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(54) **COMPOSITIONS AND METHODS FOR TISSUE REPAIR**

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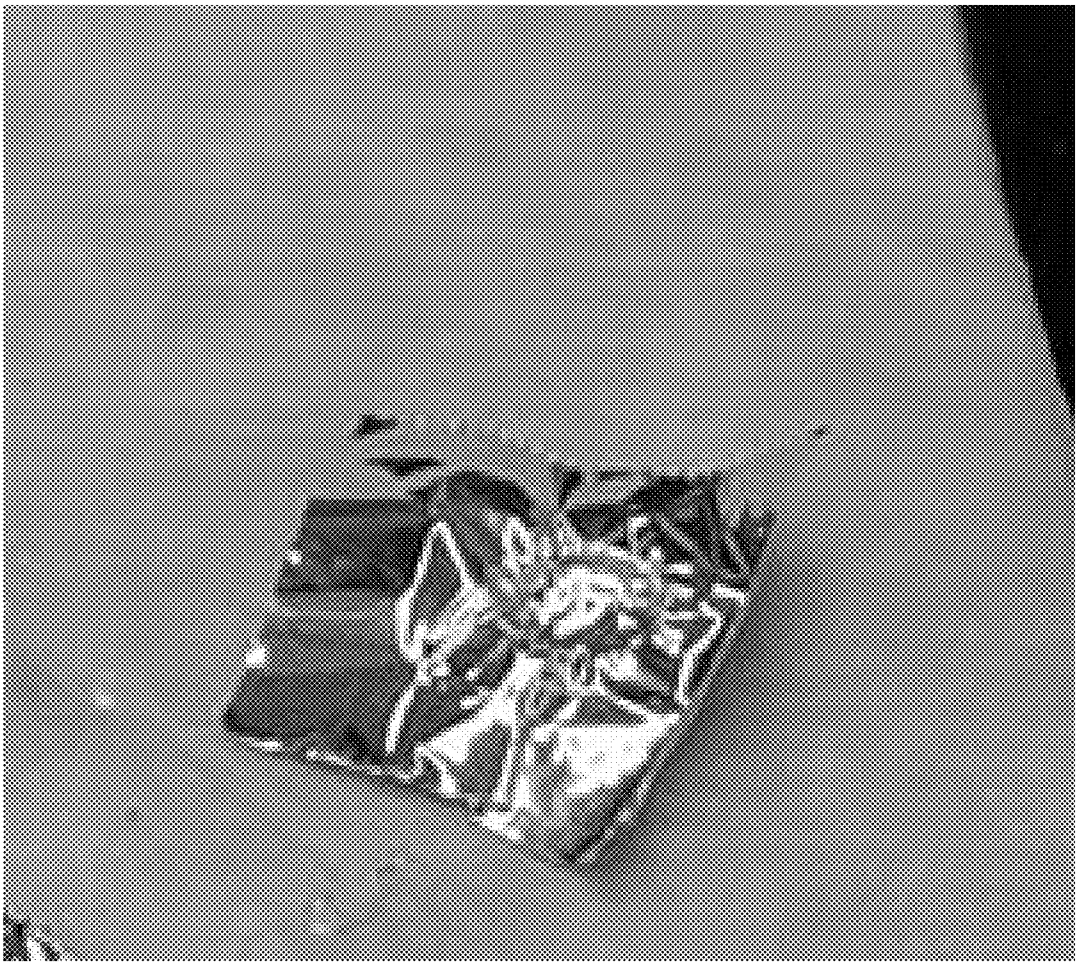
(57) **ABSTRACT**

Implants for repairing tissue defects, such as cartilage tissue defects, and methods of their preparation and use are disclosed. A mold of a tissue defect is prepared by pressing upon the defect a substrate having shape memory, such as aluminum foil. The mold, which has contours substantially conforming to those of the defect, is removed from the defect, and tissue particles are added to the mold ex vivo. A biological carrier such as biocompatible glue is also added to the mold. The combination of tissue particles and the biological carrier thereby form an implant, which retains its shape after separation from the mold. The implant can be transferred to the tissue defect, with contours of the mold matching corresponding contours of the defect.



