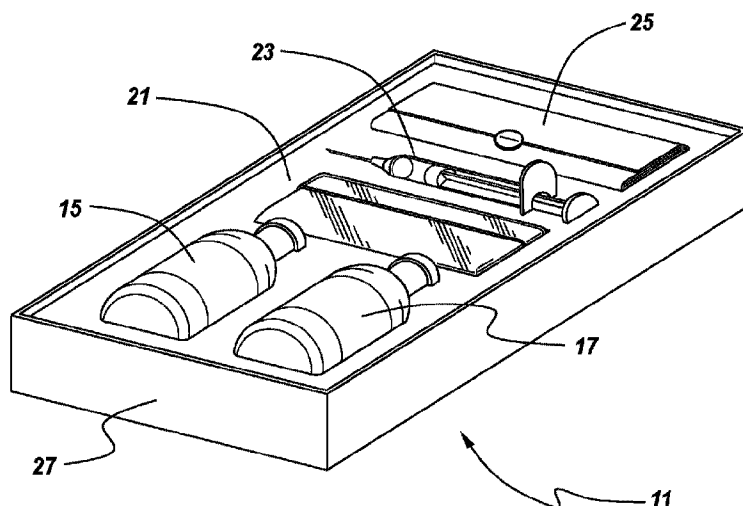




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AND METHODS OF MAKING THE SAME



(57) Abrégé/Abstract:

Methods are disclosed for the collection and processing of amniotic material in animals. These methods involve collection of amniotic material directly during parturition or caesarian section in animals for the processing of regenerative wound treatments and tissue repairs without culturing or utilization of any excess manipulation of tissue.

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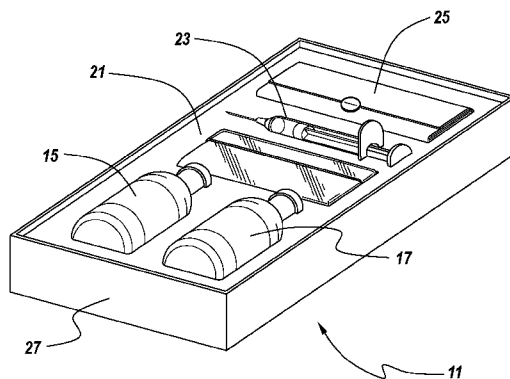


Fig. 1

(57) Abstract: Methods are disclosed for the collection and processing of amniotic material in animals. These methods involve collection of amniotic material directly during parturition or caesarian section in animals for the processing of regenerative wound treatments and tissue repairs without culturing or utilization of any excess manipulation of tissue.

**ARTICLES OF MANUFACTURE CONTAINING DECELLULARIZED AND RECONSTITUTED
FETAL TISSUE AND METHODS OF MAKING THE SAME**

FIELD OF THE INVENTION

5 The present disclosure provides processed fetal tissues and cells suitable for
promoting healing and methods of using these fetal tissues and cells to promote healing at
the site of an injury.

BACKGROUND OF THE INVENTION

10 Wounds and injuries in animals and humans can sometimes be difficult to treat.
Failure to adequately treat and promote healing can place the animal or individual in
jeopardy with potential loss of function in an effected limb or amputation, or even loss of
life.

 It would be useful to have methods and articles of manufacture that promote
healing in animals and humans. As used herein, unless the context requires otherwise, the
term “animal” encompasses and includes humans.

SUMMARY OF THE INVENTION

15 Embodiments of the present invention feature methods and articles of manufacture
that promote healing in animals.

 The fetal tissue and cellular compositions disclosed herein have many advantages

for use in promoting healing of injuries including immune privilege, an absence of associated ethical issues, and no requirement of invasive procedures for harvesting the cells and tissues. In addition, the treatment regimen disclosed herein is remarkably effective at promoting the rapid healing of open wounds within one to two weeks.

5 One embodiment is directed to an article of manufacture. The article comprises a dried particulate mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal. The dried particulate mixture is capable of reconstitution to form a reconstituted medicament for administration to the subject animal to promote healing.

10 By way of example, without limitation, a therapeutically effective amount of the reconstituted medicament is applied by injecting at or around the periphery of an internal or external injury, or applied to the subject animal by way of dropper or spray, used as a wash, powder or dressing for wounds, surgically implanted, or the like to promote healing. The treatment may also be applied by way of nebulization or oral. For example, without
15 limitation, applications to an eye of a subject animal feature drops.

 In one aspect, one milliliter of reconstituted medicament is the amount of particulate matter obtained from the mechanically decellularized amnion of about $1.5 \cdot 10^{-2}$ cm^3 to $5 \cdot 10^{-2}$ cm^3 of amnion. In one aspect, the mechanically decellularized amnion is filtered to contain particles of less than about 100 microns in diameter. In one aspect,
20 particulate suspension containing particles of less than about 20-100 microns in diameter is well suited for the treatment of the eye injury.

 Embodiments of the present invention feature a particulate mixture comprising particles. The particles have an approximate diameter of less than 500 μ , or less than 400 μ , or less than 300 μ , or less than 200 μ , or less than 150 μ , or less than 100 μ , or less
25 90 μ , or less than 80 μ , or less than 70 μ , or less than 60 μ , or less than 50 μ , or less than 40 μ , or less than 30 μ , or less than 20 μ , or less than 10 μ . In other embodiments, the particles have a diameter of more than 10 μ , or more than 20 μ , or more than 30 μ , or more than 40 μ , or more than 50 μ , or more than 60 μ , or more than 70 μ , or more than 80 μ , or more than 90 μ , or more than 100 μ , or more than 200 μ , or more than 300 μ , or more than
30 400 μ , or more than 500 μ . In other embodiments, the particles have an approximate

diameter of about 500 μ , or about 400 μ , or about 300 μ , or about 200 μ , or about 150 μ , or about 100 μ , or about 50 μ , or about 25 μ .

In certain embodiments, the article comprises a dried particulate mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal, and fetal cells obtained from one or more animals compatible with a subject animal to form a particulate cellular suspension medicament. The particulate cellular suspension medicament is administered to a subject animal to promote healing.

By way of example, without limitation, a therapeutically effective amount of the particulate cellular suspension medicament can be injected around the periphery of an internal or external injury to promote healing, applied subject animal by way of dropper or spray, used as a wash, powder or dressing for wounds, surgically implanted, or the like. For example, without limitation, applications to an eye of a subject animal feature drops.

A therapeutically effective amount of the particulate cellular suspension medicament comprises about 10^1 to 10^{20} cells per mL of medicament. Other embodiments feature about 10^3 to about 10^7 cells per mL.

A further embodiment of the article further comprises a fetal tissue wrap. The tissue wrap can comprise amnion tissue obtained from one or more animals compatible with a subject animal. The wrap is constructed and arranged for placement in juxtaposition with a site of injury to promote healing.

The wrap, comprising fetal tissue, can be air-dried for about 1 minute to about 48 or more. In another embodiment the wrap is air-dried for about 1 hour to about 12 hours. In another embodiment, the wrap is air-dried for about 1 hour to about 6 hours. In another embodiment, the wrap is air-dried for about 1 hour to about 3 hours. In another embodiment, the wrap is air-dried for about 1 hour to about 2 hours.

In another aspect of the invention, one embodiment features a kit for the treatment of a subject animal. One kit comprises a dried particulate mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal that can be capable of reconstitution to form a reconstituted medicament.

A further embodiment of the kit comprises fetal cells obtained from one or more animals compatible with a subject animal that are compatible with a particulate mixture obtained from the mechanical decellularization of amnion isolated from one or more animals. The fetal cells are applied in cooperation or concurrently with the reconstituted
5 medicament or form a combined medicament comprising a particulate cellular suspension and/or amniotic liquid medicament for administration to the subject animal to promote healing.

