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(54) PARTICULATE MATERIALS FOR TISSUE MIMICS

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(52) **U.S. Cl.**

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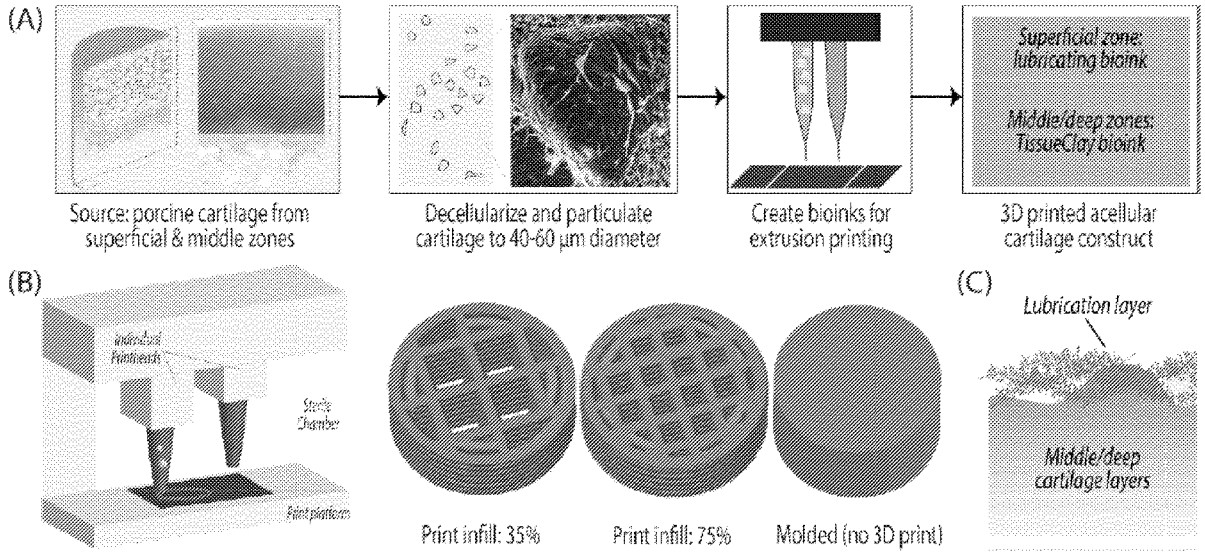
(57) **ABSTRACT**

A two-component biomaterial and method that replicates both the structural complexity and diverse molecular composition necessary to create a tissue's form and function. It is an objective of the current invention to use the unique combination material and methods herein to provide a pharmaceutical composition, a medical device, a tissue regeneration scaffold, as well as a scaffold for 3D organ culture (tissue on a chip, lab grown meat, research stem cell differentiation) comprising a significant amount of acellular tissue particles packed tightly and held together via cross-linking between the acellular particles and a thiolated protein.



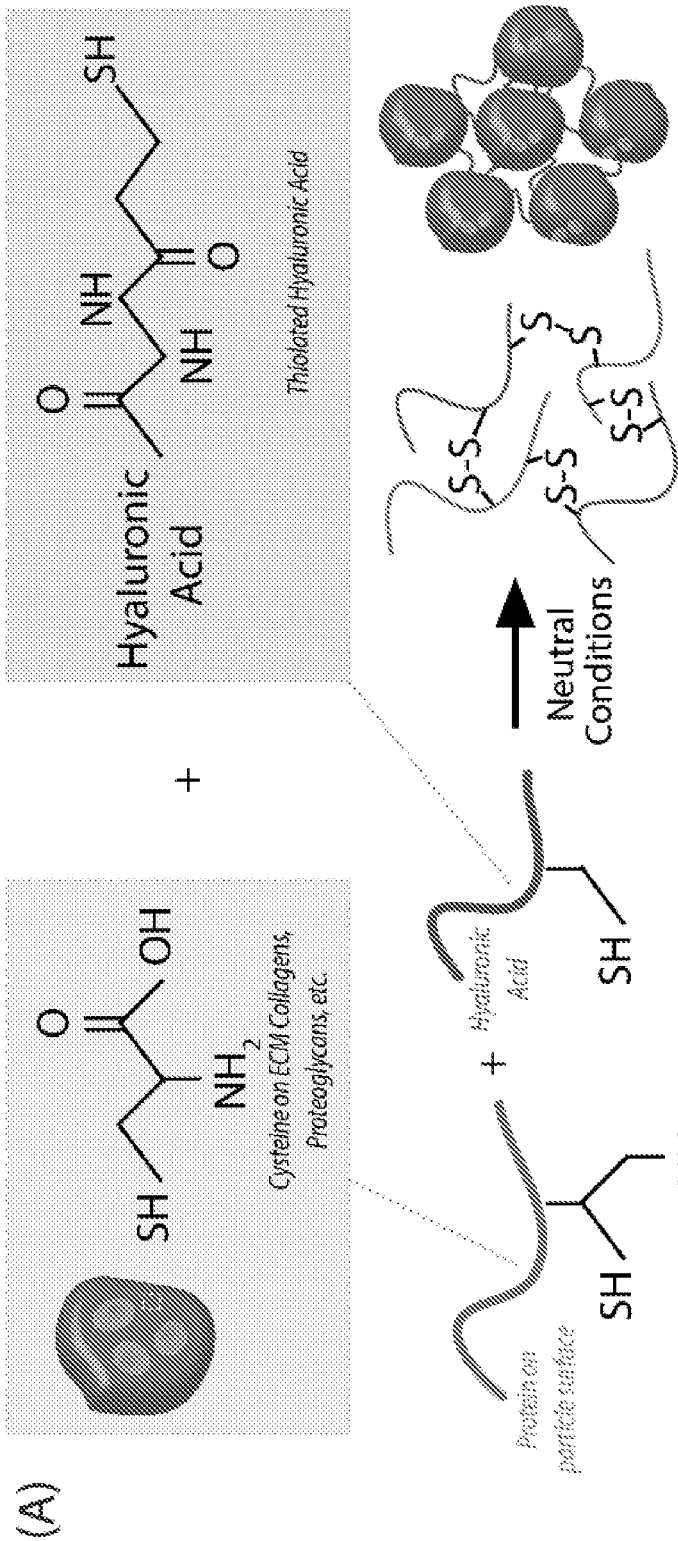

(A)
Cysteine on ECM Collagens, Proteoglycans, etc.
Thiolated Hyaluronic Acid
Hyaluronic Acid
Neutral Conditions
Protein on particle surface
Hyaluronic Acid