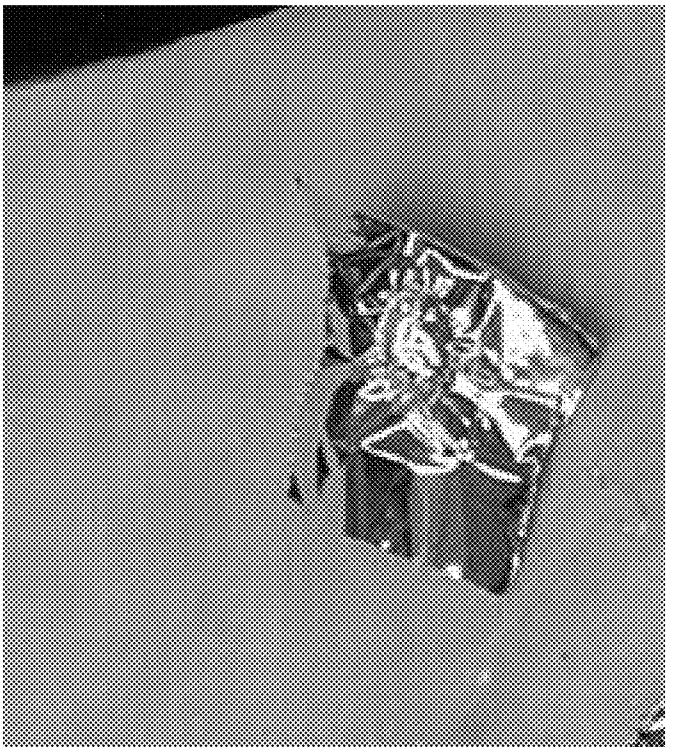


FIG. 1

FIG. 2

FIG. 3

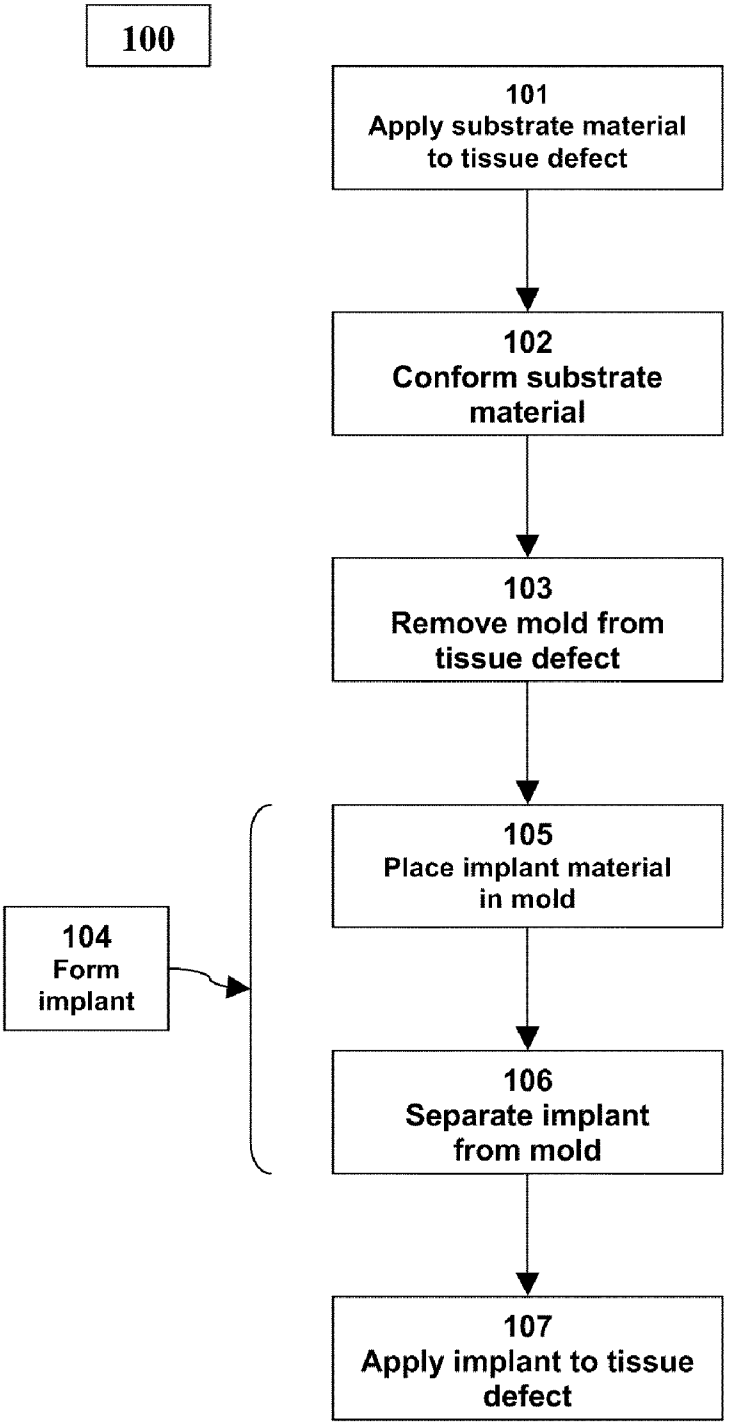

100
101
Apply substrate material to tissue defect
102
Conform substrate material
103
Remove mold from tissue defect
105
Place implant material in mold
104
Form implant
106
Separate implant from mold
107
Apply implant to tissue defect

FIG. 4

COMPOSITIONS AND METHODS FOR TISSUE REPAIR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Ser. No. 60/911,429 filed on Apr. 12, 2007, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A COMPACT DISC

[0003] Not Applicable.

INTRODUCTION

[0004] The need for implants for repairing tissue defects, such as cartilage tissue defects, is ongoing. Materials have been developed to fill spaces comprising tissue defects, but have typically been two-dimensional, comprising, for example, a flat sheet cut to approximate the dimensions of a tissue defect, or a layer of cells grown in vitro, from which a two dimensional sheet of cells is applied to a tissue defect.

[0005] U.S. Pat. No. 5,067,964 to Richmond et al. discloses an articular cartilage repair piece and methods of forming. This patent discloses a repair piece which includes a backing layer of non-woven, felted fibrous material which is conformable to flat and curved surfaces.

[0006] U.S. Pat. Nos. 5,655,546 and 6,179,871 to Halpern disclose methods for repairing a cartilage defect, comprising the provision of apertures in the cartilage by drilling holes at the base of the cartilage defect. The holes may enter the mesenchymal depot. In these methods, a porous scaffold material containing a plurality of magnetic particles is introduced into the apertures. Subsequently and sequentially, magnetically-tagged cartilage growth promoting materials such as chondrocytes or growth factors are injected into the area of the defect.

[0007] U.S. Pat. No. 6,443,988 to Felt et al. discloses methods and apparatuses for repairing a tissue site. The method involves use of a curable polyurethane adapted to be mixed at time of use in order to provide a flowable composition and initiate cure.

[0008] U.S. Pat. Nos. 6,511,958 and 6,514,514 to Atkinson et al. disclose devices and products for repair of cartilage lesions, comprising a cartilage repair matrix suitable for conforming to a cartilage defect and a cartilage-inducing composition on or within the matrix.

[0009] U.S. Pat. Nos. 6,626,945, 6,632,246 and 6,852,125 to Simon et al describe cartilage plugs made from a biocompatible artificial material. These plugs are made according to pre-determined shapes.

SUMMARY

[0010] The aforementioned patents do not describe a biological implant having surface contours substantially conforming to those of a tissue defect such as a cartilage defect. In view of these considerations, the present inventors have developed methods, compositions, apparatuses and kits for repairing a tissue defect such as a cartilage defect.

[0011] In some embodiments of the present teachings, these methods comprise forming, on a tissue defect of a subject, a mold conforming to the contours of at least a portion of the tissue defect, removing the mold from the defect, forming in the mold an implant comprising one or more tissue particles and a biocompatible carrier, whereby the implant has a shape substantially conforming to the contours of at least the portion of the tissue defect, separating the implant from the mold, and applying the implant to at least the portion of the tissue defect.

[0012] In various aspects of the method and apparatus, the mold comprises for example a pliable substrate having shape memory, which in one embodiment is a sterile substrate. The substrate having shape memory is for example a metal foil such as aluminum foil, a plastic, or a polymer such as a polyurethane or a curable plastic. The substrate may be a material permeable to liquid. The one or more tissue particles are in one embodiment initially suspended in a liquid medium which can be a cell, tissue or organ storage medium. In one embodiment, forming the implant includes removing an amount of the liquid medium from the mold. The liquid medium may be removed by introducing one or more perforations in the mold, thereby promoting draining of the liquid medium from the mold. Alternatively, the mold is made of a substrate material initially having perforations therethrough, or of a substrate material that is permeable to liquid. The liquid medium may be further removed by contacting the mold with an absorbent pad. In one embodiment the method further comprises distributing the tissue particles substantially uniformly in the mold. The biocompatible carrier can include at least one biocompatible polymer such as a fibrinogen, a fibrin, a thrombin, a type I collagen, a type II collagen, a type III collagen, a gelatin, a fibronectin, a laminin, a hyaluronic acid, a hydrogel, a pegylated hydrogel or a chitosan. The biocompatible carrier may include at least one biocompatible adhesive such as a fibrin adhesive. The implant may further comprise at least one bioactive agent such as a growth factor. The growth factor can be selected for example from among a TGF-β, a bone morphogenetic protein, a growth differentiation factor, ADMP-1, a fibroblast growth factor, a hedgehog protein, an insulin-like growth factor, a platelet-derived growth factor, an interleukin, a colony-stimulating factor, an EGF and an activin. The at least one bioactive agent may be a bioactive peptide.

[0013] In one aspect, forming in the mold an implant comprises forming an implant including a first layer including the one or more tissue particles and a second layer comprising the biocompatible carrier. The second layer may further include at least one bioactive agent, which can be a growth factor which can be selected from among a TGF-β, a bone morphogenetic protein, a growth differentiation factor, ADMP-1, a fibroblast growth factor, a hedgehog protein, an insulin-like growth factor, a platelet-derived growth factor, an interleukin, a colony-stimulating factor, an EGF and an activin. The at least one bioactive agent in a second layer of the implant can be a bioactive peptide. In another aspect of the method, applying the implant to the tissue defect includes inserting the implant at the tissue defect. Applying the implant to the tissue defect can further comprise affixing the implant to the tissue defect. Inserting the implant at the tissue defect can include inserting the implant using a minimally invasive surgical technique, such as arthroscopically. In one embodiment, the

biocompatible carrier can be a biocompatible gel, such as a starch gel, an agarose gel, a polyacrylamide gel or a combination thereof. The carrier that includes at least one biocompatible polymer may be autologous to the subject. In various aspects, the subject of treatment using the method is a non-human mammal, or is a human in need of treatment of the tissue defect. In one aspect, the contours of the tissue defect being treated are three-dimensional. In one aspect the contours of the tissue defect can be substantially cylindrical.

[0014] In another aspect, a method of repairing a tissue defect includes forming, on a tissue defect in a subject, a mold having a surface conforming substantially to contours of the tissue defect, wherein the mold comprises a bioabsorbable substrate having shape memory, removing the mold from the tissue defect, forming within the mold an implant comprising at least a first layer, wherein the first layer comprises one or more tissue particles and a biocompatible carrier, whereby the implant has a shape substantially conforming to the contours of the tissue defect; and applying the mold and implant to the tissue defect. The bioabsorbable substrate having shape memory is sterile. The bioabsorbable substrate having shape memory is for example a plastic, which can be a bioabsorbable polymer such as a bioabsorbable polyester. The bioabsorbable polymer can be a polyester selected from among a polylactic acid, a polyglycolic acid, and a co-polymer comprising a polylactic acid and a polyglycolic acid. In one aspect, the method may further include distributing the tissue particles substantially uniformly throughout the first layer. The second layer includes for example at least one biocompatible carrier. The second layer can include at least one bioactive agent such as a growth factor.