A further embodiment of the kit comprises a tissue wrap obtained from one or more animals compatible with a subject animal. The tissue wrap comprises amnion tissue
10 constructed and arranged for placement in juxtaposition with the site of injury. The tissue wrap is applied in cooperation or concurrently with the reconstituted medicament or a combined medicament comprising a particulate cellular or acellular suspension medicament with or without mechanically decellularized amnion and/or amniotic liquid for administration to the subject animal to promote healing.

A further embodiment of the present invention is directed to a method of
15 promoting healing in a subject animal. One embodiment of the present method features the steps of administering to the site of an injury at least one of the group consisting of a reconstituted medicament, a reconstituted amnion suspension with or without cells and a tissue wrap all of which have been previously described.

For example, without limitation, in one embodiment, a method comprises the step
20 of applying a reconstituted medicament. The reconstituted medicament is made from a dried particulate mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal.

Another method features the step of applying, by way of injection, a particulate
25 cellular suspension medicament to the periphery of the injury. The particulate cellular suspension medicament suspension comprises particles derived from the mechanical decellularization of amnion obtained from one or more animals compatible with the subject animal, and isolated amniotic fluid cells obtained from the one or more animals compatible with the subject animal.

30

In one aspect, the method further comprises the step of applying a tissue wrap to the site of the injury. The wrap comprises amnion tissue compatible with the subject animal constructed and arranged for placement in juxtaposition with the site of injury. The amnion tissue is air-dried for about 1 minute to about 48 hours or more. In another
5 embodiment the amnion tissue is air-dried for about 1 hour to about 12 hours. In another embodiment, the amnion tissue is air-dried for about 1 hour to about 6 hours. In another embodiment, the amnion tissue is air-dried for about 1 hour to about 3 hours. In another embodiment, the amnion tissue is air-dried for about 1 hour to about 2 hours.

In one aspect of the method, the site of injury is debrided prior to applying one or
10 more of the group consisting of a cellular reconstituted suspension, or a reconstituted acellular suspension medicament and a tissue wrap.

A further embodiment is directed to a method of making a dried particulate mixture of mechanically decellularized obtained from one or more animals compatible with a subject animal. The dried particulate mixture is capable of reconstitution to form a
15 reconstituted medicament for administration to the subject animal to promote healing. The method comprises the step of mechanically decellularizing amnion tissue to form particles capable of reconstitution.

A further embodiment is directed to a method of making a particulate cellular suspension medicament. The method comprises the steps of providing a dried particulate
20 mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal, and fetal cells obtained from one or more animals compatible with a subject animal to form a particulate cellular suspension medicament and forming a particulate cellular suspension. The particulate cellular suspension medicament is administered to a subject animal to promote healing.

A further embodiment of the present invention features methods of making a tissue wrap, a particulate mixture medicament and a particulate cellular suspension medicament. One embodiment of the method of making the tissue wrap comprises the steps of applying amnion tissue to a support to form a supported amnion. The supported amnion is next air dried to form the tissue wrap which is placed in a suitable containment means until applied
25
30 to a site of injury.

These and other features and advantages will be apparent upon viewing the Figures that are briefly described below and upon reading the detailed description that follows.

BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 depicts a kit embodying features of the present invention;
 Figure 2 depicts a tissue wrap embodying features of the present invention; and,
 Figure 3 shows a tissue wrap embodying features of the present invention in a
 container.

DETAILED DESCRIPTION

10 Unless otherwise defined herein, scientific and technical terms used herein have
 the meanings that are commonly understood by those of ordinary skill in the art. In the
 event of any latent ambiguity, definitions provided herein take precedence over any
 dictionary or extrinsic definition. Unless otherwise required by context, singular terms
 shall include pluralities and plural terms shall include the singular. The use of the term
15 “including”, as well as other forms, such as “includes” and “included”, is not limiting.

 It is noted here that as used in this specification and the appended claims, the
 singular forms “a”, “an”, and “the” also include plural reference, unless the context clearly
 dictates otherwise.

 The term “about” or “approximately” means within 10%, and more preferably
20 within 5% (or 1% or less) of a given value or range.

 As used herein, the term “isolated cell” refers to a cell that has been removed from
 its in-vivo location.

 As used herein, the term “decellularization” refers to a process that removes cells
 from a tissue while preserving the native ultrastructure and composition of the
25 extracellular matrix (ECM). For example, an amnion particulate mixture can be obtained
 by decellularizing a fetal tissue comprising amnion.

There are a number of methods of decellularization of tissue known in the art, including, but not limited to, chemical agents, hypotonic and hypertonic solutions, detergents (e.g., Triton-X™), alcohols, solvents (e.g., tributyl phosphate (TBP), biologic agents (e.g., collagenase, trypsin, lipase, nucleases, α -galactosidase), non-enzymatic agents (e.g., chelating agents such as EDTA or EGTA), physical agents (e.g., temperature, force and pressure, non-thermal irreversible, mechanical, electroporation (NTIRE) (see, for example, Crapo *et al.*, *Biomaterials*. 2011; 32(12): 3233–3243). In certain embodiments, one or a combination of the aforementioned methods may be used to decellularize a tissue. However, methods that preserve the complex composition and three-dimensional ultrastructure of the extracellular matrix (ECM) are preferred.

In one embodiment, a tissue is mechanically decellularized, e.g., by cryofractionation, a procedure in which a tissue is frozen and ground in a cryomill to produce a mixture of particles. Such particles are obtained from the cryofractionation of about 0.5 cm², or about 1 cm², or about 1.5 cm², or about 2 cm², or about 2.5 cm², or about 3 cm², or about 3.5 cm² or about 4 cm², or about 4.5 cm² to about 5 cm² of amnion or more. The amnion can have a thickness of from about 500 μ m to 50 μ m, or from 400 μ m to about 50 μ m, or from about 300 μ m to 50 μ m, or from about 200 μ m to about 50 μ m, or from about 150 μ m to about 50 μ m, from about 100 μ m to about 50 μ m or from about 50 μ m to about 25 μ m or less. In another embodiment, the amnion has a thickness of about 500 μ m, or about 400 μ m, or about 300 μ m, or about 200 μ m, or about 150 μ m, or about 100 μ m, or about 50 μ m, or about 25 μ m or less.

As used herein, 1 μ m refers to a micrometer or 10⁻⁶ meters.

The term “amnion” refers to a thin, cellular, extra-embryonic membrane that forms the inner membrane of a closed sac surrounding and protecting an embryo in reptiles, birds, and mammals. The sac contains the fetus and amniotic fluid, in which the embryo is immersed, nourished and protected. Typically, the amnion is a tough, transparent, nerve-free, and nonvascular membrane consisting of two layers of cells: an inner, single-cell-thick layer of ectodermal epithelium and an outer covering of mesodermal, connective, and specialized smooth muscular tissue. In the later stages of pregnancy, the amnion expands to come in contact with the inner wall of the chorion creating the appearance of a

thin wall of the sac extending from the margin of the placenta. The amnion and chorion are closely applied, though not fused, to one another and to the wall of the uterus. Thus, at the later stage of gestation, the fetal membranes are composed of two principal layers: the outer chorion that is in contact with maternal cells and the inner amnion that is bathed by amniotic fluid. The amnion has multiple functions, e.g., as a covering epithelium, as an active secretory epithelium, and for intense intercellular and transcellular transport.

As used herein, the term “tissue” refers to an aggregate of similar cells and associated extracellular matrix (ECM) forming a definite kind of organized material with a specific function, in a multicellular organism.

As used herein, an “amnion tissue” refers to the isolated cellular, extra-embryonic amnion membrane that is detached from the chorion. In one embodiment, the amnion tissue is air-dried. In another embodiment, the amnion is air-dried for about 60 to about 90 minutes or more at ambient temperature (i.e. about 18 to 24 ° C).

As used herein, a “particulate mixture” refers to the powder or particles obtained from the cryofractionation of amnion.