FIG. 1

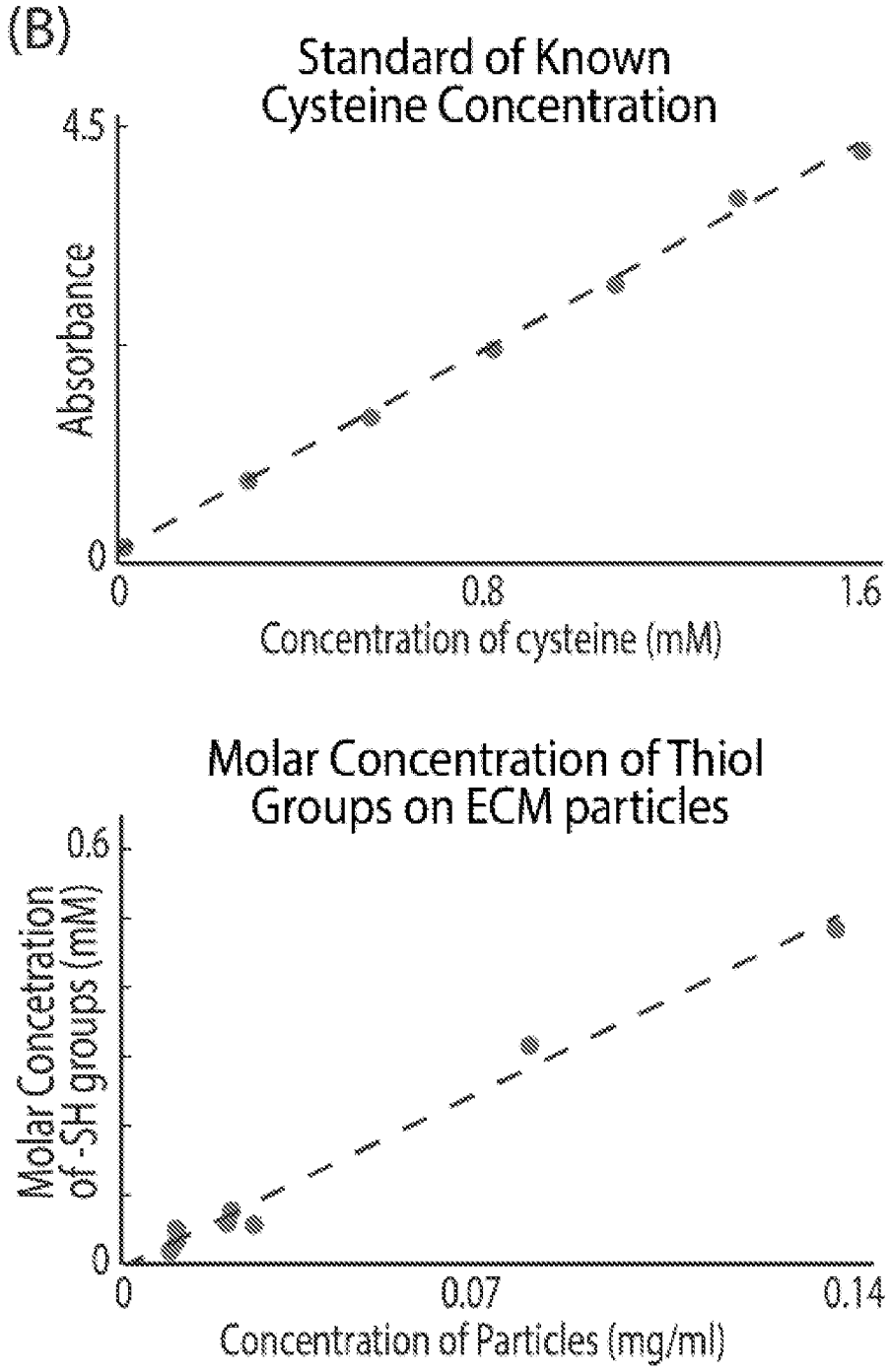


FIG. 1 - continued

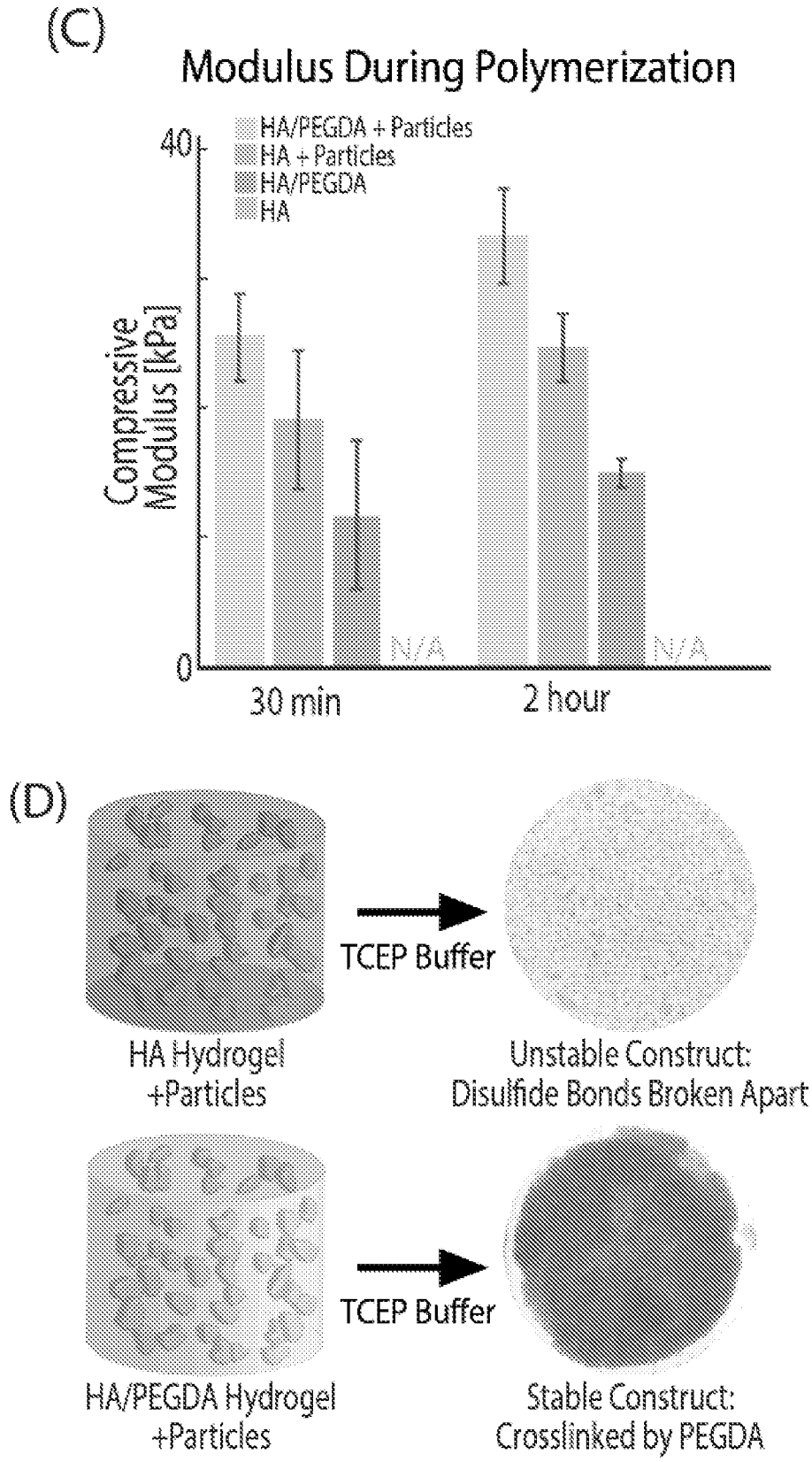


FIG. 1 - continued

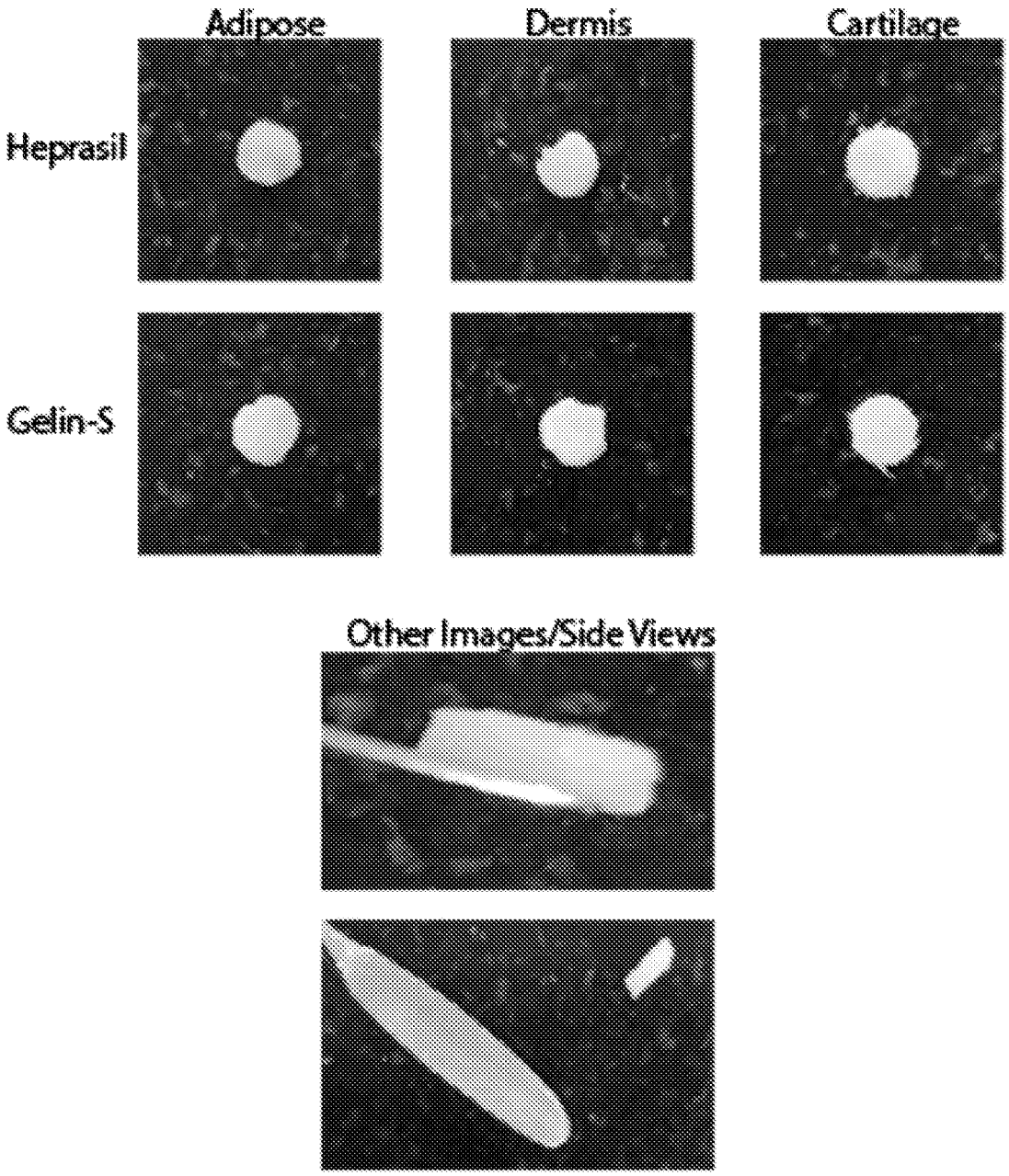

Adipose
Dermis
Cartilage
Heprasil
Gelin-S
Other Images/Side Views

FIG. 2

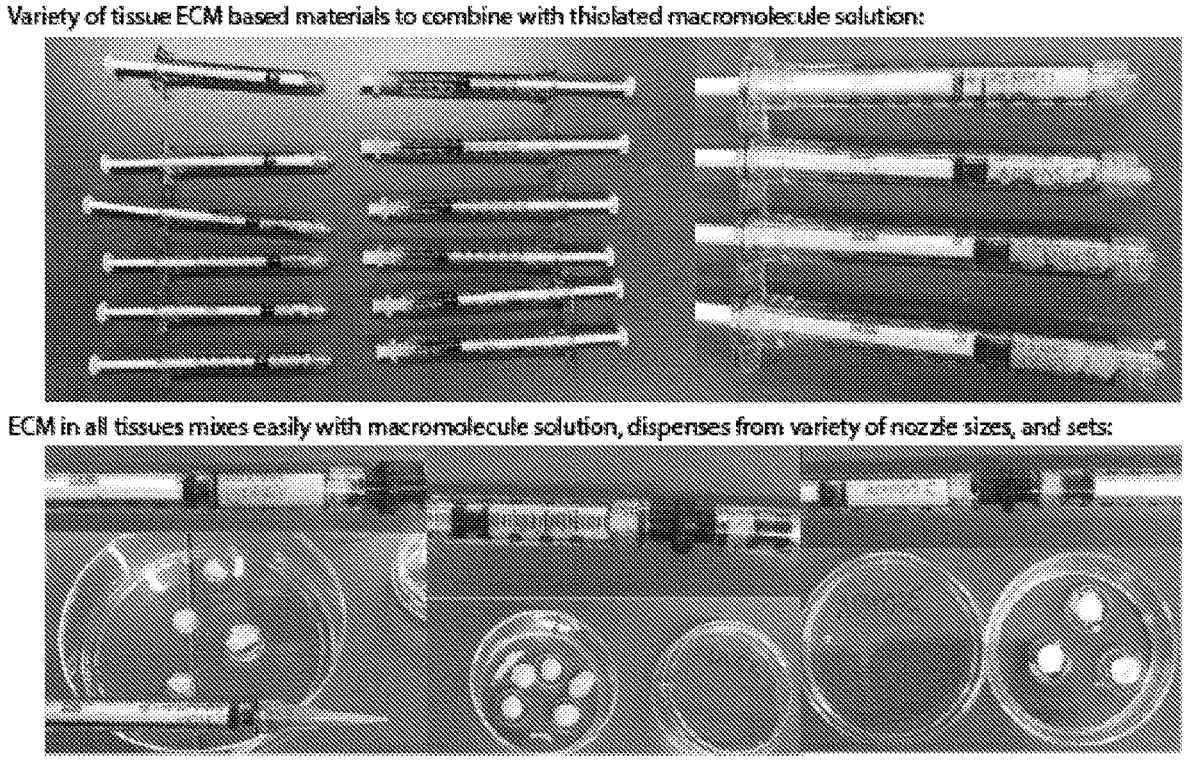

Variety of tissue ECM based materials to combine with thiolated macromolecule solution:
ECM in all tissues mixes easily with macromolecule solution, dispenses from variety of nozzle sizes, and sets:

FIG. 3

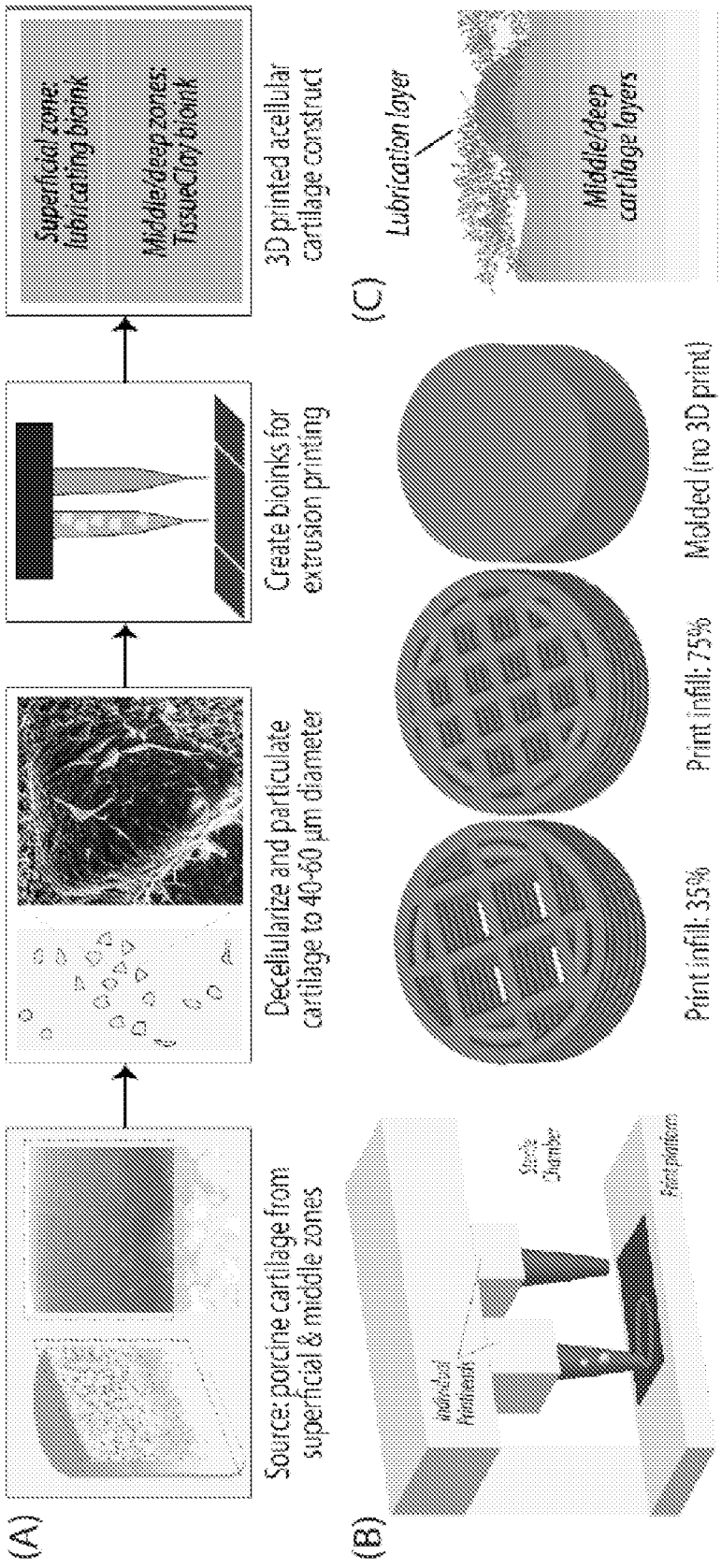
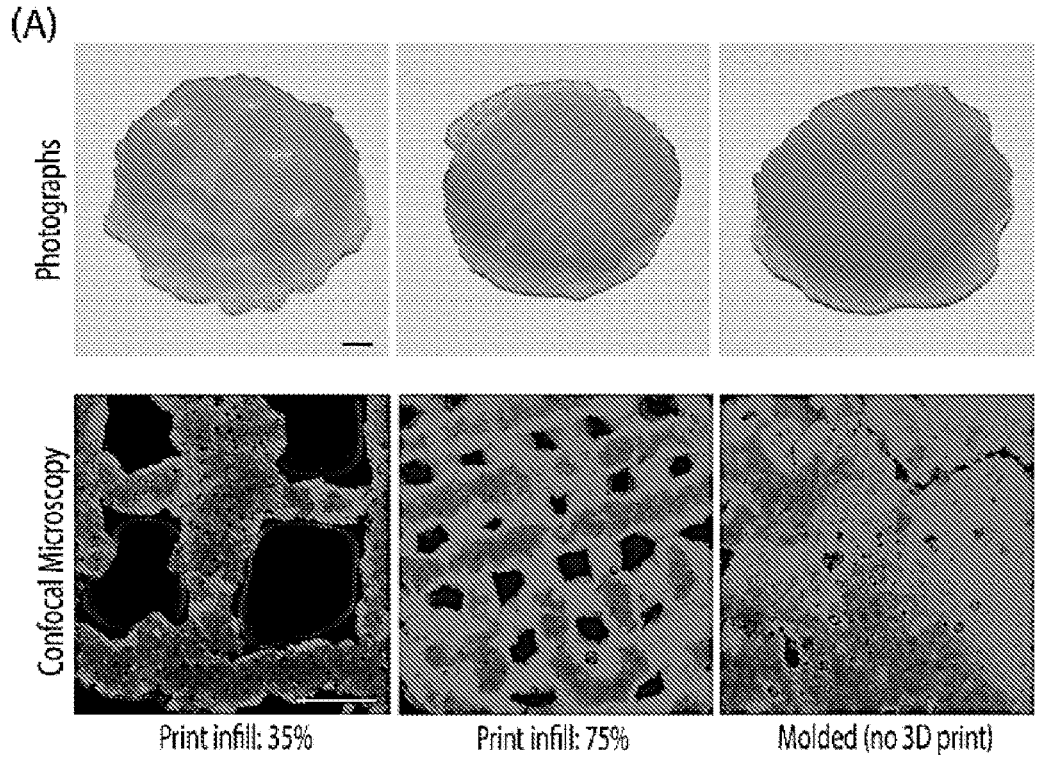
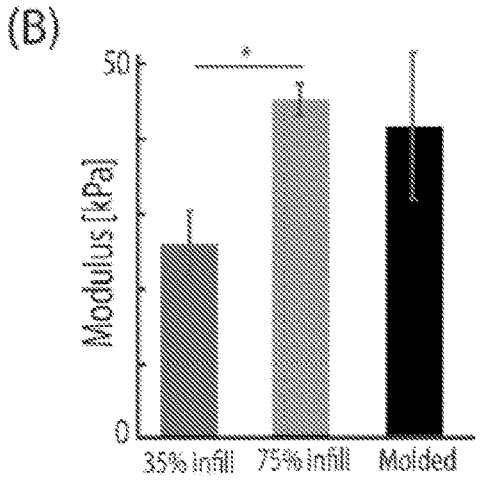