[0015] In another aspect a method is provided for repairing a tissue defect in a subject, which includes forming, on the tissue defect a mold having a surface substantially conforming to the contours of at least a portion of the tissue defect, removing the mold from the tissue defect, forming within the mold an implant comprising one or more tissue particles and a biocompatible carrier, whereby the implant has a shape substantially conforming to the contours of the portion of the tissue defect, applying the mold and implant together to the portion tissue defect, and separating the implant from the mold after applying to the tissue defect. Applying the implant to the tissue defect may include inserting the implant at the tissue defect and may further include affixing the implant to the tissue defect. Inserting the implant at the tissue defect may include inserting the implant using a minimally invasive surgical technique such as arthroscopically.

[0016] In another aspect, apparatus is provided for forming an implant for repairing a tissue defect, the apparatus including a mold having a shape substantially conforming to the contours of a tissue defect of a subject, and a composition including one or more tissue particles and a biocompatible carrier, the composition disposed in the mold wherein the shape of the composition substantially conforms to the contours of the tissue defect. The mold is formed from a substrate having shape memory such as for example a metal foil such as aluminum foil, a plastic, or a polymer such as a polyurethane or a curable plastic. The substrate may be for example a sterile substrate. The substrate may be a material permeable to liquid. The substrate may be a bioabsorbable substrate having shape memory such as a bioabsorbable polymer including a bioabsorbable polyester which may be selected from a polylactic acid, a polyglycolic acid, and a co-polymer comprising a polylactic acid and a polyglycolic acid. The mold may have one or more perforations therethrough for draining excess liquid medium. The tissue particles can be cartilage tissue particles such as neocartilage particles, juvenile cartilage particles, cadaver cartilage particles, or may be selected from among bone tissue particles, liver tissue particles, renal tissue particles, neuronal tissue particles, muscle tissue particles, adipose tissue particles, and a combination thereof. The apparatus includes in some aspects the tissue particles distributed substantially uniformly in the mold. The biocompatible carrier may include at least one biocompatible adhesive such as a fibrin adhesive. The biocompatible carrier can include at least one biocompatible polymer such as a fibrinogen, a fibrin, a thrombin, a type I collagen, a type II collagen, a type III collagen, a gelatin, a fibronectin, a laminin, a hyaluronic acid, a hydrogel, a pegylated hydrogel or a chitosan. A biocompatible polymer may be autologous to the subject. The biocompatible carrier may include at least one biocompatible adhesive such as a fibrin adhesive. The biocompatible carrier may include a biocompatible gel selected from a starch gel, an agarose gel, a polyacrylamide gel and a combination thereof. In one aspect, the apparatus includes an implant having a first and a second layer. The second layer can include at least one bioactive agent such as a growth factor as described elsewhere herein. The at least one bioactive agent may be a bioactive peptide.

[0017] In another aspect, a method of forming an implant for repairing a tissue defect includes providing a substrate having shape memory, forming the substrate into a mold of a tissue defect of a subject, wherein the mold substantially conforms to contours of at least a portion of the tissue defect, removing the mold from the tissue defect, and disposing in the mold a composition comprising at least one biological agent and a biocompatible carrier, thereby forming the implant having a shape substantially conforming to the contours of at least the portion of the tissue defect. The at least one biological agent can be a pharmaceutical compound. The biological agent is for example a plurality of tissue particles such as cartilage particles. The cartilage tissue particles may be neocartilage particles, juvenile cartilage particles, cadaver cartilage particles, or may be selected from among bone tissue particles, liver tissue particles, renal tissue particles, neuronal tissue particles, muscle tissue particles, adipose tissue particles, and a combination thereof. The method may further comprise mixing within the mold at least one bioactive agent. The bioactive agent can be a growth factor selected from among a TGF-β, a bone morphogenetic protein, a growth differentiation factor, ADMP-1, a fibroblast growth factor, a hedgehog protein, an insulin-like growth factor, a platelet-derived growth factor, an interleukin, a colony-stimulating factor, an EGF and an activin. The at least one bioactive agent may be a bioactive peptide. The at least one bioactive agent may be a plurality of cells which may be selected from chondrocytes, osteoblasts, mesenchymal stem cells, neuronal cells, T cells, B cells, neuronal cells, liver cells, mesenchymal stem cells, adipocytes, renal cells, lung cells or a combination thereof. The method may include growing the plurality of cells ex vivo.

[0018] In another aspect, an implant for repairing a tissue defect of a subject includes a biological agent and a biocompatible carrier, wherein the shape of the implant substantially conforms to the contours of at least a portion of a tissue defect of a subject. The biological agent is for example a plurality of tissue particles such as cartilage particles. The cartilage tissue particles may be neocartilage particles, juvenile cartilage par-

ticles, cadaver cartilage particles, or may be selected from among bone tissue particles, liver tissue particles, renal tissue particles, neuronal tissue particles, muscle tissue particles, adipose tissue particles, and a combination thereof. The at least one biological agent may be a population of cells such including for example a plurality of chondrocytes, fibroblasts or tendoncytes, or a combination thereof. The population of cells may include a plurality of cells selected from among bone cells, liver cells, and kidney cells. The at least one biological agent may include a pharmaceutical compound. In one aspect the implant includes a first layer comprising the tissue particles. The implant may further include at least one bioactive agent such as a growth factor or a bioactive peptide as described elsewhere herein. The implant may further include a second layer. The second layer includes for example at least one biocompatible carrier such as a biocompatible polymer. The second layer may further include at least one bioactive agent such as a growth factor or a bioactive peptide. The biocompatible carrier of the second layer may include a biocompatible polymer such as a biocompatible gel selected from among a starch gel, an agarose gel, a polyacrylamide gel and a combination thereof.

[0019] In another aspect, a kit for forming an implant for repairing a tissue defect includes a pliable substrate having shape memory and which can be shaped to substantially conform to the contours of at least a portion of a tissue defect thereby serving as a mold of at least the portion of the tissue defect in a subject, a biological agent and a biocompatible carrier. The biological agent is for example a plurality of tissue particles such as cartilage particles or other tissue particles as described elsewhere herein. The tissue particles may be provided in a liquid medium and the pliable substrate material having shape memory and the biocompatible carrier are all as described elsewhere herein. The kit may further include at least one perforation tool such as a scalpel, an awl, a pin, a needle or a forceps for perforating the mold to remove an amount of liquid medium after introducing liquid medium to the mold. The kit may further include at least one absorbent pad also for removing liquid medium. The kit may also further include at least one biocompatible adhesive such as a fibrin adhesive and may include at least one bioactive agent as described elsewhere herein. The kit may further an amount of calcium chloride.

DRAWINGS

[0020] FIG. **1** is a flow diagram illustrating a method for repairing tissue defects according to the present teachings;

[0021] FIG. **2** illustrates an aluminum foil mold of the present teachings;

[0022] FIG. **3** illustrates formation of an implant in a mold of the present teachings; and

[0023] FIG. **4** illustrates application of an implant of the present teachings to a cartilage defect.

DETAILED DESCRIPTION

[0024] The following examples are provided for illustrative purposes only and are not to be construed as limiting the invention's scope in any manner. The description of an article, a composition, or a method in an example does not imply that the described article or composition has, or has not, been produced, or that that the described method has, or has not, been performed, regardless of verb tense. The methods and compositions described throughout this specification utilize laboratory techniques well known to skilled artisans and can be found in commonly available laboratory manuals describing for example use of biomolecules, cell culture and handling techniques, and use of antibodies.

[0025] The present inventors disclose herein molds and implants for repairing tissue defects, as well as methods of forming such molds and implants, and kits for forming and using the molds and implants.

