METHODS OF MANUFACTURING
BIOACTIVE GELS FROM
EXTRACELLULAR MATRIX MATERIAL

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This patent is subject to a terminal disclaimer.

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U.S. PATENT DOCUMENTS


FOREIGN PATENT DOCUMENTS

JP 2013-500065 1/2013

OTHER PUBLICATIONS

Kentner et al., “Quantification of FGF-2, VEGF, & GAGs in MatriStem MicroMatrix UBM Biomaterial,” BMES Fall Meeting, Hartford, CT 1 page (2011).

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FIG. 1

1.0 M NaOH WITH VARYING SOLUBILIZING TIMES

100 mM NaOH WITH VARYING SOLUBILIZING TIMES

100 mM NaOH WITH VARYING DWELL TIMES

1 HOUR 2 HOUR 4 HOUR 8 HOUR 24 HOUR 48 HOUR

EGF-2 CONCENTRATION (pg/mg)
METHODS OF MANUFACTURING BIOACTIVE GELS FROM EXTRACELLULAR MATRIX MATERIAL

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of a U.S. application Ser. No. 14/811,993, filed on Jul. 29, 2015, which is a continuation application of U.S. application Ser. No. 14/332,465, filed Jul. 16, 2014, now granted U.S. Pat. No. 9,119,831, issued on Sep. 1, 2015, which is a continuation application of U.S. application Ser. No. 14/174,980, filed Feb. 7, 2014, now granted U.S. Pat. No. 8,802,436, issued on Aug. 12, 2014, which claims priority to and benefit of U.S. Provisional Application No. 61/762,437, filed Feb. 8, 2013, the entire content of each is incorporated by reference herein for all purposes.

GOVERNMENT SUPPORT

This invention was supported by grant no. NCC 9-58 from the National Space Biomedical Research Institute through NASA. The Government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods of manufacturing bioactive gels from extracellular matrix material and their uses for restoration of tissues in a patient.

BACKGROUND

Biologic scaffolds composed of extracellular matrix material (ECM) have been used for the repair of a variety of tissues including the lower urinary tract, esophagus, myocardium and musculotendinous tissues, often leading to tissue-specific constructive remodeling with minimal or no scar tissue formation.

Although uses of ECM as scaffolds for preclinical and clinical tissue engineering and regenerative medicine approaches to tissue reconstruction are very promising, challenges remain in the process to manufacture bioactive gels from ECM, which retain their bioactivity.

The methods of manufacturing bioactive gels from ECM described in the prior art require the use of enzymes and are time-consuming because they require aggressive purification steps, which are deleterious to bioactivity, tedious to perform or are time consuming, and which increase the regulatory burden (e.g. FDA approval), are avoided.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows FGF-2 content (pg/mg) of gels following various solubilization conditions in NaOH according to embodiments of the invention. All gels not marked with a % w/v were done at 7.0% w/v UBM to NaOH. All gels in FIG. 1 were done at 7.0% w/v UBM to NaOH.

FIG. 2 shows VEGF content (pg/mg) of gels following various solubilization conditions in NaOH according to embodiments of the invention. All gels not marked with a % w/v were done at 7.0% w/v UBM to NaOH. All gels in FIG. 1 were done at 7.0% w/v UBM to NaOH.

DETAIL DESCRIPTION

The present invention is directed to methods of manufacturing bioactive gels from ECM, i.e., gels which retain sufficient bioactivity to positively assist tissue repair by decreasing the time needed for repair, decreasing scar tissue formation, and improving restoration of the injured tissue to its pre-damaged native structure and function as compared to injured tissues not treated with the bioactive gel according to the invention. The gel invention and methods of making described herein serves as scaffolds for preclinical and clinical tissue engineering and regenerative medicine approaches to tissue reconstruction. Bioactivity in the ECM gel according to the invention is in the range of about 0 to 100%, 25-75%, 10-25%, less than 10%, less than 5% or less than 1% of the bioactivity of one or more bioactive molecules in the native ECM from which the gel was derived. As will be described in detail below, these manufacturing methods take advantage of a new recognition that bioactive gels from ECM can be created by solubilizing a particularized ECM in a basic (greater than pH 7) environment, which when neutralized with acid provides bioactive gels.