(A)
Source: porcine cartilage from superficial & middle zones
Decellularize and particulate cartilage to 40-60 μm diameter
Create bioinks for extrusion printing
Superficial zone: lubricating bioink
Middle/deep zones: TissueCity bioink
3D printed acellular cartilage construct
(B)
Print infill: 35%
Print infill: 75%
Molded (no 3D print)
(C)
Lubrication layer
Middle/deep cartilage layers

FIG. 4


(A)
Photographs
Confocal Microscopy
Print infill: 35%
Print infill: 75%
Molded (no 3D print)


(B)
Modulus (kPa)
50
0
35% infill
75% infill
Molded

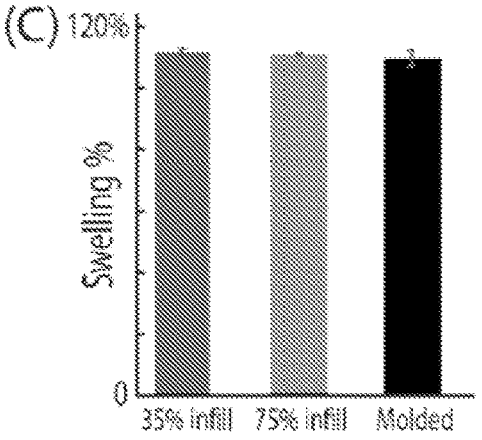

(C)
Swelling %
120%
0
35% infill
75% infill
Molded

FIG. 5

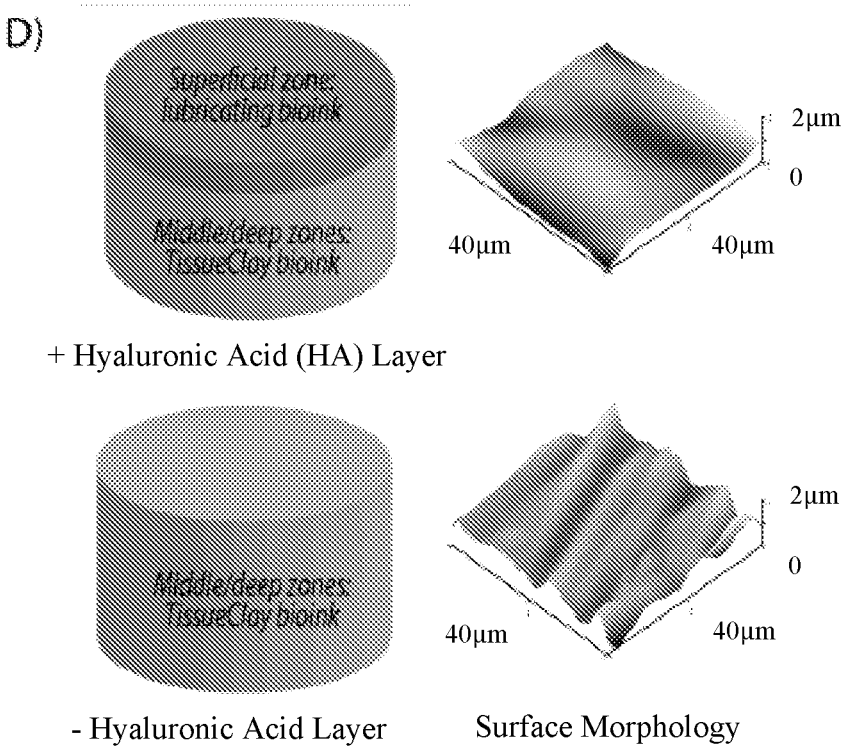


FIG. 5 - continued

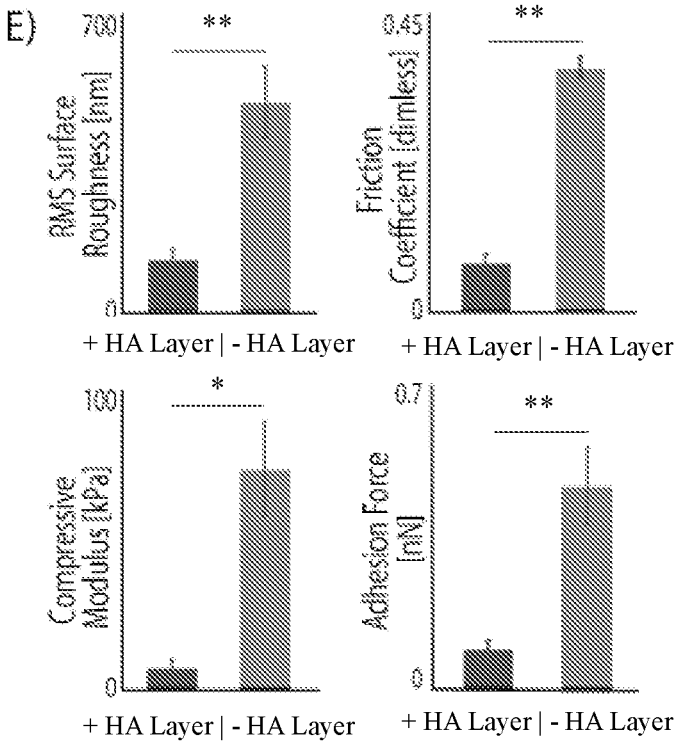


FIG. 5 - continued

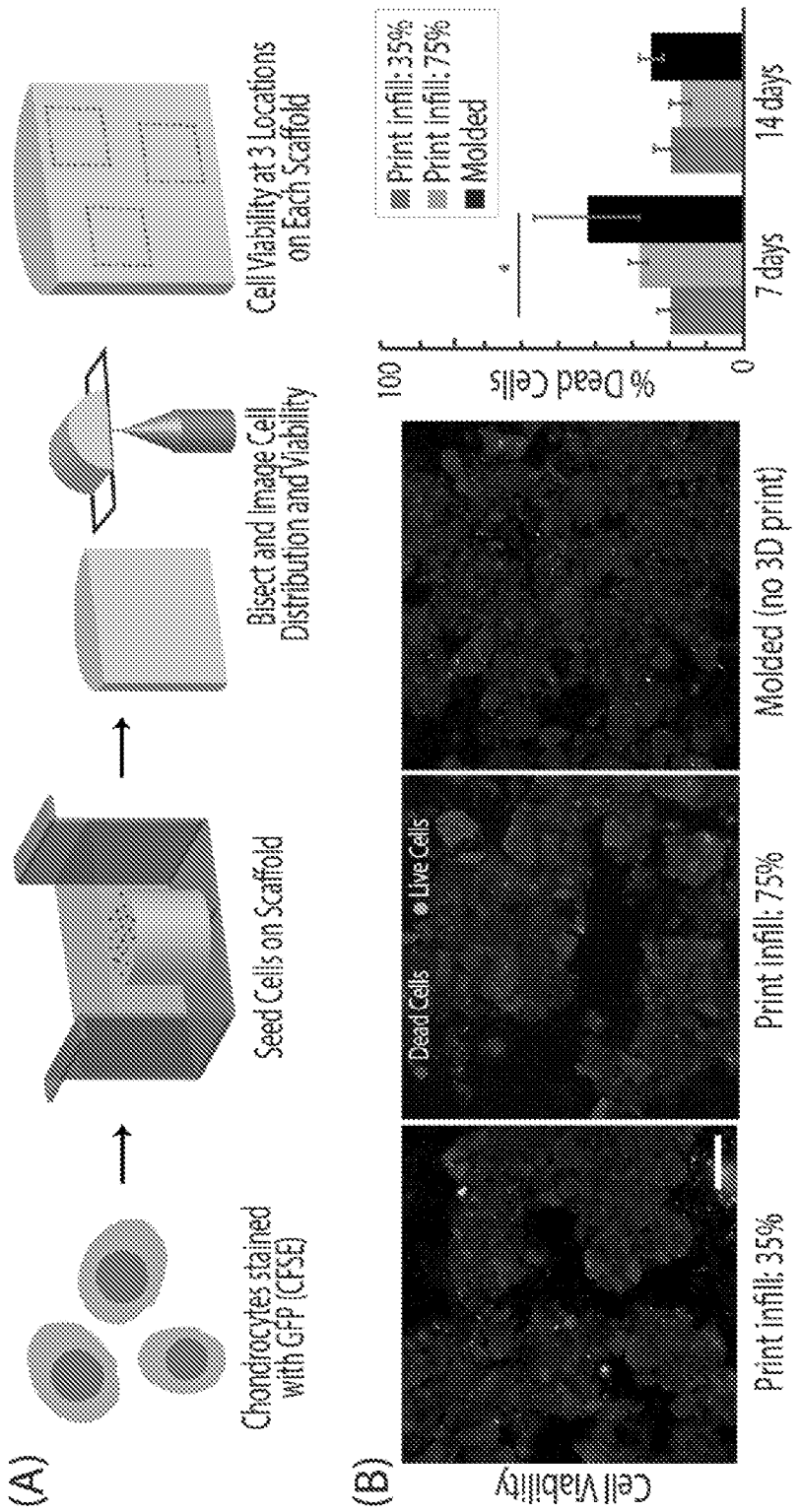

(A)
Chondrocytes stained with GFP (CFSE)
Seed Cells on Scaffold
Bisect and Image Cell Distribution and Viability
Cell Viability at 3 Locations on Each Scaffold
(B)
Cell Viability
Dead Cells
Live Cells
Print infill: 35%
Print infill: 75%
Molded (no 3D print)
% Dead Cells
100
0
7 days
14 days
Print infill: 35%
Print infill: 75%
Molded