[0026] In various embodiments of the present teachings, these methods comprise forming, on a tissue defect of a subject, a mold having a surface substantially conforming to contours of a tissue defect. In one embodiment, a tissue defect is, for example, a cartilage tissue defect such as an injury to a hyaline cartilage comprised by a subject, for example a torn knee cartilage. The tissue defect may alternatively be in any bodily tissue susceptible to repair or treatment of the defect using an implant, including musculoskeletal hard and soft tissues, oral and maxillofacial tissues, dermal tissues, and solid organ tissues. In the following detailed description, it should be understood that reference is made to cartilage tissue for illustrative purposes only and should not be taken as limiting the present mold, implant and related methods of making and using to cartilage tissue alone. It is contemplated that the present mold, implant and related methods of making and using will be comparably useful in repairing or treating other hard and soft bodily tissues.

[0027] In various configurations, a mold can be made of a pliable substrate material having shape memory, such as, without limitation, a polymer or a metal foil such as aluminum foil, a wax or other malleable material capable of retaining a particular shape The substrate material may alternatively be a liquid or fluid material such as a hydrogel or a wax in liquid phase capable of transitioning to a solid phase upon a triggering event such as exposure to a chemical agent or exposure to a change in temperature. For example, certain hydrogels are known which have a fluid phase that transitions to a solid or semi-solid phase upon exposure to a cross-linking agent. Wax in liquid phase can be disposed in the mold and then the combination cooled so that the wax resolidifies. It should be understood that in describing suitable pliable substrate materials as having shape memory, suitable substrate materials include those having the ability to adapt or conform to at the three-dimensional configuration of at least a portion of a tissue defect, and then to return to the shape conformation if briefly deformed upon removal from the defect after conforming thereto. The substrate may be a material permeable to liquid, such as liquid that may be part of a liquid storage medium for cells or tissue.

[0028] In the case of a metal foil, a medical caregiver such as a physician or surgeon can manually, with or without a surgical instrument, press the foil on to a tissue defect, such that the substrate material is substantially juxtaposed to contours of the defect (see examples below). In various configurations, a substrate material such as a foil can initially be sterile (e.g., as a result of autoclaving), and initially can be substantially flat. A foil can be of any available thickness and any convenient initial size and shape, such as, in non-limiting example, a square or rectangle of from about 5 cm to about 10 cm a side, or a circle of about 5 cm to about 10 cm diameter. In various configurations, a mold substrate such as a metal foil can have a thickness of from about 5 microns to about 200 microns; from about 10 microns to about 100 microns, from about 12 microns to about 30 microns, or from about 13 microns to about 25 microns. In some configurations, after a

foil is molded to a tissue defect, the mold can have contours corresponding to those of the defect; it can further comprise walls that can aid in keeping added materials within the mold during formation of an implant.

[0029] In some aspects, a polymer such as a pliable plastic (such as a putty) can provide the substrate material for forming a mold. In these cases a medical caregiver such as a physician or surgeon can manually or otherwise mechanically using surgical instruments press the plastic on to the defect, such that the plastic is substantially juxtaposed to contours of the defect. If necessary, the material can be flattened by methods known to persons of skill in the art prior to application of the material to a tissue defect. Alternatively, an amount of a pliable solid substrate material such as a metal foil, meshed metal or plastic can initially be configured in a compact folded or pleated configuration for delivery to the site of the defect in vivo, and then mechanically expanded and applied to the defect site in order to conform thereto. For example, an amount of a pliable solid substrate material such as a metal foil may be configured in a "pleated skirt" configuration or any other folded or pleated configuration that achieves sufficient compaction of the material for it to be delivered to the defect site in vivo through limited access routes, for example using laparoscopic surgical catheters, and then expanded at the defect site so that it may be applied and conform to the defect site.

[0030] In other aspects, a curable plastic or other suitable liquid material such as a wax or a hydrogel can be applied to a tissue defect as a liquid, e.g. by flowing through a syringe. A curable plastic can be, for example, a polyurethane described in U.S. Pat. No. 6,443,988 to Felt et al., or an epoxide plastic in which a monomer is mixed with a catalyst prior to applying to applying to a tissue defect, and which polymerizes while in contact with the defect. In yet other examples, a plastic can be a bioabsorbable polymer, such as a polyester. In various configurations, a polyester can comprise a polylactic acid, a polyglycolic acid, and/or a co-polymer comprising a polylactic acid and a polyglycolic acid.

[0031] In an illustrative embodiment, following formation of the mold which has contours substantially conforming to the contours of a tissue defect, the mold is removed from the defect. Removal is accomplished manually, or otherwise mechanically with the aid of tools or surgical instruments such as forceps, or may be accomplished or assisted by exposure to a chemical or physical stimulus. For example, exposure to an increase in temperature can be used to accomplish or assist removal of a wax mold by partially melting the mold to the point that the mold is released from the implant. Alternatively, in the case of certain hydrogels, exposure to a chemical agent such as a cationic composition can break bonds between the mold and the implant. Because the mold comprises a substrate material having shape memory, if the mold is deformed during removal from the tissue defect, the mold regains the contours of the defect upon which it was formed. An implant can then be formed within the mold ex vivo.

[0032] In one embodiment, formation of the implant is accomplished by introducing into the mold a suitable biological agent such as tissue particles, cells, collagen, extracellular matrix (ECM) or tissue-engineered scaffold material. For example, the biological agent can be cartilage particles such as those described in U.S. Patent application publication 2005152882 (application Ser. No. 11/010,779 of Kizer et al., filed Dec. 13, 2004). The choice of what type of biological agent to use for forming the implant for a particular application may take into account, for example, whether a need exists for a longer shelf-life for the implant once formed. Use of tissue-engineered scaffold for forming the implant may be especially well-suited for applications where it is anticipated that the implant will remain in storage for a period of time. The scaffolding may later be populated with cells just before or at about the time the implant is being prepared for actual implantation.

[0033] In various embodiments, tissue particles or other biological agents or both are added to the mold along with liquid medium in which the particles or other agents are carried or suspended and maintained. Excess liquid medium can then be removed from the mold by aspiration, or, in some configurations, by piercing the mold to introduce one or more apertures so that the liquid can drain, or by a combination of aspiration and piercing. In various configurations, perforations can be introduced to the mold using any sharp tool or implement, such as, without limitation, a scalpel, a forceps, a needle, a pin or an awl. In other embodiments, the mold is formed of a substrate material that is perforated or is a mesh material that does not require additional piercing or perforation for draining excess liquid medium. Alternatively, the mold is formed of a substrate material that is otherwise permeable to liquid, such as a porous or semi-porous membrane, so that when tissue particles or cells in a liquid storage medium are introduced to the mold, excess liquid medium drains through the material without the need for perforations.

[0034] In some aspects, draining of liquid through perforations or mesh material can be promoted by contacting the mold with an absorbent body, such as, without limitation, a surgical sponge, paper towel, gauze or pad.

[0035] In other aspects, the tissue particles or other biological agent for forming the implant may be disposed in the mold without excess liquid medium. For example, excess liquid medium may be removed from tissue particles or other biological agent prior to disposing in the mold. Alternatively, the tissue particles or other biological agent may not have required combination with excess liquid medium in the first instance in order to be suitable for disposition in the mold. In either case, it will be appreciated that while excess liquid medium may assist in achieving a more uniform distribution of the tissue particles or other biological agent in the mold, excess liquid is not necessarily required for the distribution to be sufficient for formation of the implant. In particular, in certain cases autologous tissue that has been removed from the subject may be especially suitable for forming the implant, and such tissue may exist in a form such as a tissue core or plug reasonably well-suited to being disposed in the mold without the need to add liquid medium. In various embodiments, a biocompatible carrier is added to the mold. The carrier is added to the mold before, during or after adding the biological agent such as tissue particles, cells, collagen, ECM or a tissue-engineered scaffold. In various configurations, a carrier can be added to a mold as a liquid. Such a carrier can embed the one or more tissue particles, and can also form a solid. In various aspects, a liquid carrier can fill space within the mold and therefore can have a shape substantially conforming to the contours of the mold. Because the mold has a shape substantially conforming to the contours of the defect, the carrier can have a shape substantially conforming to contours of the tissue defect. In various aspects, a carrier can be a biological adhesive such as a fibrin glue. As used herein, "fibrin" and "fibrin glue" include fibrin generators such as mixtures of fibrinogen and thrombin (Gibble, J W