As used herein, the term “fetal tissue” refers to extra-embryonic tissues including, but not limited to, amnion, chorion, yolk sac, the allantois, umbilical cord and/or fetal placenta (villous chorion).

As used herein, the term “fetal cells” refers to cells resident in the extra-embryonic tissues including, but not limited to, amnion, chorion, yolk sac, the allantois, umbilical cord, fetal placenta (villous chorion) and/or amniotic fluid. In certain embodiments, the term “fetal cells” refer to isolated fetal cells.

In certain embodiments, the term “fetal cells” refers to unfractionated cells of the amniotic fluid including epithelial and/or amniotic fluid or membrane-derived mesenchymal stem cells (see U.S. Patent Publication No. US 2013/0230924.

The term “injury” means a pathological condition, such as, by way of example, without limitation, a wound, incision, a break in the skin, bone, tendon, ligament, muscle, neoplasia, eye, and soft tissues, an inflammation, infection, or other disease condition.

The term “promoting healing” refers to causing a favorable result compared to no treatment. The favorable result comprises any one or more of the following such as reduction of scarring, reduction of inflammation, regrowth of normal tissue or growth of scar tissue, improved load bearing on a limb movement, closure of wound, reduction in
 5 infection and reduction in mortality associated with the underlying pathology.

The term “compatible with a subject animal” denotes the origin of the tissue as being from the same species or closely related species.

In other embodiments, the term “compatible with a subject animal” refers to an xenograft, i.e., a tissue graft from different species.

10 In another embodiment, the term “compatible with a subject animal” refers to allografts, i.e., a tissue from one individual to another of the same species with a different genotype.

As used herein, an “animal” refers to living multi-cellular vertebrate organisms, a category that includes, for example, mammals, birds, reptiles, and amphibians. The term
 15 mammal includes both human and non-human mammals. Similarly, the term “subject animal” includes both human and non-human subjects. The term “subject animal” refers to an individual human or animal which has a site of injury or disease.

As used herein, a non-human animal can refer to a mammal including, but not limited to, a domesticated animal such as a dog, a racing dog, sheep, a pig, a goat, cattle,
 20 a zebu, a cat, a guinea pig, a donkey, water buffalo, including “river buffalo” and “swamp buffalo”, a horse, a racing horse, a dromedary camel, a yak, a bactrian camel, a llama, an alpaca, a ferret, a mouse, a bali cattle, a gayal, a rabbit, a rat and a lab rat, a silver fox or a hedgehog.

In certain embodiments, a non-human animal can refer to mammals kept in zoos
 25 including, but not limited to, zebra, gazelle, wolves, wild swine (pigs & hogs), wild cattle, warthogs, vervet monkeys, two-toed sloths, tree pangolins, tigers, tapirs, tamandua or lesser anteaters, takins, sun bears, striped hyena, spotted hyena, spiral-horned antelope, somali wild ass, snow leopards, small cats, sloth bears, singing dogs, siamang, serval, sea lions, rock hyrax, rhinoceros, reindeer, red pandas, pygmy
 30 marmosets, pygmy hippopotamus, przewalski's horses, pronghorns, prairie dogs,

porcupines, polar bears, painted dogs, otters, oryx, orangutan, okapi, ocelot, nubian ibex, Nile lechwe, naked mole-rats, mountain lions (puma, cougar), monkeys, meerkat, mangabey, mandrill, lynx and bobcats, lions, leopards, lemur, jaguars, honey badgers (ratel), hippos, hamadryas baboons, guenon, guanaco, gorillas, giraffe, giant pandas, giant anteaters, gelada baboons, fossa, fishing cats, elephants, echidna, dhole, coquerel's sifaka, clouded leopards, chimpanzees, cheetahs, tigers, caracals, capybara, camels, brown bears, bonobos, binturongs, bat-eared fox, bats, armadillos, antelope, andean (spectacled) bears and agouti.

In certain other embodiments, a non-human animal can refer to mammals considered by the World Wildlife Fund to be endangered including, but not limited to, the amur leopard, black rhino, cross river gorilla, javan rhino, mountain gorilla, pangolin, saola, south china tiger, sumatran elephant, sumatran orangutan, sumatran rhino, sumatran tiger, vaquita, western lowland gorilla, yangtze finless porpoise, african wild dog, amur tiger, asian elephant, bengal tiger, black spider monkey, black-footed ferret, blue whale, bonobo, bornean orangutan, borneo pygmy elephant, chimpanzee, eastern lowland gorilla, fin whale, ganges river dolphin, giant panda, hector's dolphin, indian elephant, indochinese tiger, indus river dolphin, malayan tiger, north atlantic right whale, orangutan, sea lions, sei whale, snow leopard, Sri Lankan elephant, tigers and whales.

In certain embodiments, a non-human animal can refer to marsupials, including, but not limited to, wallabies, koalas, possums, opossums, kangaroos, bandicoots, wombats, bettongs, bilbys, quolls, quokkas and the Tasmanian devil.

The term "reconstituted" means that that an aqueous liquid is added to make the medicament. A liquid for reconstitution comprises a biocompatible solution such as normal saline, e.g. phosphate buffered saline (PBS) or amniotic fluid. A preferred liquid for reconstitution is calcium-free sterile, non-pyrogenic isotonic solution suitable for intravenous administration. For example, without limitation, one such liquid is sold under the trademark PlasmaLyte ATM in a single dose container for intravenous administration. Each 100 mL contains 526 mg of Sodium Chloride, USP (NaCl); 502 mg of Sodium Gluconate (C₆H₁₁NaO₇); 368 mg of Sodium Acetate Trihydrate, USP (C₂H₃NaO₂•3H₂O); 37 mg of Potassium Chloride, USP (KCl); and 30 mg of Magnesium Chloride, USP (MgCl₂•6H₂O). It contains no antimicrobial agents. The pH is 7.4.

The term “administering” means applying or injecting or ingesting the medicament. The term “applying” is used broadly and includes uses such as washes and implantation.

Connective soft tissue defects or injuries often occur by damage to the extra-
5 cellular matrix (ECM) that forms muscles, ligaments or tendons in mammals. Collagen is the most abundant structural protein in the connective tissue (ECM) and acts as a natural scaffold for cellular attachment in the body.

Amnion is an abundant source of collagen, as well as the other proteins, carbohydrates, lipids, hyaluronic acid, laminin, fibronectin, pluripotent mesenchymal stem
10 cells (MSC) and other complex growth factors that are essential for fetal growth and development. In particular, amnion has a complete lack of surface antigens, thus it does not induce an immune response when implanted into a ‘foreign’ body, which is in contrast to most other allograft implants. Amnion also markedly suppresses the expression of the pro-inflammatory cytokines, IL-1 α and IL-1 β (Solomon *et al.*, 2001, Br. J. Ophthalmol. 85
15 (4):444-9) and produces natural inhibitors of matrix metalloproteases (MMPs) expressed by infiltrating polymorphonuclear cells and macrophages (Hao *et al.*, 2000, Cornea, 19 (3):348-52; Kim *et al.*, 2000, Exp. Eye Res. 70 (3):329-37). Amnion also down-regulates TGF- β and its receptor expression by fibroblasts leading to the ability to modulate the healing of a wound by promoting tissue reconstruction. Furthermore, amnion has a broad
20 spectrum of antimicrobial activity against bacteria, fungi, protozoa, and viruses for reduced risk of post-operative infection.

Amnion derived tissues are therefore immune-privileged and ideally suited for regenerative medicine applications.

A “kit” is an assembly of parts, materials, and compositions of matter packaged
25 together to facilitate a treatment. Kits commonly comprise instructions for the use of the parts, materials and compositions.

Turning now to Figure 1, a kit embodying features of the present invention, generally designated by the numeral 11 is depicted. Kit 11 has the following major elements: a first vial 15, a second vial 17, a container for a tissue wrap 21, a syringe 23,
30 and instructions 25. The kit 11 is held in suitable packaging, as depicted, a box 27.