FIG. 6

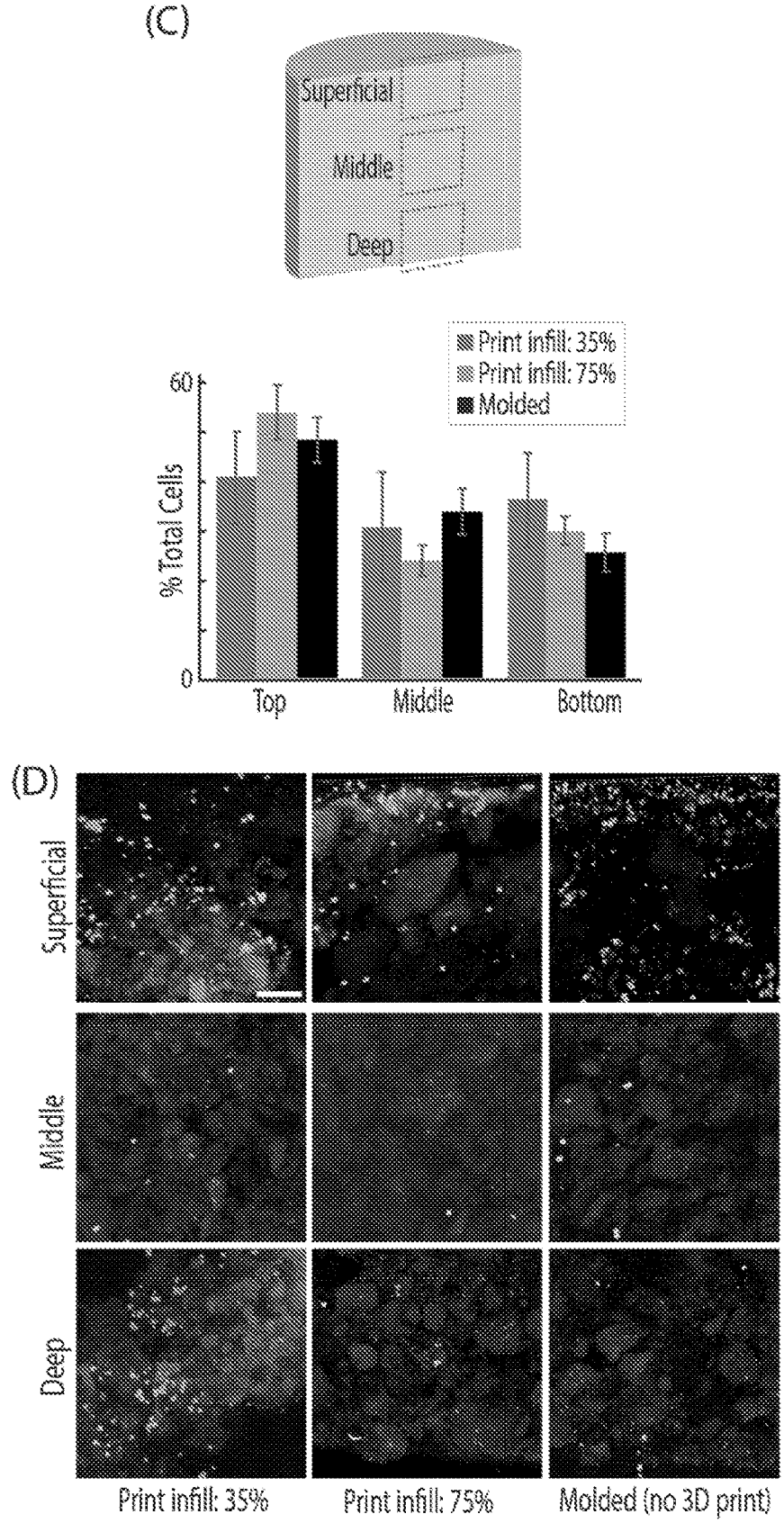


FIG. 6 - continued

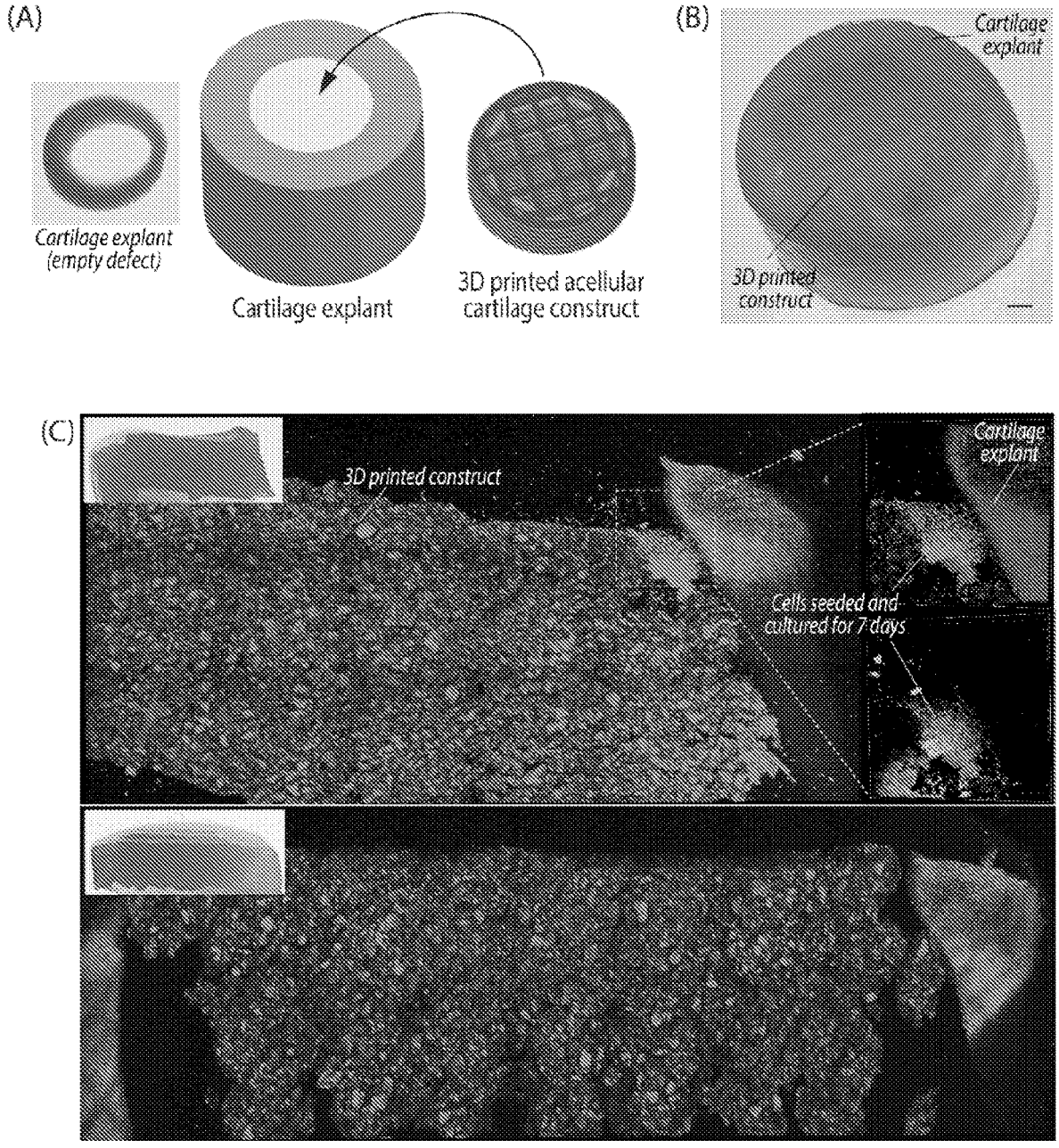

(A)
Cartilage explant (empty defect)
Cartilage explant
3D printed acellular cartilage construct
(B)
Cartilage explant
3D printed construct
(C)
3D printed construct
Cartilage explant
Cells seeded and cultured for 7 days

FIG. 7

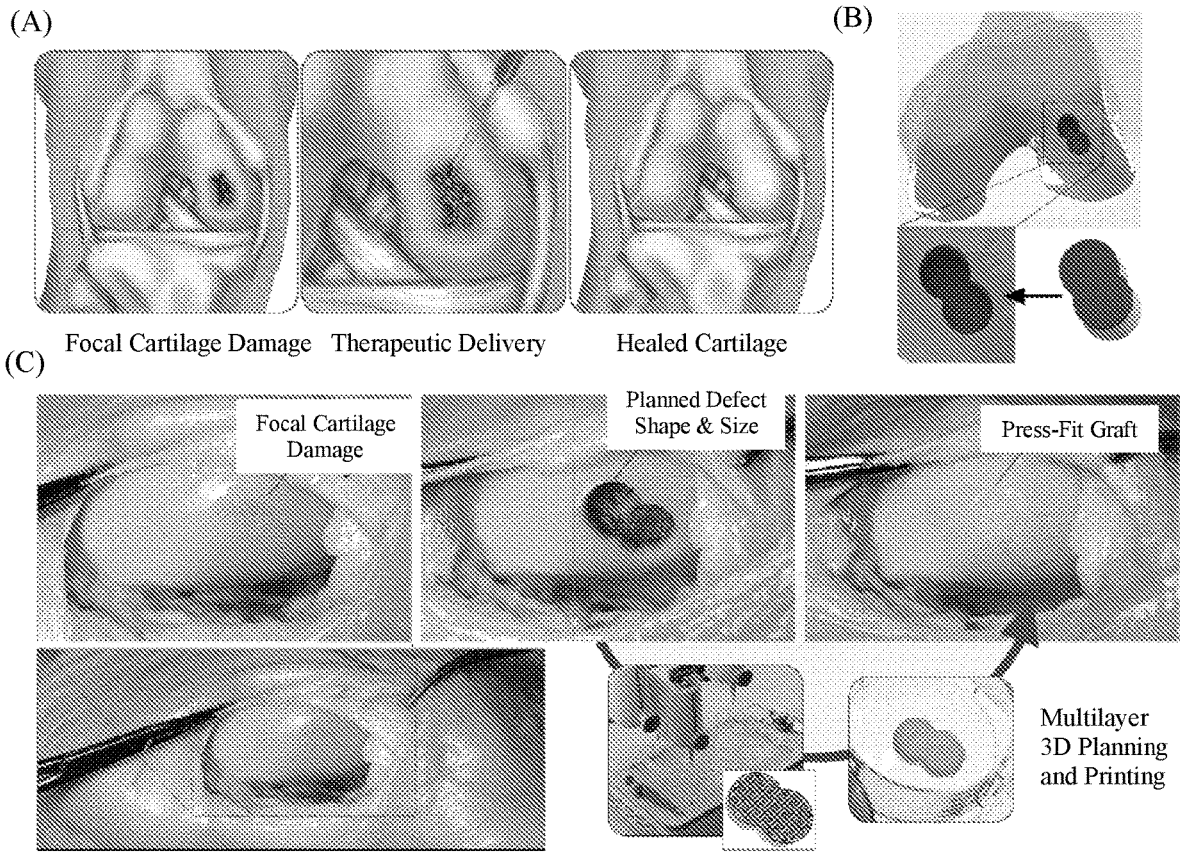

(A)
(B)
Focal Cartilage Damage
Therapeutic Delivery
Healed Cartilage
(C)
Focal Cartilage Damage
Planned Defect Shape & Size
Press-Fit Graft
Multilayer 3D Planning and Printing