In accordance with the inventive methods, the ECM may be derived from layers of native mammalian tissues including but not limited to submucosa, dermis, epithelial basement membrane, or from tissues such as aponerous, fascia, tendon, ligament, smooth and skeletal muscle and treatment site-specific ECM. The native mammalian tissue source may be porcine, bovine, ovine, allogenic, or autogenous, for example. For example, the ECM may be SIS (small intestinal submucosa), UBS (urinary bladder submucosa) or UBMM (urinary bladder matrix) or liver basement membrane.
(LBM) described in U.S. Pat. No. 6,576,265, U.S. Pat. No. 6,579,538, U.S. Pat. No. 5,573,784, U.S. Pat. No. 5,554,389, U.S. Pat. No. 4,956,178, and U.S. Pat. No. 4,902,508, each of which are incorporated by reference herein. In one embodiment of the invention, the ECM is derived from a mammalian tissue and comprises bioactive components of the extracellular matrix material that are arranged and in quantities similar to those in the tissue in its native form.

In accordance with the inventive method, the ECM derived from any one of the above sources is particularized, i.e., the size of the ECM particles are in a range of about 1 µm to about 1000 µm. In one embodiment, particularization of the ECM prior to subjecting the ECM to a basic environment provides homogeneity to the ECM, i.e., provides a more uniform composition in comparison to ECM from individual animals, decreasing the impact of inter-donor variability. In another embodiment, the particularization of the ECM facilitates in solubilizing the material in a basic environment by increasing surface area to volume ratio.

The particulate ECM product, e.g., particularized ECM, is manufactured by grinding/milling or otherwise performing a size reduction process to ECM typically but not exclusively originally provided in sheet form. The resulting particulate can be any desired range of density for example in the range of about 0.1 g/cm³-10 g/cm³, about 0.1-1.0 g/cm³-1 g/cm³ or about 1 g/cm³, and particle size for example in the range of about 1 micron-1000 microns, about 200-700 microns, about 300-600 microns, or about 400 microns.

A basic environment is provided by solutions of alkaline compounds. Alkaline compounds which could be used in accordance with the invention are metal hydroxides which include, but are not limited to, LiOH, NaOH, KOH, RbOH, and CsOH. Alkaline compounds which could be used in accordance with the invention also include weak bases, such as but not limited to, ammonia (NH₃), pyridine (C₅H₅N), hydroxylamine (H₂ONH), methylamine (NH₂CH₃) and the like. Alkaline compounds are generally used at a concentration ranging from 0.1 Molar to 1.0 Molar, although concentrations lower that 0.1 Molar or higher than 1.0 Molar are also contemplated in an embodiment of the invention.

Concentrations of particularized ECM to NaOH (w/v) are in the range of 0.1% to about 20%, in particular 0.5% to 11%, and more particular, 7%.

The solubilization step at 4°C. (i.e., digestion) of the particularized ECM can extend over a period of time ranging from a few minutes to several hours (e.g., 30 minutes to 48 hours) or days (e.g., 3-7 days), 30 minutes to 12 hours, 12-24 hours, 24 to 36 hours, 36 to 48 hours, or two to seven days. In an embodiment of the invention, it is contemplated that the time period required for the digestion step is determined by the size of the particularized extracellular matrix material and/or the concentration of the metal hydroxide used for solubilization. For example, if the concentration of an alkaline compound, such as NaOH, is low, longer incubation, i.e., longer time period for solubilization may be required. After the solubilization step in a basic solution, the solubilized ECM (i.e., the gel form) is neutralized to a neutral pH using molar concentrations, e.g., equimolar concentrations of an acid in a volume sufficient for the solubilized ECM to reach pH 6.8 to 7.4. Acids, which aid in neutralization of the ECM gel, can be selected from weak or strong acids. Selectivity of acids for the neutralization step depends on the salts which are produced when an acids reacts with the basic environment during neutralization. The resulting salt should be biocompatible. For example, in an embodiment of the invention, hydrochloric acid (HCl) is used to neutralize the basic environment created by the base NaOH because the resulting salt (i.e., NaCl) is clinically acceptable.