Suitable packaging may comprise any means for holding the collection of parts, materials and compositions. For example, without limitation, bags, wraps, containers, ties and the like. The kit 11 for ocular treatments may comprise fibrin glue [not shown] for attaching the tissue wrap 31 to the eye.

5 The first vial 15 contains a dried particulate mixture of mechanically decellularized amnion obtained from one or more animals compatible with a subject animal that upon of reconstitution forms a reconstituted medicament. The kit 11 may contain a vial containing such liquid for reconstitution [not shown] or the liquid for reconstitution may be derived from other sources.

10 The second vial contains fetal cells obtained from one or more animals compatible with a subject animal and compatible with a particulate mixture in the first vial 15. The fetal cells are applied in cooperation or concurrently with the reconstituted medicament or form a combined medicament comprising a particulate cellular suspension medicament for administration to the subject animal to promote healing. In forming a combined
15 medicament, the dried particulate mixture of the first vial 15 is reconstituted with or combined with the fetal cells of the second vial 17, supplemented as needed with further liquid for reconstitution. For example, the dried particulate mixture can be reconstituted by suspension in a solution of 50% solution for reconstitution, such as PlasmaLyte A™, and 50% amniotic fluid containing fetal cells.

20 The combined medicament is injected into or around the site of injury with syringe 23.

 The container for a tissue wrap 21 contains a tissue wrap derived from amnion tissue obtained from one or more animals compatible with a subject animal. The tissue wrap comprises amnion tissue constructed and arranged for placement in juxtaposition
25 with the site of injury. The tissue wrap is applied in cooperation or concurrently with the reconstituted medicament or a combined medicament comprising a particulate cellular suspension medicament for administration to the subject animal to promote healing.

 Turning now to Figure 2 the container for tissue wrap 21 is depicted as a transparent bag through which the tissue wrap designated by numeral 31 can be seen. As
30 seen in Figure 3, tissue wrap 31 is formed by affixing amnion tissue to a first support 33

on one side of the amnion and a second support on the other side of the amnion [not shown] and air drying the tissue for thirty minutes to three hours or more, and, most preferably, for about one hour. The supports, of which first support 33 is depicted, maintain the shape of the tissue during the drying process. The supports are preferably removed prior to placement of the tissue in container 21.

Tissue wrap 31 is removed from the container and placed over the site of injury.

Returning now to Figure 1, the dried particulate mixture is obtained from the mechanical decellularization or cryofractionation of about $1.5 \times 10^{-2} \text{ cm}^3$ to $5 \times 10^{-2} \text{ cm}^3$ of amnion /mL of reconstituted medicament and include particles greater than 20-100
 10 microns in diameter. Upon reconstitution of the dried particulate mixture by suspension in a solution of amniotic fluid and/or PlasmaLyte A™, the reconstituted medicament can be administered to the subject animal to promote the healing of superficial wounds.

A reconstituted medicament containing particles less than about 100 microns is suitable for treatment of eye injuries. The kit 11 for the treatment of eye injuries
 15 comprises a dropper for administration.

The dried particulate mixture obtained from the cryofractionation comprises about $1.5 \times 10^{-2} \text{ cm}^3$ to about $5 \times 10^{-2} \text{ cm}^3$ of amnion /mL of reconstituted medicament.

The fetal cells can comprise amniotic fluid cells and the particulate matter can be filtered to contain particles that are less than 100 microns in diameter. The fetal cells can
 20 have a concentration from 10^3 to 10^{20} /mL mesenchymal and/or epithelial stem cells. In another embodiment, fetal cells can have a concentration of 10^3 to 10^{12} /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^{12} /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^{11} /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^{10} /mL. In another
 25 embodiment, fetal cells can have a concentration of 10^4 to 10^9 /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^8 /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^7 /mL. In another embodiment, fetal cells can have a concentration of 10^4 to 10^6 /mL. In another embodiment, fetal cells can have a concentration of 10^3 to 10^6 /mL. In another
 30 embodiment, fetal cells can have a concentration of 10^3 to 10^7 /mL.

In one example, the fetal cells can have a concentration of about 0.8×10^6 to 1.2×10^6 cells / mL of the particulate cellular suspension. The dried particulate mixture can contain particles obtained from the cryofractionation of from about $1.5 \times 10^{-2} \text{ cm}^3$ to about $5 \times 10^{-2} \text{ cm}^3$ amnion per mL of the particulate cellular suspension. In one example, the
5 particulate cellular suspension comprises a particulate matter obtained from the cryofractionation about $1.5 \times 10^{-2} \text{ cm}^3$ to about $5 \times 10^{-2} \text{ cm}^3$ amnion for every 10^6 plus or minus 2×10^5 amnion fluid cells.

Embodiments of the present invention are well suited for the treatment of open wounds, superficial and deep including such wounds that expose a movable body element
10 of the animal's musculoskeletal system, for example, a ligament or tendon. Such wounds can include ligaments and tendons that are lacerated.

For example, in the event the animal is a horse and the injury may comprise chondral and fascial pathologies, tendon and ligament pathologies, surface reconstruction or articular joint pathologies. The chondral and fascial pathologies can include, for
15 example, laminitis. The tendon and ligament pathologies can include, for example, tendonitis, sport-induced superficial digital flexor tendon (SDFT) or suspensory ligament (SL) lesions. The articular joint pathologies can include, for example, navicular disease, ringbone or arthritis. The site of injury can also be an open wound that, in one example, is able to heal in about one to two weeks. In another example, healing of the site of injury
20 reduces the risk of recurrence. In one example, the risk of recurrence of the injury can be reduced by about 5 fold.

Features of the present invention are further described with respect to the following Examples. These examples feature equine subjects and materials. However, materials derived from other animals species, for example, without limitation, canine, feline, bovine,
25 porcine and other animal species materials and subjects can be prepared in a similar manner as outlined below. The equine materials have been utilized with avian, reptilian and other animal groups.

30

EXAMPLES

EXAMPLE 1: AMNIOTIC MATERIAL PROCESSING

This procedure defines the aseptic collection of amniotic material (amnion and amniotic fluid) for injection at the site of an injury.

5 Amnion Tissue

The amnion container was picked up and sampled for Bioburden. The amnion was aseptically transferred into the sterile field (laminar flow hood). The amnion transport packaging (previously disinfected, i.e. with 70% ETOH) was opened.

10 A 50mL sample of the Amnion Transport Solution was aseptically transferred into a 30 to 60mL conical tube for pre-processing bioburden testing. The vial was labeled with sample description, batch number, date and time and placed in a designated refrigerator.

(1) Amnion Preparation

The amnion from the incoming container was transferred into approximately 200 mL of Plasma Lyte-A in a sterile bioassay dish where it was gently rinsed. A piece of
15 amnion was then spread evenly on a sterile cutting board carefully avoiding any overlaps. A record was made of the amnion preparation start time. Sterile gauze or laps were used to remove any remaining debris/ blood from the surface of the amnion. The amnion was inverted and the surface of the opposite site was similarly washed. Any chorion was removed by blunt dissection to separate it from the amnion. After washing and cleaning,
20 the amnion pieces were returned to the bioassay dish containing Plasma Lyte-A. Using a sterile scissors/scalpel, the amnion was cut into 2 to 10 sections. The approximate area of each piece (50-450 cm²) was measured and recorded using a sterile stainless steel ruler.

The cleaned pieces of the amnion were placed back on the sterile cutting board and the amnion was spread out on the board taking care to not overlap. A sterile nylon mesh
25 was placed over the surface of the amnion again taking care to overlap them. The amnion and mesh were then placed onto a sterile drying rack and allowed to air dry for a minimum of one hour. Start and stop times for drying were recorded.