FIG. 8

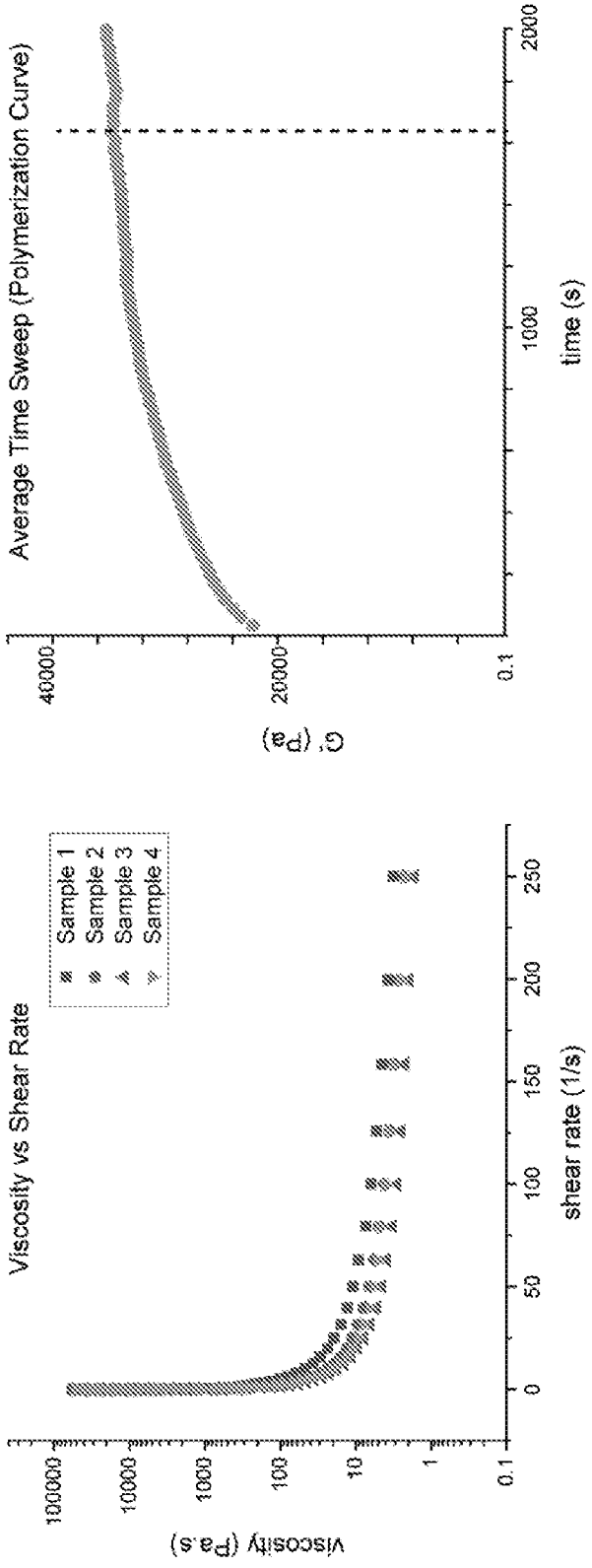

Average Time Sweep (Polymerization Curve)
G' (Pa)
40000
20000
0.1
1000
2000
time (s)
Viscosity vs Shear Rate
Sample 1
Sample 2
Sample 3
Sample 4
Viscosity (Pa.s)
100000
10000
1000
100
10
1
0.1
0
50
100
150
200
250
shear rate (1/s)

FIG. 9

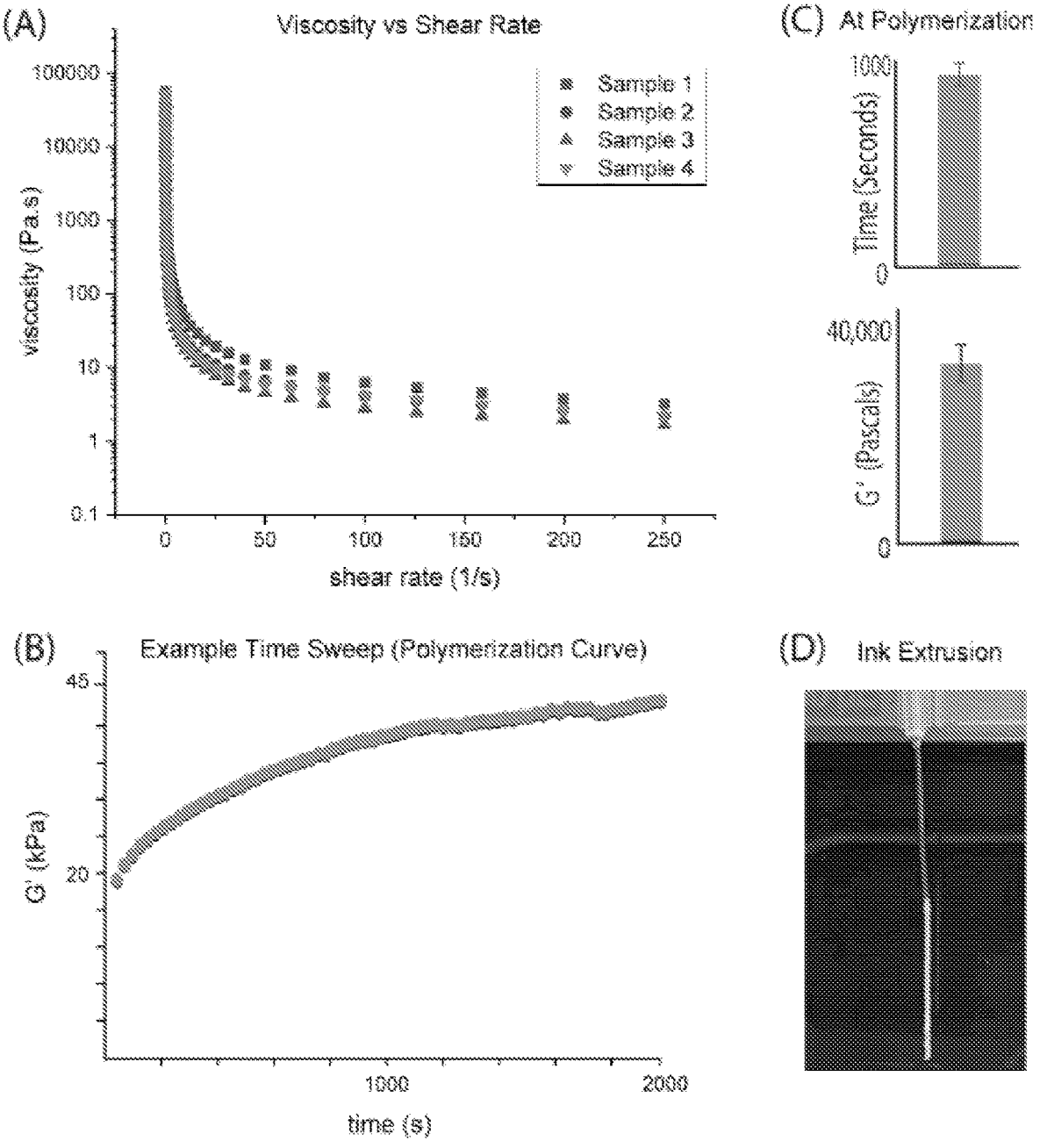

(A)
Viscosity vs Shear Rate
Sample 1
Sample 2
Sample 3
Sample 4
viscosity (Pa.s)
100000
10000
1000
100
10
1
0.1
0
50
100
150
200
250
shear rate (1/s)
(C) At Polymerization
Time (Seconds)
1000
0
G' (Pascals)
40,000
0
(B)
Example Time Sweep (Polymerization Curve)
G' (kPa)
45
20
1000
2000
time (s)
(D) Ink Extrusion

FIG. 10

PARTICULATE MATERIALS FOR TISSUE MIMICS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/125,280, filed Dec. 14, 2020, U.S. Provisional Application No. 63/263,228, filed Oct. 28, 2021, and U.S. Provisional Application No. 63/264,564, filed Nov. 24, 2021.

FIELD OF INVENTION

[0002] This invention relates to methods and devices for tissue engineering. More specifically, this invention relates to an extracellular matrix tissue particle-based biomaterials and bioinks that can be used to create or 3D print layered tissues such as an articular cartilage scaffold.

BACKGROUND OF THE INVENTION

[0003] The extracellular matrix of biological tissues is a dynamic environment that supports macroscale structure and protection for cells under physiological loading, while also providing microscale cellular contact for proliferation, differentiation, and receptor signaling. The specific structure and composition of both healthy and engineered extracellular matrix determines mechanical behavior and can guide cellular responses.

[0004] Biological tissues provide essential load bearing functions throughout the body. There are many remaining challenges in regenerative medicine, as some tissues like articular cartilage demonstrates an inability to repair following injury [Mow, V. C., Ateshian, G. A. & Spilker, R. L. Biomechanics of diarthrodial joints: a review of twenty years of progress. *J Biomech Eng* 115, 460-467 (1993)]. Tissues with high extracellular matrix content and low cell density require unique tissue engineering designs and approaches for robust and integrative repair solutions.

[0005] Healthy biological tissues are often composed of heterogenous and distinct tissue layers. For example, in the case of articular cartilage, each layer has a unique ratio of the two major extracellular membrane proteins; collagen and aggrecan. Each layer also has different cell densities. The composition of each cartilage layer and the interplay between layers allows articular cartilage to provide excellent joint lubrication and facilitates load transmission during normal movement.

[0006] Successfully replicating biological tissues with a tissue-engineered construct to repair defects following injury is an elusive challenge, despite many decades of innovation. To enable efficient and effective restoration of tissue defects, there exists a significant need to engineer a replacement scaffolds that mimic the structural properties of tissues and present natural biochemical cues for promoting cell growth and integration [Camarero-Espinosa, S., Rothen-Rutishauser, B., Weder, C. & Foster, E. J. Directed cell growth in multi-zonal scaffolds for cartilage tissue engineering. *Biomaterials* 74, 42-52 (2016)].

SUMMARY OF THE INVENTION

[0007] This invention relates generally to a biomaterial, composed of (1) decellularized and particulated extracellular matrix and (2) thiolated polymers, as well as methods for the production and use of the biomaterial. Specifically, in one aspect the invention relates to treating areas of tissue disease or damage by combining the two biomaterial components and injecting the combined material into the damaged region. The invention can be used as a medical implant/treatment or as a biomaterial for 3D cell culture and/or 3D tissue printing. Methods for manufacturing the individual components, and the combined biomaterial are also provided herein.

[0008] Many tissues are layered with specific and complex heterogenous structures to provide critical functionality of the tissue. Many engineered constructs for in vivo tissue repair, 3D printing, organoid creation, lab on a chip, in vitro analyses, and lab grown meat fail to replicate the mechanics, unique layers, or complexity of a tissue and often rely on exogenous compounds to crosslink the materials into a stable scaffold. To address the need for mechanically robust, complex, and layered scaffolds, an extracellular matrix (ECM) tissue particle-based biomaterial ink has been developed which extrudes easily, polymerizes via disulfide bonding into a mechanically robust construct, and can be layered or used alone to create reliable tissue mimics for 3D organ culture or implantable medical devices for tissue repair. The ECM-based particle bioink, pECM bioink, utilizes functionalized macromolecules combined or packed together with acellular tissue particles. When combined, the two materials crosslink via disulfide bonding to create stable tissue mimics or repair scaffolds.

[0009] In particular, the invention relates to the composite material of functionalized macromolecules that can crosslink by disulfide bonding with acellular tissue particles. The functionalized macromolecules can be natural or synthetic. The tissue particles can be sourced from any animal or human tissue or organ. The sourced tissue particles can be mechanically broken down and decellularized prior to particle functionalization or in combination with the functional macromolecules. The invention also relates to the methods of combining the composite material and using the mixture to create a tissue mimic. There are numerous uses of the biomaterial disclosed herein, including applications for clinical tissue repair, such as an injectable/implantable medical implant (e.g., as repair for cartilage, skin, muscle, bone, kidney, liver, heart), tissue/lab on a chip for testing of pharmaceutical treatments (any tissue type), organoid development using the biomaterial mixed with living cells, as a commercial 3D printed biomaterial ink that can be used in series to create layered tissue mimics, and for labs/companies to answer a variety of questions (i.e., to serve as a model for tissues, disease, regeneration, etc.) needing an ECM based, or 3D culture, tissue system, and finally as a matrix to produce lab grown meat.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

[0011] FIG. **1** is a set of images and two graphs (Labeled (A)-(D)) illustrating the native tissue-based acellular biomaterial that crosslink ECM particles to functionalized macromolecules (here showing thiolated hyaluronan) via disulfide bonding. (A) Scaffolds crosslink by disulfide bond formation between thiolation sites on the macromolecule, in this example thiolated hyaluronan, and open sulfahydryl groups on the tissue particles thereby forming a stable 3D network.

(B) By comparing to known quantities of cysteine, it was measured via Ellman's assay that particles contain sulfhydryl groups to interact with thiolated hyaluronan. (C) The addition of either particles, PEGDA, or both lead to initial cross linking and polymerization within 30 minutes, with further polymerization by 2 hours. The control containing only thiolated hyaluronan remained liquid at 2 hours, demonstrating the role the particles play in increasing the rate of crosslinking. (D) TCEP buffer is a reducing agent which breaks apart disulfide bonds. Incubation in TCEP buffer confirms that hyaluronan (HA)+particle gels crosslink via disulfide bonding.

[0012] FIG. **2** is a set of eight images. Heprasil and Gelin-S are additional types of functionalized macromolecules (in addition to the thiolated hyaluronic acid/hyaluronan shown, for example, in FIG. **1**). Heprasil is thiol-modified heparin/hyaluronic acid mix and Gelin-S is thiolated gelatin. When combined with the acellular microparticles (i.e., adipose, dermis, cartilage), all functionalized macromolecules cross-link with the acellular microparticles into stable and robust scaffolds.

[0013] FIG. **3** is a set of images illustrating that the extracellular matrix can be sourced from a wide variety of tissue types, and then decellularized, and particulated for use as an ECM particle. Once in particle form, all tissues can be combined with functionalized macromolecules (in this example they are functionalized by thiol groups), mixed, and dispensed through any needle/nozzle size (limits depend on the largest size particle embedded in the ink).