For the following exemplifications, any number of ECM products such as but not limited to one or more of isolated urinary bladder submucosa, small intestinal submucosa, dermis, for example, could be used. In the following exemplifications, UBMB, an ECM isolated from the urinary bladder and having epithelial basement membrane is used as an exemplary ECM. However the invention disclosed herein is not limited to UBMB and is applicable to any isolated ECM. In an exemplification, gels were created using various concentrations of particularized UBMB (0.5-11% w/v) solubilized in various concentrations of NaOH (0.1-1.0M). UBMB was solubilized for various time periods (1-48 hours)
in its respective concentration of UBM and NaOH at 4°C. In order to test whether the UBM could restructure after solubilization, gels were also made using various dwell periods (1-48 hours) following neutralization.

UBM gels created in the above manner were tested in vitro for bioactive molecular content. In this study, growth factor (e.g., FGF-2, CTGF, VEGF) content was analyzed. Data for FGF-2 and VEGF content following solubilization for each gel structure is shown in FIGS. 1 and 2. Lower concentration gels (1-6%) are not shown but produced similar results. As shown in FIGS. 1 and 2, FGF-2 and VEGF levels increased in these studies. In one study it was found that using 7.0% w/v UBM to various range of NaOH with 24 hours of solubilizing at 4°C and no dwell period had significantly influenced the FGF-2 and VEGF contents in the gel. FGF-2 and VEGF contents were measure by standard ELISA procedures.

What is claimed is:

1. A bioactive gel composition comprising: an extracellular matrix material (ECM) derived from the extracellular matrix of a mammal, digested in a basic solution comprising a concentration of the ECM to a base (w/v) in the range of about 0.1% to about 20%, and wherein said composition is gelled at room temperature.

2. The composition of claim 1 wherein said ECM comprises epithelial basement membrane.

3. The composition of claim 1 wherein said base is selected from the group consisting of LiOH, NaOH, KOH, RbOH, CsOH, NH₃, CsH₃N, H₂NOH, and NH₂CH₃.

4. The composition of claim 1 further comprising a bioactive component selected from the group consisting of VEGF, FGF-2, CTGF, and combinations thereof.

5. The composition of claim 1 wherein said ECM comprises submucosa.

6. The composition of claim 1 wherein said ECM is selected from the group consisting of submucosa, tunica propria, dermis, liver basement membrane, and combinations thereof.

7. The composition of claim 1 wherein the concentration of ECM to NaOH (w/v) is in the range of 0.5% to 11%.

8. The composition of claim 1 further comprising a neutralizing solution comprising a volume of an acid, sufficient to solubilize the gelled composition and bring the pH of said solubilized gel composition into a range between about 6.8 and about 7.4.

9. The composition of claim 8 wherein said composition is lyophilized.

10. The composition of claim 9 wherein said lyophilized composition is reconstituted in a solution.

11. The composition of claim 1 wherein said bioactive gel composition further comprises particularized ECM.

12. The composition of claim 1 wherein said particularized ECM comprises urinary bladder matrix (UBM).

13. The composition of claim 11 wherein said particularized ECM comprises small intestinal submucosa (SIS).

14. The composition of claim 11 wherein said particularized ECM comprises urinary bladder submucosa (UBS).

15. The composition of claim 11 wherein said particularized ECM comprises liver basement membrane (LBM).

16. The composition of claim 11 wherein said particularized ECM comprises a particle size in the range of about 200 microns to 700 microns, or about 300 microns to 600 microns.

17. The composition of claim 11 wherein said particularized ECM comprises a particle size in the range of about 200 microns to about 400 microns.

18. The composition of claim 11 wherein said particularized ECM comprises a particle size in the range of about 200 microns to about 400 microns.

19. The composition of claim 1 wherein said base comprises NaOH.

20. The composition of claim 19 wherein said base comprises 0.1-1.0M NaOH.

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