(2) Amniotic fluid

(a) Amnion Fluid Preparation

A large sterile pan was first placed into the sterile field (laminar flow hood) and filled with cold packs from a -80 °C freezer. The aspiration containers with the amniotic fluid were disinfected with 70% ethanol (ETOH), inspected for integrity and placed on the cold packs in the laminar flow hood. The source of the amniotic fluid was confirmed by looking at the Donor animal ID number. The 2.0 mL of amniotic fluid was then aseptically pipetted into a 2 mL sterile microcentrifuge tube for bioburden testing. The vial was labeled with the sample number, batch number, date and time and placed in the designated refrigerator.

Using a 50 mL sterile disposable serological pipette all the remaining amniotic fluid was transferred into 1 liter sterile disposable bottles and placed on the cold pack. The total volume of the amniotic fluid and the color were recorded.

(3) Amniotic Fluid Cell Count and Determination of the Number of Viable Cells

Each amniotic fluid bottle was gently mixed and 1.0 mL of the fluid was collected using a 1 mL micropipette and transferred into a 2 mL Eppendorf microcentrifuge tube. A total of 50 µL of cell suspension was then added to 50 µL of trypan blue (0.4%) in an Eppendorf microcentrifuge tube and vortexed for 5 seconds. The sample was placed on a rack for 5 minutes.

A Neubauer chamber (hemocytometer) was rinsed with distilled water, and then sprayed with ETOH 70% and wiped clean and dried with paper towels. A cover slip was placed on the top of the micro-grids of the chamber. The trypan blue-cell suspension was gently mixed and used to fill both sides of the hemocytometer with 10 µL by capillary action. The cells were allowed to settle down for at least 30 seconds. The hemocytometer was placed under the microscope and all cells in the four 1 mm corner squares and one 1mm center square were counted. For accuracy the total number of cells counted was greater than 100. The cells were re-counted if > 10% of the cells appeared clustered, by vigorously pipetting in the original cell suspension as well as in the trypan blue cell suspension mixture. Using a double cell counter, the number of viable and non-viable cells was determined. The cells in both chambers were counted and an average was calculated.

For the trypan blue test, live cells did not take up the dye, whereas dead (non-viable) cells did. Thus non-viable cells stained blue and viable cells remained opaque. “Ghost” cells, which appeared as flattened pale blue cells were not counted.

The number of cells was determined as follows: Each square represented a total
 5 volume of 0.1 mm^3 or $1 \times 10^{-4} \text{ mL}$ (0.1 mm depth x 1 mm width x 1 mm height = 0.1 mm^3).
 The number of cells per mL was then deduced from the average viable cell count per 1 mm square x 2×10^4 . The total cell number was therefore equal to the number of cells per mL multiplied by the original volume of sample fluid. The cells were then diluted to the desired concentration.

10 EXAMPLE 2: ASEPTIC PROCESSING OF THE AMNIOTIC MATERIAL

(1) Aseptic Cryofractionation of Amnion

After at least one hour, the amnion was removed from the drying rack and transferred into the milling chambers having an impactor. The milling chambers were placed into the Cryomill and cryofractionated using the following settings:

15 Number of Cycles: 4
 Frequency 1/s: 10 CPS_
 Precooling Time: 10 minutes
 Grinding Time: 4 minutes
 Intermediate Cooling: 3 minutes

20 Once grinding was complete, the milling chambers were allowed to warm to room temperature for approximately two hours. The start and stop times were recorded.

Approximately 50 mL of the amnion suspension solution was dispensed into each milling chamber. The inside milling chamber and the impactor were rinsed with the solution multiple times until the ground amnion (dried particulate mixture) was re-
 25 suspended and collected in the bottom of the chamber. The impactor was removed using the magnet pen. The cryofractionated amnion solution was then transferred to the amnion suspension container and placed on cold packs in the sterile field and diluted to the desired amount.

(2) Aseptic Processing of Amniotic Fluid

The amniotic fluid was aliquoted evenly into 50 mL sterile centrifuge tubes and centrifuged at 200-400 x g (1100-1600 rpm) for approximately 5-10 minutes at room temperature. The supernatant was then removed from each tube using a 25 mL sterile serological pipette. The amniotic liquid was kept in new container and the pellet was re-suspended in Plasma Lyte-A™ to a total volume of 25 mL in each tube. The re-suspended cells in any two different tubes were vortexed for approximately 3 to 5 sec and consolidated into a single tube prior to centrifugation at 200-400 x g (1100-1600 rpm) for approximately 5-10 minutes at ambient temperature. The preceding steps were repeated as necessary.

The supernatant from each tube was removed using a sterile pipette and the pellet was again re-suspended in a cell suspension solution (amniotic fluid and/or an isotonic solution, e.g., PlasmaLyte A™) to bring the volume in each tube to about 10 mL and vortexed for approximately 3 to 5 seconds. A 1 mL aliquot was removed and the cell count and viability was determined using the above-described trypan blue test.

If red blood cells were present in the amniotic fluid cell suspension, they were removed using a RBC Lysing Solution. A 10x concentration was prepared as follows: NH_4Cl (ammonium chloride) = 8.02gm + NaHCO_3 (sodium bicarbonate) = 0.84gm adjusted to a total volume of 100 mL with Millipore filtered water. 10 mL of the 10x concentrate was added to 90 mL Millipore filtered water and refrigerated until use. The amount needed of Erythrolysis solution (15 mL per tube centrifuged) was removed from the refrigerator and kept for a period of 0.5 hours in the stabilization incubator. After centrifuging the amniotic fluid at 400 x g for 10 minutes, the supernatant was removed and the pellet was re-suspended in Erythrolysis solution (minimum of 50 mL per tube). The contents of all the tubes were consolidated into one tube that was rocked for ~10 minutes at room temperature until the liquid was clear red. The cells were again centrifuged for 5 minutes at 250 to 400 x g. The supernatant was decanted. The pellet was washed with 50 mL of PBS or PlasmaLyte A™ before centrifugation again for 10 minutes at 250 to 400 x g. The washing of the pellet was repeated as needed. The amniotic fluid cells were then filtered through a 100 μm cell strainer, and re-suspended in PBS or PlasmaLyte A™. The cells were again centrifuged for 10 minutes at 250 to 400 x g. The supernatant was

decanted and the pellet was left in the 50 mL conical centrifuge tube.

(3) Procedure for Cryopreservation of Cryofractionated Amnion with Amnion Fluid Cells

Appropriate size cryovials that were previously labeled and their corresponding size of CoolCell™ freezer (CCF) racks were placed in the hood. CryoStor 10™, the Cell Suspension Solution and the Amnion Suspension Solution were also placed on cold packs in the hood. Cryostor 10™ is commercially available from Biolife Solutions.

The cell suspension solution and the amnion suspension solution were then combined into the cell suspension solution container. Using 50mL serological pipettes, the solutions were homogenized several times. The container was again placed on the cold packs on the sterile field. Empty cryovials were placed in the CCF racks on cold packs and their caps were removed inside the hood (sterile field). The mix of cell / amnion suspension solution was pipeted into an empty Amnion Suspension container and a same volume of CryoStor 10™ was added and homogenized before being placed on the cold packs. A 50 mL pipette Combipip was fitted on to a repeat pipetor set to dispensing mode. The fill volume was adjusted and 50 mL of the Cell/Amnion solution was aspirated and then adjusted to the desired dispense volume (1 or 2 mL). The cryovials in the CCF were then filled and the vial caps were replaced securely and the rack of filled vials was placed on cold packs for QC inspection.

Cell freezing was achieved by cooling the cells at a cooling rate of 1°C per minute from 4°C to -80 °C, using a passive cooling controlled-rate freezer CoolCell™ (commercially available from Biocision.)

The cell suspension in cryoprotective freezing medium was aliquoted into each of the cryovials and the cells were gently mixed to maintain a homogeneous cell suspension. The solid core of the CoolCell (black ring) at room temperature was seated in the bottom of the central cavity and the vials containing the cell suspension were placed in each well. The lid of the CoolCell™ was fully sealed and the Coolcell™ was placed into a -80°C freezer for at least 4 hours prior to transfer on dry ice to long term storage. Cell viability and QA/QC were evaluated by thawing one vial after short term storage.

