone, ligament, meniscus, tendon, muscle and combinations thereof.

15. The composition for use according to claim 14, wherein the damaged tissue is articular cartilage.

Patentansprüche

1. Zusammensetzung, die eine Vielzahl von isolierten lebensfähigen Gewebeteilchen umfasst, wobei die Teilchen Zellen mit wenigstens 80% Lebensfähigkeit und zellassoziierten extrazellulären Proteinen umfassen, wobei jede Teilchen solche Abmessungen hat, dass es in wenigstens einer Richtung kleiner als 60 \( \mu m \) ist.

2. Zusammensetzung gemäß Anspruch 1, wobei die extrazellulären Proteine eine Komponente einer extrazellulären Matrix sind.


5. Zusammensetzung gemäß einem der vorstehenden Ansprüche, wobei die Teilchen gezüchtetes Gewebe umfassen.


7. Zusammensetzung gemäß einem der vorstehenden Ansprüche, wobei die Teilchen Gewebesknorpel umfassen.


12. Zusammensetzung gemäß einem der vorstehenden Ansprüche, die weiterhin Thrombin umfasst.


14. Zusammensetzung zur Verwendung gemäß Anspruch 13, wobei das geschädigte Gewebe aus der Gruppe ausgewählt ist, die aus Knorpel, Knochen, Bändern, Meniskus, Sehnen, Muskeln und Kombi-
nationen davon besteht.


**Revendications**

1. Composition comprenant une pluralité de particules isolées de tissu viables, les particules comprenant des cellules ayant une viabilité d’au moins 80 % et des protéines extracellulaires associées aux cellules, chaque particule étant calibrée pour avoir au moins une dimension inférieure à 60 μm.

2. Composition selon la revendication 1, dans laquelle les protéines extracellulaires sont un composant d’une matrice extracellulaire.

3. Composition selon la revendication 2, dans laquelle les protéines extracellulaires sont choisies dans le groupe constitué du collagène, de l’élastine, de la fibrilline, de la fibronectine, et de la laminine.


5. Composition selon l’une quelconque des revendications précédentes, dans laquelle les particules comprennent un tissu modifié.

6. Composition selon l’une quelconque des revendications précédentes, dans laquelle les particules comprennent un tissu juvénile.

7. Composition selon l’une quelconque des revendications précédentes, dans laquelle les particules comprennent du cartilage articulaire.

8. Composition selon l’une quelconque des revendications précédentes, dans laquelle le cartilage articulaire comprend au moins un parmi le cartilage hyalin ou le cartilage fibreux.

9. Composition selon l’une quelconque des revendications précédentes, comprenant en outre au moins un parmi un adhésif, une solution tampon, ou un agent bioactif.

10. Composition selon la revendication 9, dans laquelle l’adhésif est choisi dans le groupe constitué d’une fibrine, de l’acide hyaluronique, de la colle de fibrine, d’un caillot fibrineux, du gel de collagène, du gel d’alginate, d’un adhésif gélatine-résorcine-formaline, d’un adhésif à base de moule, d’un adhésif à base de dihydroxyphénylalanine (DOPA), de chitosane, de la transglutaminase, d’un adhésif à base de poly (acides aminés), d’un adhésif à base de cellulose, d’un adhésif à base de polysaccharides, des adhésifs à base d’acrylate de synthèse, du plasma riche en plaquettes (PRP), du plasma pauvre en plaquettes (PPP), d’un caillot de PRP, d’un caillot de PPP, d’un co-succinate de monostéaroyl glycérol (MGSA), des copolymères de co-succinate de monostéaroyl glycérol / polyéthylène glycol (MGSA/PEG), de la laminine, de l’élastine, les protéoglycanes, et des combinaisons de ceux-ci.

11. Composition selon la revendication 9, dans laquelle l’agent bioactif est choisi dans le groupe constitué des hormones facteurs de croissance, des nutriments, de BMP, IGF, TGF, PDGF, et d’un échantillon prélevé par aspiration de la moelle osseuse.

12. Composition selon l’une quelconque des revendications précédentes, comprenant en outre de la thrombine.

13. Composition selon l’une quelconque des revendications précédentes, destinée à améliorer un tissu endommagé chez un mammifère.

14. Composition pour utilisation selon la revendication 13, dans laquelle le tissu endommagé est choisi dans le groupe constitué d’un cartilage, un os, un ligament, un ménisque, un tendon, un muscle, et des combinaisons de ceux-ci.

15. Composition pour utilisation selon la revendication 14, dans laquelle le tissu endommagé est celui du cartilage articulaire.
REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader’s convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 20050196460 A, Malinin [0006]
- US 20040078090 A, Binette [0006]
- US 20050288796 A, Awad [0006]
- US 20040219182 A, Gomes [0006]
- US 20040175690 A, Mishra [0006]