[0014] FIG. **4** is a set of illustrations depicting the use of ECM particle biomaterial as a bioink with layered printing for creation of cartilage mimics with mechanically-robust structure and lubrication. (A) In this illustrated process, cartilage tissue is decellularized and particulated to 40-100 μm. The resulting particles are encapsulated in hyaluronan and printed to create a mechanically robust cartilage scaffold. A top lubrication layer composed of only hyaluronan can be printed via a second nozzle to replicate lubrication surface characteristics of cartilage. (B) Scaffolds are printed at two different densities with a 22-gauge printhead tip to demonstrate the role of differentially sized macropores on nutrient diffusion, cell viability, and mechanics. For the purpose of comparison, a third group can be made by molding the scaffold, rather than printing. (C) The lubrication layer can be printed as a superficial layer to the cartilage tissue construct, where the superficial layer is composed of crosslinked hyaluronan, a polysaccharide which plays a key role in lubrication of native articular cartilage.

[0015] FIG. **5** is a set of illustrations, images and graphs demonstrating that, where biomaterial ink is 3D printed, the printing can be used to create layered tissue and specifically use unique acellular tissue inks to create tissue specific layering. When the biomaterial ink is printed via extrusion for articular cartilage, it can be used to create mechanically robust middle and deep zones with a smooth and low friction superficial zone. (A) Incorporating microtissue particles into a hyaluronan ink enables printing of a wide variety of structures. Here, scaffolds were created with a range of macropores: 1 mm, ~400 μm, no pores. (B) The addition of small macropores had no effect on the overall compressive modulus of the scaffold, measured under unconfined compression with a 10 mm flat platen. (C) The incorporation of pores did not change the swelling levels of the constructs, confirming that the swelling depends heavily on the chemical crosslinking of the ink rather than the shape of the construct. (D and E) Printing a superficial zone of HA decreased the surface roughness, friction, and adhesion significantly (surface measured on the AFM with a 2 μm spherical probe). * denotes $p<0.05$, ** denotes $p<0.001$, two-way ANOVA followed by Tukey's honest significant difference test. Scale bar in (A) is 1 mm.

[0016] FIG. **6** is a set of illustrations, images and graphs demonstrating that, where biomaterial ink is 3D printed, the printing can be used to introduce a tissue scaffold with macropores. Increasing porosity in constructs led to improved cell distribution and viability. (A, B) Chondrocytes are extracted from bovine knee joints, stained with a fluorescent proliferation dye (CFSE), and seeded on top of all constructs. The percentage of dead cells was measured by quantification of CFSE (live) or ethidium homodimer (dead) stained cells present 7- and 14-days post seeding. (C, D) Chondrocyte distribution throughout the scaffold was measured by quantification of CFSE stained cells at three tissue depths: superficial, middle, and deep after 7 days of culture. *Denotes $p<0.05$, two-way ANOVA followed by Tukey's honest significant difference test.

[0017] FIG. **7** is a set of illustrations and images showing that the biomaterial can be designed to be used in in situ to provide a 3D scaffold for studying tissue regeneration, repair, or barrier interactions/integration. The biomaterial can be designed and printed to fill a specific tissue defect and in the cartilage example shown, after 7 days the printed construct integrates and adheres to the surrounding native tissue. (A, B) Custom tissue defects are measured and inputted into CAD software to create a construct custom designed to fit the defect. Extrusion printing is used to create the custom construct and immediately introduced to the defect for polymerization. (C) After 7 days, constructs were bisected and imaged for direct observation of scaffold integrity and integration with native tissue. Scaffolds adhere to the native tissue, even with the forces exerted during bisecting. Additionally, CF SE stained cells (green—light gray in gray-scale), which were introduced after polymerization, collect where the native tissue and scaffold meet.

[0018] FIG. **8** is a set of illustrations and images showing that the 3D printing of ECM particle bioink is tunable for a wide variety of shapes and sizes and shows potential for clinical on-demand applications. (A) For clinical applications, it is essential to develop a material that can be applied to patient-specific defects, which can vary in terms of size and extent of focal cartilage damage within a complex joint. (B, C) With the ECM particle bioink, it is demonstrated that a custom defect shape can be measured, applied to a 3D CAD model, and printed via extrusion to create a multilayered construct suitable for implantation. Here, planning of a two-layer cartilage construct, using tissue particles for enhanced tissue structure in addition to an engineered lubrication layer, is printed and applied immediately to repair a joint defect.

[0019] FIG. **9** is a pair of graphs showing that the ECM particle bioink can be evaluated for printability in numerous ways. FIG. **9** shows the polymerization time (A) and shear thinning behavior (B) of the ECM particle bioink when using cartilage tissue, size 40-100 μm, and 20% thiolated hyaluronan. The rheological properties were measured using a Flow Procedure with a steady state flow step setting on Rheometer (AR-G2, TA Instruments). Experiments were carried out using parallel plates configured with 8-mm

diameter plates in stiff bearing mode at room temperature with a gap distance of 800 μm. The shear sweep was measured as a function of shear rate from 0.01 to 200 s^{-1} with no normal force, a pre-shear rate of 10 for 10s, and zero velocity. The time sweep was performed with parallel plates set at 37° C.

[0020] FIG. **10** shows the pair of graphs as shown in FIG. **9** (see FIGS. **10**(A) and **10**(B), along with two additional graphs (FIG. **10**(C) showing the modulus at polymerization of the ECM particle bioink scaffold and the time it takes to reach a crosslinked state (for the graph to flatten) and FIG. **10**(D) showing an image of the ink extrusion out of a 22 gauge nozzle used for 3D printing to demonstrate the smoothness and consistency of the extruded ink.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0021] A successful bioscaffold functions as a structural support for both cell integration and mechanical integrity. Ideally, an implantable bioscaffold is a substrate for cell attachment and proliferation, enables sufficient diffusion of nutrients and waste products through the scaffold, mimics native structural heterogeneities, and mechanically responds to normal loading on the specified tissue. Therefore, a decellularized tissue extracellular matrix (ECM) is a highly advantageous scaffold for tissue repair because it provides the same structural support as the native tissue, while additionally presenting familiar biological cues to enable cell invasion and integration from surrounding tissue (in vivo) or from introduced cells to the scaffold (in vitro).

[0022] However, in practice, the application of an intact whole decellularized tissue for tissue defect repair is limited by shape constraints and can be limited by high native tissue density (depending on the tissue needing to be replicated). The ECM from decellularized donor tissue can be broken down into small particles, and then fabricated into a composite material by densely packing particles in a hyaluronic acid/PEGDA crosslinked hydrogel to create a regenerative model for cartilage repair. The dense packing of particles closely recapitulates mechanical properties of large decellularized tissue, but also significantly improves cellular infiltration into the scaffold. While hydrogels packed with ECM particles are a promising advancement in scaffold technology, it is critical to produce a scaffold that also mimics the distinct layered structure of tissues (e.g., articular cartilage), and uses a crosslinking chemistry that does not depend on the Michael addition reaction between functionalized hyaluronic acid and PEGDA diacrylate groups.

[0023] The present invention addresses problems with prior bioscaffolds by creating a scaffold that uses a crosslinking interaction between functionalized macromolecules (natural or synthetic) and tissue particles directly.

[0024] Advanced manufacturing techniques, especially 3D printing, provide several advantages for tissue engineering and bioscaffold fabrication. A wide variety of applications utilize extrusion-based printing, enabling the tunability of the extrusion size, use of a wide range of printable materials, the capacity to create constructs with unique layers, and the ability to print custom shapes and sizes. Prior attempts have involved extrusion-based printing of conventional synthetic polymers to create tissue engineered scaffolds, as these polymers provide enhanced engineering control. Unfortunately, synthetic polymers alone generally fail to fully mimic the structure or function of most natural tissues. Alternatively, natural polymers, often composed of proteins occurring in abundance in the body such as hyaluronan, alginate, and collagen, have been used for engineered 3D printed tissue applications. Unfortunately, most natural polymer-based hydrogels rely on UV light and other extra photoinitiators to crosslink and form a stable scaffold, which additionally do not recapitulate the complex interplay between the proteins and polysaccharides found in most native tissue. To increase the polymer complexity of natural hydrogels, researchers have developed extracellular matrix-based bioinks by digesting decellularized tissue into liquid (monomeric) form. [J. S. Choi, H. J. Yang, B. S. Kim, J. D. Kim, J. Y. Kim, B. Yoo, K. Park, H. Y. Lee, Y. W. Cho, *Journal of Controlled Release* 2009, 139, 2.] In these procedures, proteases digest the tissue and break down the rigid ECM structure, which liquifies the tissue. Unfortunately, the tissue digestion also destroys the protein organization that provides the tissue with its unique structure, ECM architecture, protein interactions, and cellular attachment sites; essential components to creating an ideal regenerative tissue environment.

[0025] Here, a material composition of an acellular tissue-based biomaterial has been developed that can be injected into a defect or damaged region in a patient (human or animal) or be used as a 3D printing biomaterial resin (pECM bioink) to create architecturally robust tissue mimics for 3D organ culture or tissue-on-a-chip applications. In one embodiment, the biomaterial is sourced from purely tissue-based and natural extracellular matrix components. In other embodiments, the natural tissue matrix is crosslinked with functionalized synthetic polymers. The method and material composition taught herein leverages the sulfhydryl groups on cysteines of decellularized tissue to crosslink with functionalized macromolecues, creating disulfide bonds between the two biomaterial ink components. Unlike the biomaterial inks formed from complete tissue digestion, acellular microparticles are produced via mechanical pulverization, resulting in ECM microparticles that preserve the micro mechanical and biological properties of the original tissue. The pulverized particles can be densely packed in a hydrogel solution of functionalized macromolecules and injected/applied to any shape defect or printed via extrusion. Using techniques employed such as in viscous clay printing, a printed ECM particle biomaterial ink was developed. We demonstrate that printing with ECM particle biomaterial ink enabled the fabrication of layered tissues and the ability to print macro pores in the tissue mimics. Additionally, we illustrate the simplicity in printing and implanting custom scaffolds into a patient using a layered, and lubricated, articular cartilage scaffold printed to a custom shape and implanted into a defect.

[0026] The present invention provides tissue extracellular matrix particles obtained from a tissue or organ from an animal or human. The extracellular matrix particles are mechanically pulverized to a selected size. In various embodiments, the particles can be 0-100 μm, 100-250 μm, or 250-500 μm. The particles are decellularized using chemical means to remove the cellular components of the tissue, lyophilized, and size sorted into the different size categories. The extracellular matrix particles after decellularization include native growth factors, attachments sites for cells, which will promote cellular interaction, and regeneration cascades, and complex tissue-specific architecture.

[0027] The particles are combined with a non-ECM particle component: a polymer (either natural or synthetic, preferably natural) which is functionalized by thiolation (i.e., to have open thiol (—SH) groups), and dissolved for easy mixing with the particles. In different embodiments, the percentage of the molecular groups on the macromolecule which are functionalized, or broken apart to have open thiol groups, can fall into one of the following categories: 0-15%, 15-30%, and/or 30-60%. The functionalization percentage dictates the crosslinking density of the combination material, where a higher level of cross-linking results in smaller pores/lower material porosity and stiffer materials.

Example 1—Materials and Methods—Particulate Tissue Extracellular Matrix Crosslinked with Thiolated Polymers Creates Scaffold

[0028] Sourcing Tissue ECM:

[0029] Tissue ECM is most often obtained from an animal or a human after a terminal procedure, but can also be obtained during a procedure involving tissue replacement or tissue extraction (e. liposuction). Tissue is harvested either freshly after harvest (within 48 hours of sacrifice) or is sourced as frozen tissue (which was flash frozen within 48 hours of sacrifice or harvest). Tissue is collected from the tissue of interest using a scalpel and/or dissection scissors. It is critical to not harvest the tissue in a region where it meets a different type of tissue (at the edges) to ensure that only the desired tissue type is harvested. After harvest, tissue is cut into ~1 cm 2 pieces, prior to flash freezing and storing or tissue grinding. Tissue can be sourced from xenogenic, allogenic, or autogeneic sources and can be stored as tissue from a single source (e.g., for personalized medicine or an autograft), or can be pooled with other samples of that tissue type for more generalized use.