30

EXAMPLE 3: AMNIOTIC TISSUE WRAP PREPARATION

After confirming the amniotic tissue source and donor mare ID and recording the time of receipt, the amnion transport packaging (previously disinfected, i.e. with 70% ethanol, methanol, etc.) was aseptically transferred into the sterile field (a laminar flow hood). A sample of the Amnion Transport Solution was first transferred into a 50 mL conical tube for Bioburden testing. The vial was then labeled with sample description, batch number, date and time and placed in designated refrigerator.

(1) Amniotic Membrane Wrap Preparation

Saline was aseptically added into a second receiving pan in the sterile field (i.e. laminar flow hood) and the amnion tissue was taken from incoming receiving pan to the second receiving pan containing the sterile saline. Any remaining blood was rinsed with sterile saline. After documenting the amnion preparation start time, sterile gauze or laps was used to remove any remaining debris/ blood from the surface of the amnion. The amnion was then inverted and the other side was rinsed and washed. Any remaining chorion was removed by blunt dissection to separate it from the amnion. The amnion was kept wet with sterile saline. The tissue was blocked off by cutting away any stringy ends and checked for holes or tears. After repositioning the amnion on the cutting board with the chorion side up, the approximate area of each piece of amnion was measured and recorded in cm^2 using a sterile stainless steel ruler.

The amnion (chorion side up) was covered with a sterile mesh (e.g., a nylon mesh) wetted with sterile saline. For the purpose of this discussion, there is a first steel mesh which is denoted by a first visible indicia, the color white, and a second steel mesh, to be discussed below, which is denoted by a second visible indicia, the color blue. Any reference to the colors white or blue are directed to these visible indicia. Those skilled in the art will recognize that such visible indicia is matter of choice. The mesh was cut to size and was allowed to slightly overlap the amnion. The mesh-covered amnion was then gently lifted, turned over and placed back on the cutting board. Caution was used as to not disturb the mesh/amnion interface. The newly exposed side of the amnion was wiped with sterile wipes or gauze pads to remove any remaining blood or small tissue particles. The newly exposed side of the amnion was covered with a second steel mesh, BLUE sterile

mesh (e.g., a nylon mesh) wetted with sterile saline. The mesh was cut to size and was allowed to slightly overlap the amnion.

The amnion tissue sandwiched between the white and blue mesh was placed on the drying rack where the amnion tissue was allowed to dry for 60 to 90 minutes at ambient temperature (65° to 70° F). Caution was used as to not disturb the mesh/amnion interface. The amnion was kept unfolded and as flat as possible during this step. Additional drying racks were used as needed. The total drying time was recorded. The dried tissue was then removed from the rack(s) and laid flat on the cutting board, WHITE side up. The WHITE mesh was carefully removed from the entire sheet of amnion which was checked for holes or tears. With the BLUE mesh side up on the cutting board, each section was cut using a scalpel or rotary cutting blade and the sizes and surface areas were recorded as 5 x 5 cm, 10 x 10 cm, round 15 mm diameter and round 22 mm diameter. Those skilled in the art will recognize that the sections can be cut and sized to fit particular needs and these sizes and shapes are only exemplary.

(2) Amniotic Membrane Wrap Pre-Packing

Pouches, Sealing Test and Labeling

The sterile field was set up for packaging including a sealer for packing pouches (Sealer settings: Temperature= $177 \pm 9^{\circ}\text{C}$ ($350 \pm 15^{\circ}\text{F}$)). Three (3) empty pouches were sealed for visual inspection and retention and then labeled. Packing pouches are available from numerous vendors. One suitable pouch is sold under the trademark KAPAK™.

Amniotic Membrane Wrap Packing

Pouches were transferred onto the sterile field (previously disinfected laminar flow hood). Using sterile forceps, each individual tissue membrane was inserted into the inner pouch. Large membranes were folded if necessary. The pouches were sealed with the dried amnion tissue on a mesh (see, for example, Figures 2 and 3) and inspected for a broken seal, impurities, and defects. Upon passing the inspection, the pouches were labeled with date and packaged in large pouches according to size and stored in the refrigerator or at room temperature. The donor ID, size, date, time, and initials were documented.

EXAMPLE 4: IMPLANTATION OF CRYOFRACTIONATED AMNION AND ISOLATED AMNIOTIC FLUID CELLS

(1) Thawing vials

A container was $\frac{3}{4}$ filled with hot tap water with a thermometer and cold water was
5 added until a temperature of 37°C was reached. A vial of cryofractionated amnion with
amniotic fluid cells was taken from the -80°C freezer. Holding the cap, the vial was partial
immersed in the water bath for approximately 2 to 3 minutes with gentle agitation until the
contents were melted. The vial was removed from the water bath and the exterior was
wiped with sterile gauze saturated with 70 % ethanol. The thawed contents were then
10 ready for immediate use.

(2) Animal Restraint

A horse with an injury was adequately restrained and sedated or anaesthetized before
starting the procedure according to the veterinarian's directions. Appropriate sedation or
anesthesia caused minimal distress to the patient and prevented any long lasting
15 discomfort. Use of nose twitch was optionally required. In the case of young adults or
unbroken horses, short-acting general anesthesia was required.

(3) Implantation Procedure

The site for the product implantation was processed as a surgically prepared area.
After cleaning or clipping of any gross contamination, such as dust or mud, the complete
20 the area was scrubbed with chlorhexidine for 5 to 7 minutes and then wiped down with
alcohol swabs. Before proceeding, the area was cleaned until the alcohol swabs used on
the scrubbed area were dirt-free. Systemic antibiotics were administered prior to starting
the procedure. However, in some instances, systemic antibiotics are not necessary.

The contents of the 2 mL vial were split into 3 to 4 doses (0.50 to 0.67 mL) and
25 loaded into syringes (for example, 1 mL syringes) with sterile hypodermic needles (for
example 22 gauge, 1.5 inch needles). The appropriate leg was resting or held up and
restrained as needed. For injuries to a tendon, ligament or muscle the cryofractionated
amnion and amniotic fluid cell mix was injected in close proximity to the lesion without
penetrating the actual tendon, ligament or muscle. For injuries to articulation (i.e., joints)

the cellular particulate mixture was injected either peri-articularly or intra-articularly. After injection, the site was bandaged with sterile swabs and adhesive bandage.

EXAMPLE 5: IMPLANTATION OF CRYOFRACTIONATED AMNION AND
ISOLATED AMNIOTIC FLUID CELLS IN COMBINATION WITH
5 THE AMNION TISSUE WRAP

(1) Animal Restrain

A horse was adequately restrained as described in Example 4.

(2) Materials

A kit comprising sterile hypodermic needles (18 and 23 gauge), syringes (3 to 5
10 mL), vials of thawed cryofractionated amnion and amniotic fetal cells as well as packaged amnion tissue wrap of the appropriate size were assembled (see Figure 1) together with clippers, sterile gloves, 1% lidocaine, saline and sedatives and/or anesthetics.

(3) Wound surface preparation

A horse was treated at a veterinary clinic a severe leg severe injury caused by
15 barbed wire. The wound was initially pre-cleaned to remove dirt, scrubbed with chlorhexidine for 5 to 7 minutes and then wiped down with alcohol swabs.

(4) Sharp surgical debridement:

The veterinarian aggressively debrided the wound. This step created a relatively
clean wound bed and generated an inflammatory signal that induced the migration and
20 proliferation of stem cells and growth factors (from the amniotic material and horse's own immune system). Systemic antibiotics were administered prior to starting the procedure.

(5) Treatment of an open wound with Amniotic tissue wrap

The veterinarian determined the amount of amniotic material needed to treat the
wound by measuring the maximal length, width and depth of the wound. Amniotic tissue
25 wrap of the appropriate size was selected and applied on the wound. Treatment of an open wound with Amniotic tissue wrap and a cellular suspension of cryofractionated amnion and isolated amnion fluid cells.