and Ness, P M, Transfusion 30: 741-747,1990; Alston, S et al., Translational Research 149: 187-195, 2007). In non-limiting example, a fibrin glue can be a commercially available fibrin glue such as Tisseel® VH fibrin sealant (Baxter Healthcare Corporation, Westlake Village, Calif.), which can be prepared for use according to manufacturer's instructions. In other configurations, a biocompatible carrier can comprise at least one biocompatible polymer, such as, without limitation, a fibrinogen, a thrombin, a fibrin, a type I collagen, a type II collagen, a type III collagen, a gelatin, a fibronectin, a laminin, a hyaluronic acid, a hydrogel, a pegylated hydrogel, a chitosan or a combination thereof. In addition, in some aspects, a biocompatible polymer can be autologous to the intended recipient of an implant, and can be, without limitation, an autologous plasma protein such as autologous thrombin, autologous fibrinogen, autologous fibrin and/or autologous fibronectin. In addition or alternatively, in various configurations, a biocompatible carrier can comprise a biocompatible gel, which can be, without limitation, a starch gel, an agarose gel, a polyacrylamide gel or a combination thereof. In various configurations, a biological agent such as tissue particles within a mold can be arranged such that the particles are distributed substantially uniformly within the mold. In various configurations, instruments and tools such as forceps and needles can be used to arrange the particles. In some configurations, the particles and the carrier can together comprise a first layer, within which the particles can be distributed substantially uniformly. In various aspects, the carrier can congeal, thereby forming an implant having contours substantially corresponding to those of the mold and hence to a tissue defect. In various aspects, the distributing can be effected before or after removal of the liquid, or after addition of a biocompatible carrier (see below). In some aspects, combining a biological agent such as tissue particle and a biocompatible carrier such as a fibrin glue can form a composite. Thus, an implant or a first layer thereof can comprise particles which are immobilized and distributed substantially uniformly throughout a biocompatible carrier.

[0036] In various configurations, a second or additional layer can be formed in a mold. Such a layer can be added adjacent to a first layer, for example, by adding a layer of fibrin glue over a previously formed layer comprising fibrin glue and tissue particles. A second or additional layer can comprise components which are the same or different from those of the first layer. In various aspects, a second or additional layer can comprise at least one biocompatible carrier. In various aspects, a second layer can also comprise one or more bioactive agents such as one or more growth factors, one or more bioactive peptides, and/or cell populations. In various aspects, a second layer can comprise a biocompatible gel, and/or tissue particles such as cartilage tissue particles.

[0037] Because an implant will also retain its shape after it is formed, the implant and the mold can be separated in various aspects. Separation can be effected using methods well known to skilled artisans and will depend in part on the material used for the mold. For example, one or more slits can be made in a mold with a scalpel, and pieces of the mold can be pulled away from the implant with the aid of forceps. In some aspects, such as with a mold made of pre-perforated material, the mold may be torn or peeled away from the implant with or without the aid of a surgical instrument. In the case of a mold made of a wax for example, the mold may be melted for removal. In other aspects, certain mold materials such as for example hydrogels may be readily susceptible to being dissolved away using a chemical agent. It is further contemplated that other physical methods and tools such as those involving laser or electrocautery may be sued to remove the mold from the implant.

[0038] In some aspects, an implant can be flexible yet resilient to deformation, so that it can return substantially to its original shape following a deformation, for example after passaging through a hollow needle. Following separation of an implant from a mold, the implant can be applied to the tissue defect. In various aspects of the methods described herein, applying an implant to a tissue defect such as a cartilage defect can comprise inserting the implant at the tissue defect. In some aspects, a method can further include affixing the implant to the tissue defect. In some aspects, affixing an implant can include securing the implant to the tissue defect using methods and materials well known to skilled artisans such as, for example, biocompatible glues, sutures, staples, or pins. In non-limiting example, a biological glue can be used to affix an implant to a tissue defect after the contours of the implant are apposed to the corresponding contours of the defect.

[0039] In some alterative aspects, a mold can comprise a bioabsorbable substrate material such as a polyester. Moreover, it is envisioned that a mold formed at the site of a defect in vivo may also be withdrawn from the defect site, for example through a hollow needle, and upon returning substantially to its original shape following deformation, is then used ex vivo for formation of the implant. The mold and implant are then together introduced to the defect site through the hollow needle and together applied to the defect site. In such cases, a combination of mold and implant can be applied to a tissue defect without separating the mold from the implant. In aspects in which an implant is deformable, an implant can be administered to a subject by injecting the implant into the subject at the site of the tissue defect using a syringe and a hypodermic needle. In aspects in which a combination of a mold and implant is inserted at a tissue defect, the mold can be separated from the implant by standard surgical procedures known to skilled artisans, such as, without limitation, slicing of the mold followed by removal of the mold from the defect.

[0040] In various methods of the present teachings, tissue particles can be initially carried or suspended in a liquid medium. Such a liquid medium is, in various aspects, a cell, tissue or organ storage medium, such as, without limitation, a medium disclosed in U.S. Patent application publication 2005152882 (the disclosure of which is herein incorporated by reference in its entirety), for example Dulbecco's Modified Eagle's Medium (DMEM) or Roswell Park Memorial Institute Medium (RPMI).

[0041] In some configurations, an implant and/or a mold of the present teachings can further comprise at least one bioactive agent, such as, without limitation, a hormone, a growth factor, a pharmaceutical compound, a bioactive peptide, a nucleotide such as an RNAi molecule, a vector, a plurality of cells, and the like. The bioactive agent can be a constituent of any layer of an implant. A growth factor can be, without limitation, a TGF-β, a bone morphogenetic protein, a growth differentiation factor, ADMP-1, a fibroblast growth factor, a hedgehog protein, an insulin-like growth factor, a platelet-derived growth factor, an interleukin, a colony-stimulating factor, an EGF or an activin. A pharmaceutical compound can be, without limitation, an analgesic, an anesthetic such as a local anesthetic, or a cyclooxygenase inhibitor. A bioactive

peptide can be, without limitation, neuropeptide Y, secretin, cholecystokinin, or a cell-penetrating peptide such as penetratin, substance P or R9 or for example any bioactive peptide with antimicrobial activity. In some aspects, a bioactive agent can comprise a plurality of cells. Such cells can be, without limitation, chondrocytes, osteoblasts, mesenchymal stem cells, neuronal cells, T cells, B cells, neuronal cells, liver cells, mesenchymal stem cells, adipocytes, renal cells, lung cells and combinations thereof. In some aspects, the cells can be autologous to an intended recipient of an implant, and can be obtained directly from the intended recipient or grown in vitro prior to adding to an implant. In some aspects, the bioactive agent may comprise a nucleotide. A nucleotide can be a naturally occurring sequence of DNA or RNA, synthetic DNA or RNA, or chemically modified DNA or RNA, such as chemically modified otherwise naturally occurring RNAi molecules. In some aspects, the bioactive agent may comprise a vector, for example for introducing a genetic sequence to the implant. A vector can be for example a viral vector.

[0042] In various embodiments of the present teachings, a subject can be a mammal, which can be a human or a non-human mammal. In some configurations, a human subject can be a human in need of treatment of a tissue defect, such as a cartilage defect. In some configurations, a human subject can have a degenerate or damaged cartilage such as a hyaline cartilage comprised by a joint.

[0043] In various aspects of the present teachings, a mold and an implant formed therein can be three dimensional, such as when its shape corresponds to the contours of a tissue defect. Thus, the present teachings provide implants which are not limited to two-dimensional structures, such as films or laminates. In other aspects, a mold and implant formed therein can be substantially cylindrical in shape, such as, without limitation, when a tissue defect such as a cartilage defect is prepared by a surgeon to include a substantially cylindrical aperture. In these configurations, a caregiver such as a surgeon can, prior to forming a mold, prepare a tissue defect such as a cartilage defect for receiving an implant by removing tissue from the defect, such that a circular or cylindrical defect remains. Hence, in some configurations, the methods can include preparing a tissue defect for receiving an implant. The preparation can comprise modifying the defect to comprise a substantially cylindrical aperture. However, it will be noted that an advantage of the present mold, implant and related methods and kits is the ability to adapt the shape of the mold and ultimately the shape of the implant to any shape that helps repair the defect while also preserving the greatest amount of healthy tissue. The site-specific mold and implant can avoid the need to remove healthy tissue to adapt the shape of the defect to a particular predetermined shape of the implant. In addition, the present mold, implant, related methods and kits are well-suited for use in a "tiling" approach using multiple implants at a defect site.