[0030] Tissue Grinding:

[0031] Tissue is ground at two stages of the fabrication process: Stage 1. After extraction from the tissue or organ (while it is still composed of cellular contents) and Stage 2. After decellularization and lyophilization. At stage 1, flash frozen tissue pieces are enclosed in flash frozen stainless steel grinding jars with a free floating stainless steel sphere. The grinding jars with tissue are pulverized at a high rate of vibration (e.g., 30 cycles/second on a TissueLyser II grinder) for 30 seconds-1 min. At stage 2, after decellularization, lyophilized acellular cartilage tissue particles are pulverized further in stainless steel grinding jars (room temperature grinding jars and tissue) at the same rate (e.g., 30 cycles/second on a TissueLyser II grinder) for 60 seconds. Following the second pulverization, particles are size sorted using a vibrational sieve (e.g., one supplied by Endecott) into distinct sizes: <40 μm, 40-100 μm, 100-250 μm, and 250-500 μm.

[0032] Decellularization:

[0033] Microparticles are decellularized using a sequence of chemical and enzymatic means. After the first bulk tissue grinding, cells are broken down and stripped away from the tissue by first submerging in 2% Sodium Dodecyl Sulfate (SDS) for 8 hours under agitation at 37° C. Particles are then rinsed overnight in 1×Phosphate Buffered Saline to remove the SDS. Optionally, tissue particles can then be submerged in an enzymatic treatment (e.g., 0.1% DNase for 3 hours) under slow agitation at 37° C. to further breakdown leftover cellular and genetic material. Microparticles are rinsed in PBS, flash frozen in liquid nitrogen, lyophilized, and stored frozen at −80° C. until the second round of grinding (detailed above). The extracellular matrix is stored substantially free of cells and can be described as a collection of small particles. These particles, in combination with functional macromolecules, may be used for research purposes or sterilized, stored, and processed into an implant.

[0034] Functionalization of Polymers:

[0035] Numerous polymers can be functionalized to have open —SH groups that can perform disulfide bonding with —SH groups on tissue ECM. One example of macromolecule functionalization is the thiolation of a hyaluronic acid molecule. For this example, glucoronate carboxyl groups on hyaluronan (HA) were replaced with thiol groups following previously established protocols [D. Eng, M. Caplan, M. Preul, A. Panitch, *Acta Biomaterialia* 2010, 6, 2407]. Briefly, hyaluronan (MW 100 kDa, Lifecore Biomedical) was dissolved at 10 mg/ml in degassed milliQ water. Dithiobis propanoic dihydrazide (DTP) (Frontier Scientific) was added to the solution, the pH was lowered between 4.5 and 4.75, and then (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (ThermoFisher) was added to begin the reaction. The pH was maintained between 4.5 and 5 for 50 minutes. After 50 minutes, the reaction was stopped by raising the pH above 7. Next, dithiotreitol (DTT) (Fisher Scientific) was added, the pH was raised above 8.5, and the solution was stirred for 24 hours at room temperature. After 24 hours, the pH of the solution was lowered, the liquid transferred to dialysis tubing (10 kDa membrane cutoff, Spectrum Labs) and the solution was dialyzed in an HCl solution supplemented with 100 mM sodium chloride for 8 solution changes, then in an HCl solution without supplements for 4 solution changes. Dialysis was completed in a sealed chamber with continuous nitrogen gas bubbling into the HCl solution. With each batch of HA thiolation, the substitution rate can be confirmed to be in the defined invention ranges of 0-15%, 15-30%, or 30-60% (thiolated mmols/unthiolated mmols) using a standard Ellman's assay following manufacturer's protocol (Ellman's solution, ThermoFisher).

[0036] Combine with Functionalized Polymer:

[0037] Size sorted, and acellular lyophilized tissue particles are combined with a functionalized polymer (either purchased or prepared as described above, and either natural or synthetic) to create the composition biomaterial. Lyophilized and functionalized polymers (see above), dissolve easily when introduced to 1×PBS, and are dissolved to create an aqueous solution of twice the desired polymer concentration (for example, 20 mg/ml of macromolecule) which is neutralized to a pH of 7 and loaded into a syringe, taking care to remove any bubbles that formed in syringe loading. In a separate syringe, acellular size sorted tissue particles are mixed with 1×PBS at twice the desired final ratio (for example, 0.4 g/ml). Once ready to combine (e.g., for device delivery in a defect, for tissue bioprinting, or for 3D cell culture), syringes are attached to each other using a custom luer lock connector with cross hairs across the opening to facilitate mixing. Solutions are syringed back and forth approximately 30 times until mixing is complete and the material runs smoothly through the luer lock mixing connector. The final mixture should have a macromolecule concentration roughly half of what each individual component had (in the example throughout, it would be 10 mg/ml and particle concentration of 0.2 g/ml).

Example 2—Therapeutic and R&D Use

[0038] The invention provides acellular tissue particles (either sourced from one tissue or multiple tissues and combined) crosslinked to thiolated polymers to repair and provide a regenerative scaffold for defective, damaged, diseased, or missing tissues or components of an organ in a subject in need (either animal or human subject). In certain embodiments, the material composition may be used to treat defective or damaged organs which include, but are not limited to, articular cartilage, muscle, skin, ligaments, subcutaneous adipose beneath skin, kidney, and liver.

[0039] In particular, the biomaterial composition may be used to treat diseases, conditions, or damage that are associated with bulk tissue loss and that may benefit by a structurally mature regenerative tissue scaffold to immediately fill a volume, provide mechanical function of the damaged tissue, and promote cells to migrate into the material, attach, and proliferate in a regenerative cascade. Such diseases would include, but are not limited to facial wasting syndrome, articular cartilage damage due to injury, osteoarthritis, ulcers, large or deep burns and/or wounds, breast reconstructions, volumetric muscle loss, and impaired vocal folds.

[0040] In certain embodiments, the material composition may be used as a biomaterial ink extruded using 3D printing techniques. Applications for using this material for 3D printing would include, but are not limited to, tissue-on-a-chip, tissue mimics, 3D cell culture, personalized medicine, personalized treatment testing, drug toxicity testing, and barrier tissue crosstalk research.

Example 3—Medical Devices

[0041] The biomaterial composition can be formulated as a medical device for injection and/or permanent implantation into a subject. The medical device would use the solubilized polymer as a carrier for the extracellular matrix tissue particles to supply the material to a region of need. More specifically, the biomaterial composition will be applied to a region of defect or damage to promote regeneration and/or provide structural stability in the damaged region.

[0042] In some embodiments, the polymer can also be mixed with external growth factors, region specific anti-inflammatory drugs, or other necessary active and FDA-approved pharmaceutical agents (e.g., TGF β, IGF-1, PRP, corticosteroids, etc.), prior to being mixed with the extracellular matrix particles and injection to the desired site. The ingredients of the compositions of the invention can be supplied packaged separately and mixed prior to administration.

[0043] Thiolated polymers used to compose the biomaterial composition should be biocompatible and avoid irritation or systemic reactivity to native tissue/host. Furthermore, it is possible to combine multiple functionalized polymers (for example, hyaluronan and collagen) with the extracellular matrix particles, if desired. In preferred embodiments, a single type of functional polymer is used.

Example 4—Tissue Regeneration Scaffold

[0044] One important aspect and application of the invention is the use of extracellular matrix particles combined and crosslinked with thiolated polymers to create an ideal scaffold for tissue regeneration. In an advantageous embodiment, the scaffold is injected into an area of the body where it crosslinks and adheres to surrounding native tissue via activated functional groups on the macromolecule. The growth factor remnants in the extracellular matrix particles encourage cell migration and attachment. As the cells in the tissue and the surrounding tissue begin to multiply and/or lay down new extracellular matrix, the gaps between the tissue particles are filled in with newly grown tissue. In another embodiment, the biomaterial composition described herein can be formed in sterile conditions in vitro. Cells can be seeded in the scaffold slurry before or after crosslinking. The cellular scaffold can be maintained and cultured until it reaches a desired tissue maturity, at which point the regenerative scaffold can be implanted into the patient (e.g., as an advancement to procedures such as matrix-assisted autologous chondrocyte implantation, where tissues or cells must be cultured outside of the patient.)

Example 5—Cell Culture Substrate

[0045] Many cells require or exhibit enhanced growth on substrates other than tissue culture plastic to grow and retain proper function outside their native tissue environments. In particular, cells which rely on their surroundings and external signaling for differentiation require non-plastic substrates (e.g., stem and pluripotent cells). In certain embodiments, the biomaterial composition taught herein can be used for cell culture of a variety of cells. The tissue source of the particles will dictate the specific extracellular matrix environment of the cell culture, potentially providing signals to differentiate stem cells or maintain cells in a tissue specific phenotype. In certain embodiments, cells can be plated on the surface of the crosslinked biomaterial invention described herein. In other embodiments, the cells can be encapsulated in the thiolated polymer, mixed with the particles as described, and plated in a mold for a 3D culture environment.

Example 6—Printing for Personalized Medicine or 3D Cell Culture

[0046] CAD designs for the specific defect shape or desired 3D culture model should be loaded in the 3D printer, and the use should adjust infill density accordingly to the application. The freshly mixed biomaterial ink can be loaded into printing cartridges and the print should be started within 5-10 minutes of mixing. The ink should be kept at 4° C. to prevent preliminary crosslinking, and the print should take no more than 30 minutes.

Example 7—Scaffold for Lab Grown Meat Alternatives

[0047] In certain embodiments, the material composition may be used as a tissue specific substrate to grow meat or meat substitutes artificially in the lab. The material can be used to differentiate cells into tissue specific cells (e.g., muscle) and, as such, the scaffold promotes new tissue matrix production and potential to produce meat alternatives.

Example 8 (Part 1)—Cartilage Scaffold with Hyaluronic Acid Crosslinked to Hyaline Cartilage Particles

[0048] Cartilage microparticles, which were decellularized, morsilized, and lyophilized, were encapsulated in

thiolated hyaluronic acid (HA) hydrogels at a ratio of 0.2 g/ml, via mixing of two groups: 1. Lyophilized particles in PBS and 2. Functionalized HA, dissolved in PBS and neutralized to pH 7. HA had previously been thiolated to 22%, and dissolved easily when introduced to sterile DPBS. The hydrogels were incubated at 37° C. for 30 minutes to facilitate disulfide bond crosslinking of the open sulfide groups on the cysteine of particles with the thiol groups on the functional HA molecules to form a stable 3D structure. It is contemplated that incubation temperatures can be varied around a roughly optimal temperature of 37° C. (e.g., 32° C. to 42° C.). For example, decreasing temperatures, such as to room temperature, will require increased incubation times.

Example 8 (Part 2)—Kidney Proximal Tubule Tissue Mimic

[0049] In another example, to print a kidney proximal tubule model, kidney from a porcine source was used to make freshly prepared kidney pECM bioink (e.g., including thiolated HA), particle sizes <100 μm. The ink was printed using direct ink writing on a sterile multi-nozzle extrusion printer (BioX, Cell Ink Life Sciences), in parallel with a sacrificial ink which was used to print the tubule channels. ECM particle bioink was extruded using a pneumatic print cartridge and a 22-gauge nozzle into a custom PDMS mold. Ink was extruded with 40-60 kPa pressure (adjusted in real time to ensure print consistency between layers) at a rate of 5 mm/s. The ink was set to pre-flow and post-flow for 30 ms before and after print steps. Nozzles were calibrated prior to printing to ensure alignment of the multiple printheads and unique inks. After printing and cross-linking, scaffolds are cooled to liquefy the sacrificial inks, and perfusion is used to remove the liquid.

Example 8 (Part 3)—3D Print-Layered Bone-Cartilage In Vitro

[0050] In a further example, to print an osteochondral (bone and articular cartilage) scaffold to fill a custom knee defect, the following procedure was followed: pECM bioink (e.g., decellularized cartilage particles in PBS solution mixed with thiolated HA solution) using either bone or cartilage extracellular matrix, freshly prepared, was printed using direct ink writing on a sterile multi-nozzle extrusion printer (BioX, Cell Ink Life Sciences). 3D print designs were created in open-source CAD software (OnShape), following the measurements of the defect in the injured knee and transferred to the 3D printer for segmenting. ECM particle bioink was extruded using a pneumatic print cartridge and a 22-gauge nozzle. Ink was extruded with 70-90 kPa pressure (adjusted in real time to ensure print consistency between layers) at a rate of 2 mm/s. The ink was set to pre-flow and post-flow for 30 ms before and after print steps. Ink was printed with a 75% infill using a grid pattern. For the top lubrication layer, the superficial zone ink was printed with 3-10 kPa of pressure and a speed of 20 mm/s with no pre or post flow. Nozzles were calibrated prior to printing to ensure alignment of the multiple printheads and unique inks. Immediately post-printing, the construct was inserted into the defect region to promote polymerization post-implantation, a key process for native tissue integration. See, for example, FIG. **8**C.

Example 8 (Part 4)—3D Print Layered Scaffold In Situ

[0051] In another example, the same procedure as example 8, part 3 is followed, with printing directly into the knee defect, and polymerized (37° C., 30 minutes; 18 minutes to crosslink based on rheology, but incubated 30 minutes to allow for minor differences in environment) in the joint using all sterile components, preparation, and surgical suite.