After the application of the amnion tissue wrap as described above, the approximate volume of the open wound volume was estimated and the amount of cryofractionated amnion and isolated amnion fluid cells to be injected were determined in accordance with guidelines shown in Table 3..

5 TABLE 3: Amount of cryofractionated amnion and isolated amnion fluid cells to be injected as a function of wound volume.

Wound Area	Amount of cryofractionated amnion / amnion fluid cell suspension to use
<12.5 cm ²	1.0 mL
>12.5 cm ² but <25 cm ²	1.5 mL
> 25 cm ²	2.0 mL

After debridement, the cryofractionated amnion / amnion fluid cell suspension was injected into the peri-wound skin that appeared normal in texture, turgor, and free of infection using a 23-gauge needle on a 3 mL syringe.

10 The cell suspension was injected at a distance of approximately 5 mm from the wound edges and 10 to 15 mm deep at the 3-, 6-, and 9- and 12-o'clock positions (directly into the superficial fascia and the subcutaneous tissue of the wound), aiming the needle parallel to the wound margin at each location. To minimize discomfort and to ensure an adequate spread of the amniotic material, the cryofractionated amnion / amnion fluid cell
 15 suspension was mixed with 1% plain lidocaine in a 1:1 ratio. After the completion of the procedure, the surgical site was dressed with a nonporous dressing followed by application of a nonstick dressing and a dry sterile dressing of gauze. Five to seven days after implant of the amnion allograft, the wound was redressed, and standard wound care consisting of saline wet to dry sterile gauze dressing was resumed. If the wound proved hard to heal,
 20 additional applications of the amnion tissue wrap and/or cryofractionated amnion / amnion fluid cell suspension were made using the same technique at 14- to 21-day intervals until the wound healed. Concomitant therapies were given i.e. wound offloading, and/or edema control as recommended by the veterinarian.

(6) Healing of wound

25 The wound was assessed and showed striking improvement. Generally, treatment shows 2.5 times the rate of healing.

EXAMPLE 6: TREATMENT OF EYE INJURIES WITH THE AMNION TISSUE WRAP

(1) Animal Restrain

A horse was adequately restrained as described in Example 4.

5 (2) Eye surface preparation

Corneal ulcers are the most common eye problem in horses. A veterinarian located the foreign material and determined the wound severity (size of the corneal lesion) using visual inspection or fluorescein dye to highlight any subtle corneal defect or scratch. The area around the eye was cleaned to remove debris or excessive liquid using sterile gauze.

10 (3) Application of the amniotic tissue wrap

An appropriate size of amniotic tissue wrap (ie. 15 or 22 mm diameter discs) was selected. The dehydrated amniotic tissue wrap was positioned over the tip of the index finger and applied directly to the eye surface while opening the eye lids with the other hand (if needed). The membrane attached to the eye surface by hydrostatic tension (glue or
15 sutures may be used to assist to insure placement in challenging environments.).

EXAMPLE 7: TREATMENT OF EYE INJURIES WITH A SUSPENSION OF CRYOFRACTIONATED AMNION

(1) Animal Restrain

A horse was adequately restrained as described in Example 4.

20 (2) Eye surface preparation

The eye surface and surrounding area was prepared as described in Example 6.

(3) Application of eye drops of cryofractionated amnion

Eye drops were given either alone or in conjunction with the amniotic tissue wrap application described in Example 6. Vials of cryofractionated amnion suspended in saline
25 (ie. PlasmaLyte A™ and/or Amniotic Fluid). were thawed (36 to 37°C or 97 to 98°F water bath) for at least 5 min. The vial was gently rotated upside down to mix solution. 2 drops of the suspension were applied to the injured eye twice a day (4 drops total per day) over the membrane or ocular surface. Treatment lasted for a week or until the suspension was

used up. The suspension was pH balanced to prevent any irritation to the eye.

The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and
5 accompanying figures. Such modifications are intended to fall within the scope of the invention described herein.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows

1. An article of manufacture comprising:

(i) a particulate mixture of mechanically decellularized and dried fetal tissue; wherein the particulate mixture is obtained from one or more animals compatible with a subject animal;

(ii) fetal cells obtained from the one or more animals that are compatible with the particulate mixture; and

(iii) an air dried fetal tissue wrap obtained from the one or more animals compatible with the subject animal, the fetal tissue wrap constructed and arranged for placement in juxtaposition with a site of injury, wherein the air dried fetal tissue wrap is dried at a temperature between 65° F and 70° F, wherein the air dried fetal tissue wrap obtained comprises amnion tissue,

wherein combining the particulate mixture and the fetal cells forms a medicament comprising a particulate cellular suspension for administration to the subject animal,

wherein the subject animal is a non-human animal.

2. The article of manufacture of claim 1, wherein the fetal tissue and/or fetal cells comprises amnion.

3. The article of manufacture of claim 1, wherein the fetal tissue comprises amnion.

4. The article of manufacture of any one of claims 1 to 3, wherein the particulate mixture is obtained from the mechanical decellularization of $1.5-5 \times 10^{-2} \text{ cm}^3$ of amnion/mL of fetal tissue.
5. The article of manufacture of any one of claims 1 to 4, wherein the particulate mixture comprises particles greater than 100 microns in diameter.
6. The article of manufacture of any one of claims 1 to 4, wherein the particulate mixture contains particles of less than 100 microns in diameter.
7. The article of manufacture of any one of claims 1 to 6, wherein the particulate cellular suspension comprises $0.8-1.2 \times 10^6$ amnion fluid fetal cells/mL of the particulate cellular suspension.
8. The article of manufacture of any one of claims 1 to 7, wherein a surface of the fetal tissue wrap is in contact with a mesh.
9. The article of manufacture of claim 8, wherein the fetal tissue wrap is free of chorion.
10. The article of manufacture of any one of claims 1 to 9, wherein the medicament is formulated for administration to the subject animal at a site of injury that exposes a movable

body element of the animal's musculoskeletal system, wherein the movable body element is a muscle, joint, ligament, or tendon, wherein the injury comprises a laceration.

11. The article of manufacture of claim 10, wherein the medicament is formulated for administration to the subject animal at a site of injury caused by a chondral pathology, a fascia pathology, a tendon pathology, a ligament pathology, a surface reconstruction pathology, or an articular joint pathology.

12. The article of manufacture of any one of claims 1 to 11, wherein the fetal cells comprise mesenchymal cells at a concentration between 10^3 to 10^{12} /mL.