[0044] In some embodiments of the present teachings disclosing implants comprising a mixture of at least one biological agent and a biocompatible carrier, as well as methods for forming such implants, a biological agent can comprise a plurality of tissue particles, such as cartilage particles. In other aspects, a biological agent can comprise a population of cells. A population of cells of these embodiments can comprise, without limitation, a population of chondrocytes, a population of fibroblasts, a population of tendoncytes, a population of bone cells such as osteoblasts, a population of kidney cells, a population of lymphocytes such as T lymphocytes or B lymphocytes, a population of hepatocytes, and/or a population of stem cells such as mesenchymal stem cells or embryonic stem cells, or a combination of any such cell populations. In various configurations, the inserting and the affixing of an implant to a tissue defect can be accomplished using methods well known to skilled artisans, such as inserting the implant using a minimally invasive surgical technique (MIS), such as arthroscopically. An MIS, for example, includes a mini-open arthrotomy with minimal disruption of the surrounding joint structure, typically involving a smaller incision than incisions required in regular open cartilage repair surgeries. In other aspects, the present mold, implant and related methods of forming and using same contemplate their application in situations involving defects to bone tissue underlying all or a portion of the tissue defect, for example the cartilage tissue defect. For example, alternative materials such as engineered bone tissue and/or trabecular metal may be used to reconstruct a bone tissue defect underlying a cartilage tissue defect.

[0045] FIG. **1** is a flow diagram illustrating the steps in a method **100** for repairing tissue defects according to the present teachings. By using an in vivo molding approach to determine the shape and dimensions of the implant, the method provides implants that are very well fit to the specific tissue defect. In a first step **101**, a surgeon, technician or other individual trained in orthopedic surgical techniques, applies the substrate material having shape memory, to all or a portion of the tissue defect to be repaired. The tissue defect is, for example, a cartilage tear in a major joint such as the knee. Thus the mold is prepared in vivo in the subject having the tissue defect. The process of applying the substrate material to all or a portion of the tissue defect, for example by manually pressing with or without aid of a surgical instrument, conforms **102** the substrate material to the contours of the tissue defect or portion thereof.

[0046] Depending on the substrate material being used, a wait period (not itemized in FIG. **1**) may be required in order to allow the substrate material to undergo processes necessary for the material to retain the contours of the tissue defect or portion thereof to which the substrate material has been applied. For example, if the substrate material is a curable plastic or polymerizing plastic, typically a period of time that varies with the type of plastic used should elapse before the material attains properties sufficient to retain the geometry of the tissue defect. Commercially available epoxies and other curable or polymerizing plastics for such purposes are well known and should be used according to manufacturer's instructions for the process of curing or polymerizing. If the substrate material being used is a metal foil, the foil immediately retains the geometry of the tissue defect to which it has been firmly applied. In any case, once the individual preparing the mold ascertains that the substrate material has attained sufficient shape memory of the tissue defect or portion thereof, the individual removes **103** the mold from the tissue defect, typically by simply using gloved fingers or with a sterile surgical instrument. The implant will be formed ex vivo using the mold formed in vivo.

[0047] To form the implant **104**, at least a first layer of implant material is placed **105** into the mold. The implant material includes at least a biological agent such as tissue particles. The tissue particles, e.g. cartilage particles, may be further maintained in a liquid medium as described elsewhere herein. The liquid medium can facilitate delivery of the tissue particles into the mold. If a liquid medium is used, it is

removed from the mold by aspiration, or by piercing or perforating the mold to drain the liquid after the tissue particles are placed in the mold. In one embodiment, the substrate material is perforated to start with, or is a mesh type material sufficient to initially receive an implant material including a liquid constituent, and then to allow the liquid constituent to drain from the mold after a short period of time during which the implant material is distributed within the mold. In another illustrative embodiment, a biocompatible carrier is also added to the mold to form a first layer of the implant. It should be noted however that the biocompatible carrier may be added to the mold before, during or after placing the tissue particles in the mold. For example, in one aspect fibrin can be combined directly with cartilage tissue particles with or without medium. In alternative embodiments, forming the mold may also include adding a bioactive agent or agents to the mold, thereby forming another layer of the implant, or as part of the first layer. As described elsewhere herein, adding a bioactive agent or agents may include adding growth factors or hormones, pharmaceutical compounds, bioactive peptides, nucleotides, vectors, or other cells.

[0048] Referring again to FIG. **1**, ultimately the implant is separated from the mold **106**, and the implant is applied **107** to the tissue defect or portion thereof to which the implant now conforms. It should be noted that the implant may be applied to the tissue defect together with the mold first, and the mold then separated from the implant after implantation, or the mold may be removed from the implant before the implant is applied to the tissue defect. In either case, the implant comprising at least the tissue particles and a biocompatible carrier, is made contour-specific for the particular tissue defect and therefore very well fit to the tissue defect. It is believed that the high fit specificity of the implant improves recovery speed and outcomes.

[0049] In another aspect, the present teachings encompass a kit for forming the aforementioned mold and implant for repairing a tissue defect. The mold materials and implant materials may be advantageously provided in kit form including separately packaged amounts of each type of material. In a kit the amounts of each material can be for example amounts sufficient for the treatment or repair of a defect of a predetermined size. In one aspect, a kit includes an amount of the pliable substrate for forming the mold as described herein, and the biological agent such as a plurality of cells or tissue particles. In another aspect, the kit may further comprise an amount of the biocompatible carrier. Alternatively, a kit can include an amount of the pliable substrate and an amount of the carrier, with the biological agent such as tissue particles or cells to be provided from another source. In an exemplary embodiment of a kit, each component of the kit is packaged separately in sterile packaging or in packaging susceptible to sterilization. The biological agent such as cells or tissue particles may be in a container such as a glass or plastic vial and may further be carried or suspended in a liquid storage medium suitable for maintaining cells, tissues or organs. In any kits containing cells or tissue particles in liquid storage medium, the kits may further include an absorbent material in the form for example of a pad or wipe for absorbing excess liquid medium from the mold after the biological agent has been introduced to the mold. The pliable substrate can be wrapped or sealed separately in a paper or plastic wrapper, sterilized blister pack or the like. The biocompatible carrier, for example fibrin, can also be contained in a separate glass or plastic vial or other compact container. The kit may optionally further include one or more syringes or other delivery device(s) for introducing the implant material and carrier to the mold. Kits may optionally further include one or more additional containers each storing a bioactive agent such as growth factor or a pharmaceutical agent that may be added to the implant. The kit further includes, for example, printed instructions for forming the mold and the implant and for using the implant to repair a tissue defect. All elements of the kit are provided together in suitable amounts in a box or other suitable packaging.

EXAMPLES

[0050] The following examples are provided for illustrative purposes only and are not to be construed as limiting of claim scope. The description of an article, a composition, or a method in an example does not imply that the described article or composition has, or has not, been produced, or that that the described method has, or has not, been performed, regardless of verb tense. The methods and compositions described throughout this specification utilize laboratory techniques well known to skilled artisans such as can be found in routinely available laboratory manuals.

Example 1

[0051] This example illustrates formation of a mold.

[0052] In this example, a sterile piece of aluminum foil having sufficient moldability and rigidity (shape memory) is applied by physician to a cartilage injury. In this case, the physician uses her fingers or a surgical instrument such as a spatula to press the aluminum foil into the cartilage defect so that the foil is in extensive contact with the base and side walls of the defect. This shaping results in a mold which can be used to form an implant which matches the size and shape of the defect (FIG. **1**).

Example 2

[0053] This example illustrates formation of an implant of the present teachings using a mold.