Example 8 (Part 5)—Dermis Scaffold with Gelatin Crosslinked to Dermis Particles

[0052] In another example, porcine dermis tissue was ground into small pieces, which were decellularized, morsilized, and lyophilized. The decellularized particles were combined with a Gelin-S (sourced from Advanced Biomatrix), which is a form of thiolated gelatin, at a ratio of 0.2 g/ml, via mixing of two groups: 1. Lyophilized particles in PBS and 2. Gelin-S, dissolved in PBS and neutralized to pH 7. The hydrogels were incubated at 37° C. for 30 minutes to facilitate disulfide bond crosslinking of the open sulfide groups on the cysteine of particles with the thiol groups on the gelatin molecules to form a stable 3D structure composed of dermis ECM and collagen I, with extracellular matrix components that would provide for healthy skin following wound repair.

Example 8 (Part 6)—Bone or Cartilage Scaffold with Heparin Sulfate

[0053] In another example, porcine bone and hyaline cartilage tissue was ground into small pieces, which were decellularized, morsilized, and lyophilized. The decellularized particles were combined with Heprasil (sourced from Advanced Biomatrix), which is a combination of thiolated heparin sulfate and thiolated hyaluronon, at a ratio of 0.2 g/ml, via mixing of two groups: 1. Lyophilized particles in PBS and 2. Heprasil, dissolved in PBS and neutralized to pH 7. The hydrogels were incubated at 37° C. for 30 minutes to facilitate disulfide bond crosslinking of the open sulfide groups on the cysteine of particles with the open thiol groups in the Heprasil solution. Scaffolds were created in this way for both bone and hyaline cartilage tissue engineering applications. The particle-filled solution can be easily applied to either bone or cartilage defects (using bone or cartilage decellularized tissue, respectively) and creates an environment for osteogenesis or chondrogenesis of the surrounding native pluripotent cells.

[0054] The present invention provides a composition of the material components to be utilized in creating a tissue mimic and a combined material/tissue mimic. Furthermore, the present invention provides methods of combining and forming the composition material and methods of using the combination material to mimic tissue in a variety of application cases including, but not limited to, pharmaceuticals, medical devices, 3D printing biomaterial inks, and tissue specific organ cultures.

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Definitions

[0068] As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

[0069] A "safe and effective amount" refers to the quantity of a component that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention.

[0070] As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[0071] The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[0072] The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

[0073] Other than in the operating examples, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages such as those for amounts of materials, times and temperatures of reaction, ratios of amounts, values for molecular weight (whether number average molecular weight ("M_n") or weight average molecular weight ("M_w"), and others in the following portion of the specification may be read as if prefaced by the word "about" even though the term "about" may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0074] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Furthermore, when numerical ranges of varying scope are set forth herein, it is contemplated that any combination of these values inclusive of the recited values may be used.

[0075] As used herein, the term "comprising" is intended to mean that the products, compositions and methods include the referenced components or steps, but not excluding others. "Consisting essentially of" when used to define products, compositions and methods, shall mean excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components would not exclude trace contaminants and pharmaceutically acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other components or steps.

[0076] By suitable for implantation, such as into a patient (human or animal), it is meant that the biocompatible material is sterile/terminally sterilized, endotoxin free, and noncytotoxic. By suitable for use with a 3D printer it is meant that the composition provides a smooth flow on extrusion, can be extruded with a pressure range of 20-100 kPa, and polymerizes after extrusion. The present invention is not to be limited in scope by the specific embodiments described which are intended as illustrations of certain aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the claims.

[0077] The advantages set forth above, and those made apparent from the foregoing description, are efficiently attained. Since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[0078] All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

[0079] It will be seen that the advantages set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope

of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[0080] It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween. Now that the invention has been described,

What is claimed is:

1. A composition comprising tissue extracellular matrix microparticles in combination with functionalized polymers, wherein the polymers are adapted to cross-link to the microparticles thereby forming a biocompatible scaffold.

2. The composition of claim **1**, wherein the polymers are functionalized by thiolation.

3. The composition of claim **2**, wherein the thiolated polymers are non-synthetic natural materials.

4. The composition of claim **3**, wherein the non-synthetic natural materials can be selected from the group consisting of collagen, gelatin, hyaluronan, heparin, and combinations thereof.

5. The composition of claim **1** further comprising a compound selected form the group consisting of external growth factors, region specific anti-inflammatory drugs, and FDA-approved pharmaceutical agents.

6. The composition of claim **5** wherein the external growth factors, region specific anti-inflammatory drugs, or FDA-approved pharmaceutical agents are selected from the group consisting of TGF β, IGF-1, PRP and corticosteroids.

7. The composition according to claim **1**, wherein the functionalized polymers are synthetic materials.

8. The composition according to claim **7**, wherein the synthetic materials are selected from the group consisting of PEG, PEGDA, and combinations thereof.

9. The composition according to claim **8**, wherein the polymers have a thiolated percentage selected from the group consisting of 0-15%, 15-30%, 30-60% and combinations thereof.

10. The composition according to claim **1**, wherein the microparticles are natural and sourced from tissues or organs.

11. The composition according to claim **10**, wherein the natural microparticles can be sourced from tissue selected from the group consisting of liver, kidney, dermis, cartilage, bone, muscle, ligament, tendon and adipose.

12. The composition according to claim **1**, wherein the tissue microparticles are sourced from xenogenic, allogenic, or autogenic sources.

13. The composition according to claim **1**, wherein the tissue microparticles are in the size range selected from the group consisting of less than about 100 μm, 100-250 μm, and/or 250-500 μm in the direction of their largest diameter.

14. The composition according to claim **1**, wherein the tissue microparticles are decellularized, devitalized, and/or lyophilized tissue microparticles.

15. The composition according to claim **1**, wherein the tissue microparticles are thiol-functionalized through chemical means to add open thiol groups thereby enhancing crosslinking when combined with the functionalized polymers.

16. The composition according to claim **1**, wherein the tissue microparticles and thiolated polymers are crosslinked via disulfide bridges between the thiols on macromolecule and thiols on cysteines or amino acids occurring in the tissue microparticles.

17. The composition according to claim **1**, wherein the biocompatible material is implanted into a patient.

18. The composition according to claim **1**, wherein the biocompatible material is implanted into an animal.

19. The composition material of claim **1**, wherein the biocompatible material is used with a 3D printer.

20. A method for reconstructing a tissue mimic comprising the steps of:

providing acellular microparticles produced via mechanical pulverization of an original tissue source, thereby producing ECM microparticles that preserve the micro mechanical and biological properties of the original tissue;

densely packing the pulverized particles in a hydrogel solution comprising thiolated polymers; and

incubating the resulting hydrogel solution at about 37° C. to facilitate crosslinking between the tissue particles and thiolated polymers.

21. The method for reconstructing a tissue mimic according to claim **20** using a biocompatible composition of thiolated macromolecules and tissue microparticles crosslink into a biocompatible scaffold.

22. The method for reconstructing a tissue mimic according to claim **20** further comprising the step of 3D printing or molding the packed hydrogel solution to create a tissue mimic.

23. The method for reconstructing a tissue mimic according to claim **22** further comprising the step of implanting the tissue mimic into a region of defective, diseased, and injured tissue using the biocompatible composition.

24. The method for reconstructing a tissue mimic according to claim **20** wherein the original tissue source is processed to yield decellularized, devitalized, and/or lyophilized tissue microparticles.

25. The method for reconstructing a tissue mimic according to claim **24** wherein the processed tissue microparticles are arranged in a structure through particle packing to mimic the tissue following the processing steps.

26. The method for reconstructing a tissue mimic according to claim **24** wherein the processed tissue microparticles are arranged in a structure through tissue layering to mimic the original tissue following the processing steps.

27. The method for reconstructing a tissue mimic according to claim **20** wherein the packed hydrogel solution is arranged in a structure to enable cellular infiltration, recellularization, and viability by crosslinking the composition with macromolecules functionalized to <60% to ensure pore size of the scaffold is not less than 10% the diameter of the nucleus of the tissue to be reconstructed, wherein the particles packed together to contact each other at or near percolation threshold so that cells have tissue attachment locations and respond to innate growth factor signaling.

28. A method for reconstructing a tissue mimic that can be 3D printed using a variety of methods, including laminated or extrusion processes.

29. A method for reconstructing a tissue mimic that can be used as an organ-on-a-chip for personalized medicine, drug efficacy studies, toxicity evaluation, and treatment efficacy by combining the composition of any one of claims **1-19** and molding or 3D printing a tissue mimic using the combined material.

30. A method for reconstructing a tissue mimic that enables cellular infiltration and recellurization into the biocompatible material of any one of claims **1-19** by crosslinking the composition with macromolecules functionalized to <60% to ensure pore size of the scaffold is not less than 10% the diameter of the nucleus of the tissue to be reconstructed, wherein the particles packed together to contact each other at or near percolation threshold so that cells have tissue attachment locations and respond to innate growth factor signaling.

31. A method of preparing a tissue mimic composition comprising the steps of:

providing cartilage microparticles, wherein the cartilage has been decellularized, morsilized, and/or lyophilized;

combining the cartilage microparticles with thiolated hyaluronic acid (HA) to form a hydrogel precursor solution; and

incubating the hydrogel precursor solution at 25° C. to 42° C. for 10 minutes to 90 minutes to facilitate disulfide bond crosslinking between sulfide groups on the cartilage microparticles and sulfide groups on the thiolated HA, thereby forming a tissue mimic composition.

32. The method of preparing a tissue mimic composition according to claim **31** further comprising the step of injecting the hydrogel precursor solution into a tissue void in a subject.

33. The method of preparing a tissue mimic composition according to claim **32** wherein the solution is injected into the void using a 3D printing device and the deposition pattern is custom printed following measurement of the defect in the subject.

34. The method of preparing a tissue mimic composition according to claim **33** further comprising the step applying a lubrication layer consisting essentially of hyaluronan to a surface of the tissue mimic composition, whereby the lubrication layer mimics the surface characteristics of natural cartilage.

35. The method of preparing a tissue mimic composition according to claim **31** wherein the hydrogel precursor solution is incubated at about 37° C. for about 30 minutes.

36. The method of preparing a tissue mimic composition according to claim **31** wherein the microparticles have a size range of about 40-100 μm.

37. The method of preparing a tissue mimic composition according to claim **31** wherein the macromolecules have a thiolation percentage of about 15-30%.

38. The method of preparing a tissue mimic composition according to claim **31** wherein the macromolecules have a thiolation percentage of about 20-25%.

39. A method of preparing a tissue mimic composition comprising the steps of:

providing tissue microparticles, which have been decellularized and morselized;

combining the tissue microparticles with a thiolated macromolecule to form a hydrogel precursor solution; and

incubating the hydrogel precursor solution at about 25° C. to about 42° C. for about 10 minutes to about 2 hours to facilitate disulfide bond crosslinking between sulfide groups on the tissue microparticles and sulfide groups on the thiolated macromolecule, thereby forming a tissue mimic composition.

40. The method of preparing a tissue mimic composition according to claim **39** wherein the macromolecule is a macromolecule selected from the group consisting of thiolated collagen, thiolated gelatin, thiolated hyaluronan, thiolated heparin, and combinations thereof.

41. The method of preparing a tissue mimic composition according to claim **39** wherein the tissue is bone tissue, cartilage tissue or combinations thereof.

42. The method according to claim **39**, wherein the tissue microparticles are in the size range selected from the group consisting of less than about 100 μm, 100-250 μm, and/or 250-500 μm in the direction of their largest diameter.

43. The method of preparing a tissue mimic composition according to claim **39** further comprising the step of injecting the hydrogel precursor solution into a tissue void in a subject.

44. The method of preparing a tissue mimic composition according to claim **43** wherein the solution is injected into the void using a 3D printing device and the deposition pattern is custom printed following measurement of the defect in the subject.

45. The method of preparing a tissue mimic composition according to claim **44** further comprising the step applying a lubrication layer consisting essentially of hyaluronan to a surface of the tissue mimic composition, whereby the lubrication layer mimics the surface characteristics of natural cartilage.

46. The method of preparing a tissue mimic composition according to claim **39** wherein the hydrogel precursor solution is incubated at about 37° C. for about 30 minutes.

47. The method of preparing a tissue mimic composition according to claim **39** wherein the microparticles have a size range of about 40-100 μm.

48. The method of preparing a tissue mimic composition according to claim **39** wherein the macromolecules have a thiolation percentage of about 15-30%.

49. The method of preparing a tissue mimic composition according to claim **39** wherein the macromolecules have a thiolation percentage of about 20-25%.

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