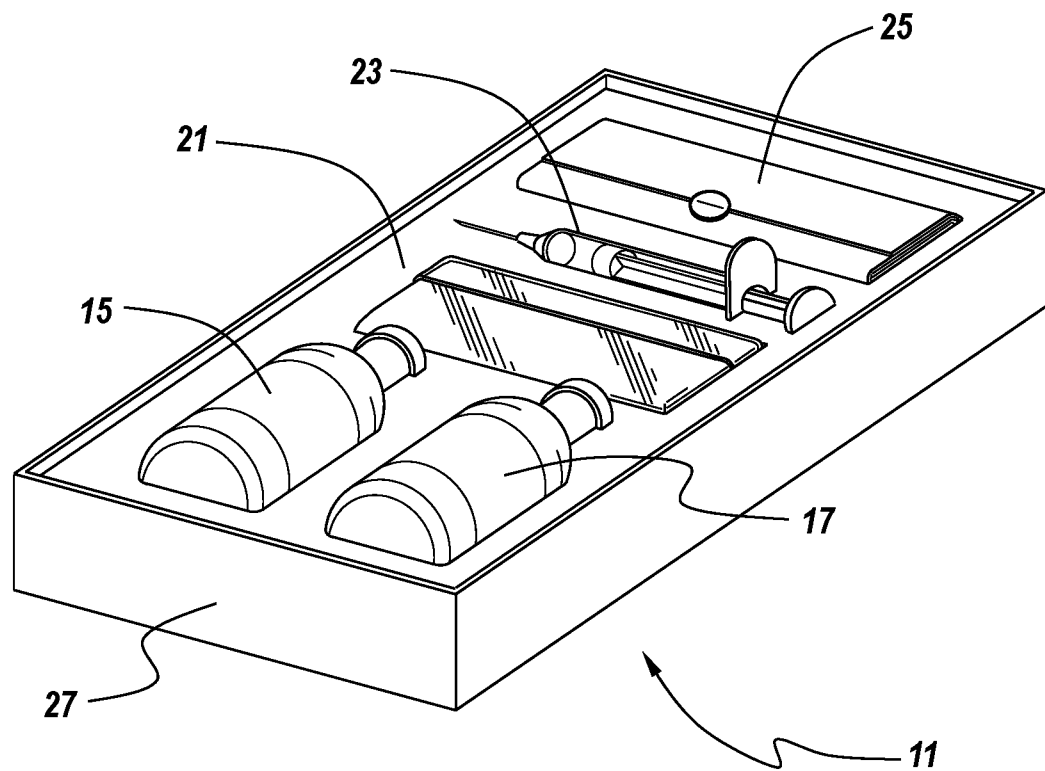
13. The article of manufacture of any one of claims 1 to 11, wherein the fetal cells comprise epithelial stem cells at a concentration between 10^3 to 10^{12} /mL.

14. A method of obtaining a particulate mixture from animal fetal tissue, comprising:
(i) decellularizing at least a portion of the animal fetal tissue by cryofractionation;
and
(ii) drying the decellularized fetal tissue to form the particulate mixture.

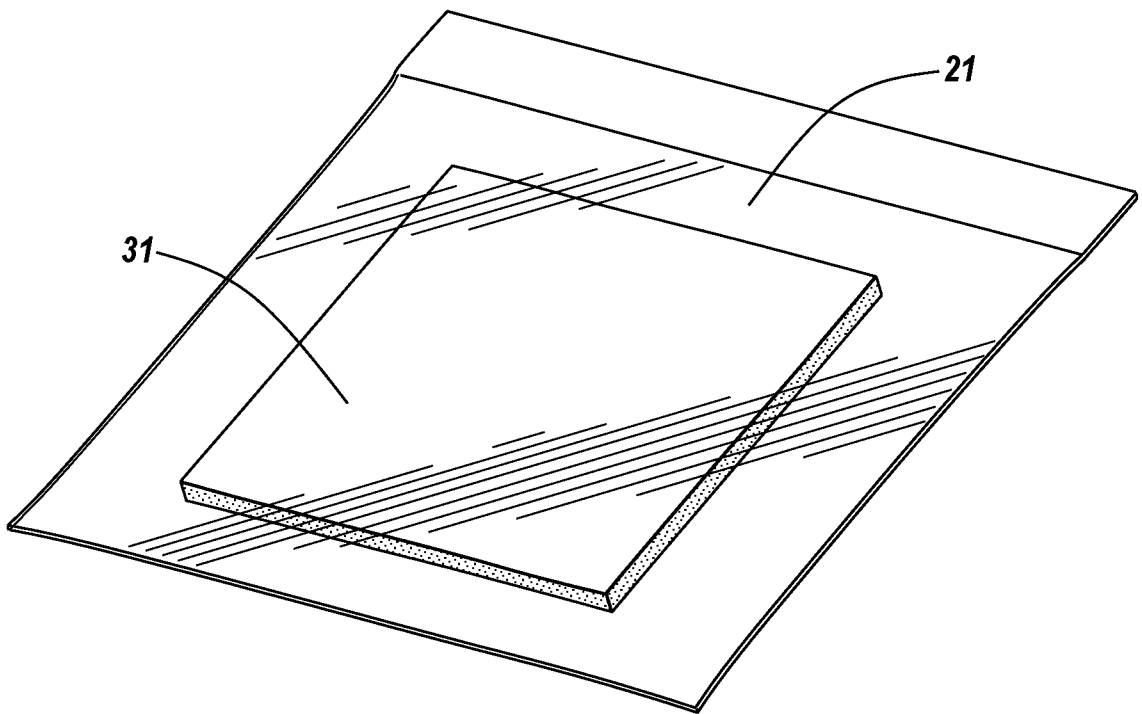
15. The method of claim 14, further comprising reconstituting the particulate mixture through adding a biocompatible solution to the particulate mixture, wherein adding the biocompatible solution creates a reconstituted particulate mixture comprising fetal cells.

16. The method of claim 14 or 15, wherein the cryofractionation comprises grinding the animal fetal tissue at a temperature below 0°C in at least two cycles separated by at least three minutes.
17. The method of any one of claims 14 to 16, wherein the fetal tissue comprises amnion fluid.
18. The method of any one of claims 14 to 17, wherein the fetal tissue comprises fetal cells comprising mesenchymal cells at a concentration between 10^3 to 10^{12} /mL.
19. The method of any one of claims 14 to 17, wherein the fetal tissue comprises fetal cells comprising epithelial stem cells at a concentration between 10^3 to 10^{12} /mL.
20. The method of any one of claims 14 to 17, wherein the cryofractionation produces particles of about 0.5 cm^2 .

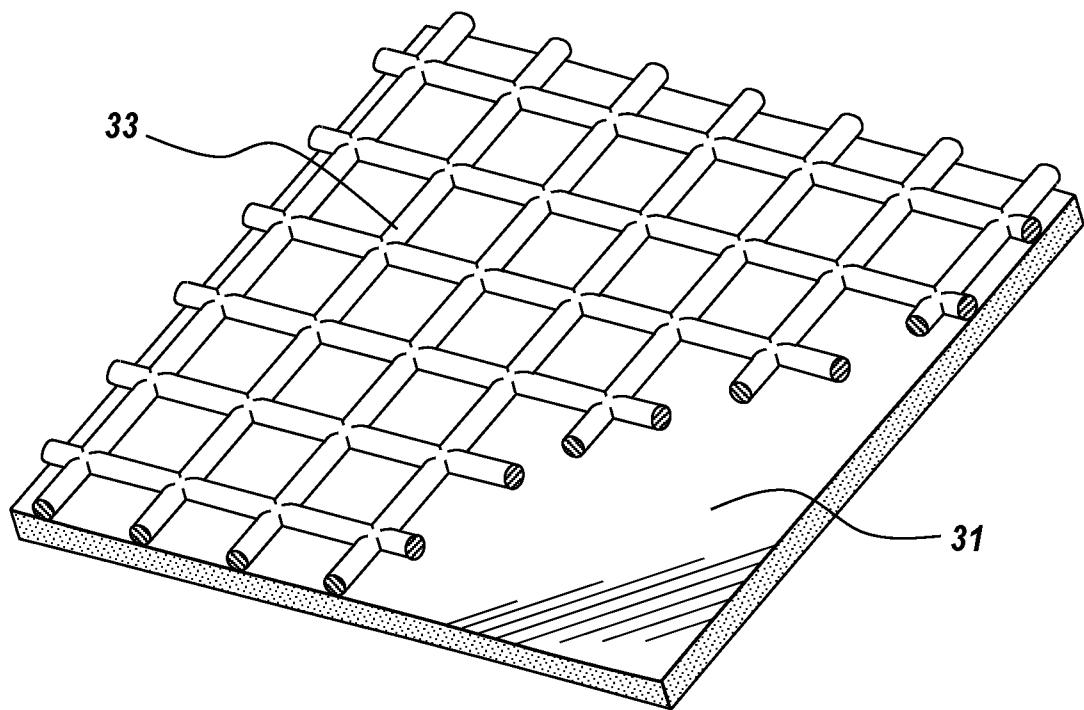
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**Fig. 1**

2/3

**Fig. 2**

3/3

**Fig. 3**