[0054] In this example, a syringe is used to transfer cartilage tissue fragments and storage medium to the base of an aluminum foil mold of a cartilage defect, such as the mold illustrated in Example 1. Excess fluid is removed by aspiration, and also by introducing holes in the base of the mold using a scalpel. The mold is contacted with an absorbent sterile pad to absorb liquid through the holes. However, enough fluid remains so that the particulate tissue pieces are not clumped together (FIG. **2**). The pieces distribute evenly across the surface of the base of the foil mold. A layer of fibrin is then gently added to embed the cartilage particles within the mold. More fibrin is then overlaid on the first layer to increase the thickness of the implant. The skirt of the foil mold is then gently pulled to straighten the foil, or pulled or peeled away using a surgical instrument such as a forceps This action releases the implant from the foil mold, which is now available for implantation at a cartilage defect.

Example 3

[0055] This example illustrates formation of an implant in an alternative aspect of the present teachings.

[0056] Example 2 above describes formation of a implant using a multiple step process including first the introduction of cartilage tissue fragments to the mold followed by introduction of fibrin to the mold. In this example, an implant is

formed in a "single-step" process. Cartilage tissue fragments are combined with fibrin and the combination is introduced to the mold, also using a syringe. A pharmaceutical agent such as a growth factor is optionally added to the combination before introducing the combination the mold. This approach is well-suited for carrier materials that have a well-defined and predictable setting time.

Example 4

[0057] This example illustrates transfer of an implant to a tissue defect.

[0058] In this example, a cartilage defect in a cadaver is exposed. A fibrin adhesive is applied to the base of the cartilage defect, and the implant of Example 2 is gently lifted with a spatula and placed into the defect such that contours of the defect and the implant correspond (FIG. **3**). The implant is then gently pressed against the defect and pulled with forceps so that the implant fits snugly in the defect. Optionally, more fibrin is then added around and over the implant to fill the defect to the extent needed.

Example 5

[0059] This example illustrates transfer of an implant to a tissue defect.

[0060] In this example, a cartilage defect in a cadaver is exposed. A fibrin adhesive is applied directly to the intended contact surface of the implant of Example 2, and the implant of is gently lifted with a spatula and placed into the defect such that contours of the defect and the implant bearing the previously applied layer of fibrin adhesive correspond. The implant is then gently pressed against the defect and pulled with forceps so that the implant fits in the defect. More fibrin is optionally then added around and over the implant to fill the defect as may be needed.

Example 6

[0061] This example illustrates a protocol for treating a cartilage defect.

[0062] In this example, an autologous fibrin adhesive is prepared at least one week in advance of surgery. Alternatively, a commercially prepared fibrin as obtained off the shelf. A medial or lateral parapatellar mini-arthrotomy is performed using a tourniquet (which need not be inflated). The defect area is marked with a sterile surgical marker. The cartilage tissue is removed within the defect area with a curette, thereby creating a well-defined vertical defect perimeter. The defect base is then cleared to remove the calcified cartilage layer, taking care to avoid violating the subchondral cortical bone plate. If subchondral bone bleeding occurs, it must be stopped before implantation of an implant of the present teachings. The defect and surrounding tissue are irrigated frequently with normal saline during the surgery.

[0063] Sub-chondral bleeding: methods for controlling and stopping subchondral bleeding include: a) use of neuro-patties soaked with a dilute 1:1000 epinephrine and sterile saline solution; b) direct application of thrombin to the site of bleeding; c) electrocautery using a needle-tipped electrocautery device to cauterize only the bleeding points, not the entire base of the defect. Electrocautery can be used, particularly if bleeding is especially difficult to control, for example in a patient who had previous marrow stimulation.

[0064] Defect sizing: with the aid of a sterile flat-ended rod, a sterile thin foil is pressed into the defect so that the outer shape of the foil fits snugly into the defect base and vertical wall. The approximate surface area of the defect is measured to determine the approximate amount of cartilage particles needed. If DeNovo® NT particles (Isto Technologies, Inc., St. Louis, Mo. and Zimmer, Inc., Warsaw, Ind.) are used, one pack of particles is needed for each about 0.5 to about 3.0 cm^2 defect or for an average of about 2.5 cm^2 defect. The foil mold is removed from the defect and placed on sterile gauze or a sterile absorbent plant. The defect may be documented photographically, including a ruler showing two dimensions at 90 degrees to each other.

[0065] Implant Preparation: A clear sterile piece of plastic tubing, at least 30 mm in length, is attached to a sterile syringe with a luer tip orifice of <1 mm. The lid of a package of DeNovo® NT particles is opened, and the cartilage tissue particles and medium are aspirated using the syringe. The cartilage particles will be contained within the tubing, while the storage medium will be aspirated into the syringe barrel. The cartilage and the storage medium are transferred to the foil old. Excess storage medium is aspirated by aspiration until only a shallow pool of the medium remains. This is done so that the cartilage pieces are not clumped together. The tissue pieces are then distributed evenly across the surface base of the foil mold with the tip of the syringe. Remaining liquid is removed by aspiration while avoiding further movement of the cartilage pieces. A sharp tip tool such as a scalpel is also used to make small perforations in the foil at various locations to allow excess liquid to be absorbed by an absorbent sterile gauze or pad underlying the foil mold. A layer of fibrin glue is then gently applied to embed the particulate tissue pieces. More fibrin is then applied, such that the tissue/fibrin composite fills to about ¾ the depth of the mold. The fibrin is then set for 5-10 minutes in accordance with fibrin preparation instructions. The edges of the foil mold are then gently pulled to straighten the foil so that the tissue/fibrin implant separates from the vertical walls of the foil mold. The implant can then be lifted from the mold base using a sterile flat instrument such as a spatula. The implant is now ready for implantation.

[0066] Alternatively, the implant is prepared using the single-step process in which the fibrin is added directly to the cartilage particles in the syringe tubing by aspirating the fibrin after any excess storage medium is aspirated. The fibrin may otherwise be added directly to the cartilage particles and medium in the tubing and syringe in the case where no excess storage medium is aspirated. In either case, the cartilage tissue particles together with the fibrin may be gently agitated using the action of the syringe plunger within the syringe barrel to promote gentle mixing of the fibrin with the cells and any remaining medium. The mixture of cartilage particles, fibrin and any remaining medium is then introduced across the surface base of the foil mold with the tip of the syringe. Remaining steps for removal and use of the implant thus formed are as described above.

[0067] Fixation of an implant into a cartilage defect: Initially, the defect area and the implant are gently dried using sterile surgical gauze. A very thin layer of fibrin glue, approx. 0.01-0.1 ml to cover then entire base of the defect. The implant is then placed on the defect, ensuring a matched fit between the contours of the defect and those of the implant. The implant is then gently held in close contact with the base and edges of the defects (e.g., using a finger). The implant should be recessed by approximately 0.5 mm relative to surrounding native cartilage. The implant is held against the

defect and gently stretched (using instruments such as a pair of surgical forceps) so that the implant fits snugly to the defect wall. The fibrin is allowed to cure for at least 5 minutes, and care must be taken not to manipulate or dislodge the implant during the curing. The transfer of the implant to the defect can be documented photographically.

[0068] Wound closure: Based upon the physician's judgment and standard of care, drains can be inserted within the wound site. The joint capsule, fascial layers and skin can be closed using standard suture and surgical techniques.

[0069] It is to be understood that the specific embodiments of the present teachings as set forth herein are not intended as being exhaustive or limiting, and that many alternatives, modifications, and variations will be apparent to those of ordinary skill in the art in light of the foregoing examples and detailed description. Accordingly, the present teachings are intended to embrace all such alternatives, modifications, and variations that fall within the spirit and scope of the following claims.

[0070] All publications, patents, patent applications and other references cited in this application are herein incorporated by reference in their entirety as if each individual publication, patent, patent application or other reference were specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A method of repairing a tissue defect, the method comprising:

a) forming, on a tissue defect, a mold conforming to the contours of at least a portion of the tissue defect; removing the mold from the defect;

b) forming in the mold an implant comprising: i) one or more tissue particles and ii) a biocompatible carrier, whereby the implant has a shape substantially conforming to the contours of at least the portion of the tissue defect;

c) separating the implant from the mold; and

d) applying the implant to at least the portion of the tissue defect.

2. The method of claim **1**, wherein the mold comprises a pliable substrate having shape memory.

3. The method of claim **2**, wherein the substrate having shape memory is a metal foil.

4. The method of claim **2**, wherein the substrate having shape memory is a plastic.

5. The method of claim **2**, wherein the substrate having shape memory is a polymer.

6. The method of claim **1**, wherein the one or more tissue particles are initially suspended in a liquid medium.

7. The method of claim **6**, wherein forming the implant comprises removing an amount of the liquid medium from the mold.

8. The method of claim **6** further comprising introducing one or more perforations in the mold, thereby promoting draining of the liquid medium from the mold.

9. The method of claim **1**, wherein the biocompatible carrier is selected from the group consisting of a fibrinogen, a fibrin, a thrombin, a type I collagen, a type II collagen, a type III collagen, a gelatin, a fibronectin, a laminin, a hyaluronic acid, a hydrogel a pegylated hydrogel and a chitosan.

10. The method of claim **9**, wherein the biocompatible carrier comprises at least one biocompatible adhesive.

11. The method of claim **10**, wherein the biocompatible adhesive is a fibrin adhesive.

12. The method of claim **1**, wherein the implant further comprises at least one bioactive agent.

13. The method of claim **12**, wherein the at least one bioactive agent is a bioactive peptide.

14. The method of claim **1**, wherein the implant comprises a first layer comprising one or more tissue particles and a second layer comprising a biocompatible carrier.

15. The method of claim **1**, wherein the applying the implant to the tissue defect comprises inserting the implant at the tissue defect.

16. The method of claim **15**, wherein the applying the implant to the tissue defect further comprises affixing the implant to the tissue defect.

17. The method of claim **15**, wherein the implant is inserted using a minimally invasive surgical (MIS) technique.

18. The method of claim **15**, wherein the applying the implant to the tissue defect further comprises affixing the implant to the tissue defect.

19. The method of claim **15**, wherein the implant is inserted using a minimally invasive surgical (MIS) technique.

20. The method of claim **31**, wherein the implant is inserted arthroscopically.

21. The method of claim **1**, wherein the tissue defect is a cartilage defect.

22. The method of claim **1**, wherein the tissue particles are cartilage tissue particles.

23. An apparatus for forming an implant for repairing a tissue defect, the apparatus comprising:

a) a mold having a shape substantially conforming to the contours of a tissue defect of a subject; and

b) a composition comprising one or more tissue particles and a biocompatible carrier, the composition disposed in the mold wherein the shape of the composition substantially conforms to the contours of the tissue defect.

24. The apparatus of claim **23**, wherein the mold comprises a substrate having shape memory.

25. The apparatus of claim **24**, wherein the substrate having shape memory is a metal foil.

26. The apparatus of claim **24**, wherein the substrate having shape memory is a plastic.

27. The apparatus of claim **24**, wherein the substrate having shape memory is a polymer.

28. The apparatus of claim **23**, wherein the mold has one or more perforations therethrough.

29. The apparatus of claim **23**, wherein the biocompatible carrier comprises at least one biocompatible adhesive.

30. The apparatus of claim **23**, wherein the biocompatible carrier comprises a biocompatible adhesive.

31. The apparatus of claim **30**, wherein the biocompatible adhesive comprises a fibrin adhesive.

32. The apparatus of claim **23**, wherein the biocompatible carrier comprises a biocompatible gel.

33. The apparatus of claim **23**, wherein the implant further comprises at least one bioactive agent.

34. The apparatus of claim **33**, wherein the at least one bioactive agent is a growth factor.

35. The apparatus of claim **23**, wherein the implant comprises a first layer and at least a second layer.

36. The apparatus of claim **23**, wherein the subject is a human.

37. The apparatus of claim **23**, wherein the tissue defect is a cartilage defect.

38. The apparatus of claim **37**, wherein the tissue particles are cartilage tissue particles.

39. A method of forming an implant for repairing a tissue defect, the method comprising:

a) forming a substrate having shape memory into a mold of a tissue defect of a subject, wherein the mold substantially conforms to contours of at least a portion of the tissue defect;

b) removing the mold from the tissue defect; and

c) disposing in the mold a composition comprising at least one biological agent and a biocompatible carrier, thereby forming the implant having a shape substantially conforming to the contours of at least the portion of the tissue defect.

40. The method of claim **39**, wherein the at least one biological agent comprises a plurality of tissue particles.

41. The method of claim **39**, wherein the at least one biological agent comprises a population of cells.

42. The method of claim **41**, wherein the population of cells comprises a plurality of chondrocytes.

43. The method of claim **39**, wherein the biological agent comprises a plurality of tissue particles.

44. The method of claim **43**, wherein the plurality tissue particles comprise juvenile cartilage particles.

45. The method of claim **39**, further comprising introducing one or more perforations in the mold.

46. The method of claim **39**, wherein the biocompatible carrier comprises at least one biocompatible adhesive.

47. The method of claim **46**, wherein the biocompatible adhesive is a fibrin adhesive.

48. The method of claim **39**, further comprising mixing into the composition in the mold at least one bioactive agent.

49. The method of claim **48**, wherein the at least one bioactive agent is a growth factor.

50. The method of claim **48**, wherein the at least one bioactive agent comprises a plurality of cells.

51. The method of claim **39**, further comprising separating the implant from the mold.

52. An implant for repairing a tissue defect of a subject, comprising a biological agent and a biocompatible carrier, wherein the shape of the implant substantially conforms to the contours of a mold wherein the mold substantially conforms to at least a portion of a tissue defect of a subject.

53. The implant of claim **52**, wherein the at least one biological agent comprises a plurality of tissue particles.

54. The implant of claim **52**, wherein the at least one biological agent comprises a population of cells.

55. The implant of claim **54**, wherein the population of cells comprises a plurality of chondrocytes.

56. The implant of claim **52**, wherein the biocompatible carrier comprises at least one biocompatible adhesive.

57. The implant of claim **56**, wherein the biocompatible adhesive is a fibrin adhesive.

58. The implant of claim **57**, wherein the implant further comprises at least one bioactive agent.

59. The implant of claim **58**, wherein the at least one bioactive agent is a growth factor.

60. The implant of claim **52**, wherein the implant comprises a first layer and at least a second layer.

61. The implant of claim **60**, wherein the second layer comprises at least one biocompatible carrier.

62. The implant of claim **52**, wherein the tissue defect is a cartilage tissue defect.

63. The implant of claim **62**, wherein the tissue particles are cartilage tissue particles.

64. A kit for forming an implant for repairing a tissue defect, comprising:

a) a pliable substrate having shape memory and which can be shaped to substantially conform to the contours of at least a portion of a tissue defect, thereby serving as a mold of at least the portion of the tissue defect in a subject;

b) a biological agent; and

c) a biocompatible carrier.

65. The kit of claim **64**, wherein the biological agent comprises a plurality of tissue particles.

66. The kit of claim **64**, wherein the pliable substrate having shape memory is a metal foil.

67. The kit of claim **64**, wherein the pliable substrate having shape memory is a plastic.

68. The kit of claim **64**, wherein the pliable substrate having shape memory is a polymer.

69. The kit of claim **64**, wherein the biological agent comprises one or more tissue particles in a liquid medium.

70. The kit of claim **69**, wherein the liquid medium is a cell, tissue or organ storage medium.

71. The kit of claim **64**, wherein the biocompatible carrier comprises at least one biocompatible adhesive.

72. The kit of claim **71**, wherein the biocompatible adhesive is a fibrin adhesive.

73. The kit of claim **64**, further comprising at least one bioactive agent.

74. The kit of claim **73**, wherein the at least one bioactive agent is a growth factor.

75. The kit of claim **64**, wherein the tissue particles are cartilage particles.

76. The kit of claim **75**, wherein the cartilage particles are selected from the group consisting of neocartilage particles, juvenile cartilage particles, cadaver cartilage particles and a combination thereof.

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