(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 August 2018 (09.08.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/144496 A1

(51) International Patent Classification:
A61K 38/39 (2006.01) *C07K 14/78* (2006.01)
A61L 27/24 (2006.01)

(21) International Application Number:
PCT/US2018/016069

(22) International Filing Date:
31 January 2018 (31.01.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/452,564 31 January 2017 (31.01.2017) US

(71) Applicant: **GENIPHYS, LLC** [US/US]; 10307 Oak Ridge Drive, Zionsville, Indiana 46077 (US).

(72) Inventor: **VOYTIK-HARBIN, Sherry L.**; 10307 Oak Ridge Drive, Zionsville, Indiana 46077 (US).

(74) Agent: **BALL, Rebecca L.**; BARNES & THORNBURG LLP, 11 South Meridian Street, Indianapolis, Indiana 46204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

(54) **Title:** METHODS AND COMPOSITIONS FOR MATRIX PREPARATION

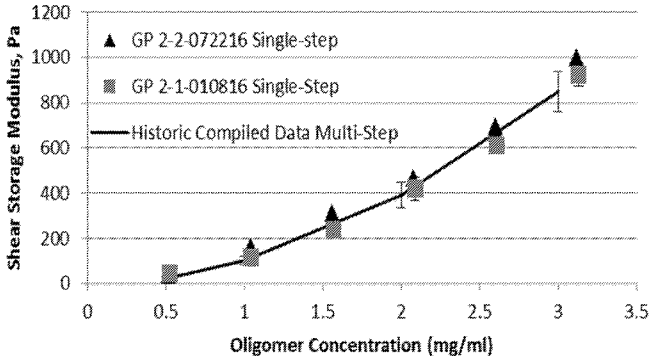

FIG. 1

(57) **Abstract:** The invention relates to methods of preparing matrices, and compositions therefor. In particular, the invention relates to methods of preparing collagen matrices, and compositions therefor, including kits and graft compositions.

WO 2018/144496 A1

[Continued on next page]

Published:
— *with international search report (Art. 21(3))*

METHODS AND COMPOSITIONS FOR MATRIX PREPARATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 62/452,564 filed on January 31, 2017, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE DISCLOSURE

This invention relates to methods of preparing matrices, and compositions therefor. In particular, the invention relates to methods of preparing collagen matrices, and compositions therefor, including kits and graft compositions.

BACKGROUND AND SUMMARY

The extracellular matrix plays a crucial role in the function of tissues and organs, such as communication between cells, differentiation during embryogenesis, wound healing, adhesion, and cell migration and proliferation. Extracellular matrix graft constructs obtained from natural sources or made synthetically can be used as tissue graft compositions, in both solid and injectable forms, for remodeling tissues *in vivo* or for *in vitro* applications, such as for research purposes. The principal component of the extracellular matrix is collagen. Some solubilized collagen compositions, including purified collagen compositions, have matrix-forming capability and can also be used as tissue graft compositions, in both solid and injectable forms, for remodeling tissues *in vivo* or for *in vitro* uses. In fact, such collagen-based matrices have broad spanning research and medical applications, including wound and hemostatic dressings, use as surgical implants, substrates for tissue engineered medical products, delivery vehicles for therapeutic cells or molecules, and as three-dimensional *in-vitro* tissue systems for basic research, including drug development and toxicity testing.

To ensure a high level of manufacturing consistency of collagen-based matrices and low lot-to-lot variability in the functional properties of collagen for use in making collagen-based matrices for both medical and research applications, standardized procedures and reagents are needed for induction of collagen self-assembly and for customization of collagen-based matrices.

Accordingly, the inventors have developed a robust method with a single mixing step for polymerization of collagen, with reagents that mimic physiologic conditions to support supramolecular self-assembly of collagen as observed in the body. In addition, the method and

the reagents used in the method described herein are based on physiologic responses such as the viscoelastic properties of self-assembled matrices (for example, shear storage modulus as measured in oscillatory shear and matrix stiffness) as a function of collagen concentration in the polymerization reaction. The methods and compositions described herein produce collagen-based matrices that have properties similar to conventional multi-step polymerization methods involving multiple mixing steps for collagen polymerization including mixing a collagen composition with a buffer solution and then mixing with, for example, with a base such as NaOH to induce polymerization, and then mixing optionally with other reagents. The methods and compositions developed by the inventors have resulted in highly predictable and reproducible in-vitro cell and in-vivo host responses to self-assembled collagen matrices. In another aspect, the inventors have developed methods for sterilizing the collagen, collagen compositions, collagen matrices, collagen solutions, and lyophilized collagen described herein by using ultraviolet radiation, while maintaining polymerization capabilities (e.g., shear storage modulus). The maintenance of polymerization capabilities is contrary to previous studies reporting that ultraviolet radiation negatively affects collagen fibril formation (Mentor et al., Photodermatology, Photoimmunology & Photomedicine, 2001; Miyata et al., Biochem. Biophys. Acta, 1971; Sudoh and Noda, Connect. Tissue Res., 1972). In one embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix.

In another embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen by mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix wherein the buffer solution does not contain magnesium ions or manganese ions.

In yet another embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, wherein the collagen in the collagen solution polymerizes to form the matrix.

In still other embodiments, a collagen matrix prepared according to any of the preceding methods is provided. In yet another embodiment, a kit comprising a collagen composition and a buffer solution is provided. In another embodiment, a kit comprising lyophilized collagen, a hydrochloric acid solution, and a buffer solution is provided.

Several additional embodiments are described by the following enumerated clauses. Any applicable combination of these embodiments is also contemplated.

1. A method for preparing a matrix, said method comprising polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix.

2. The method of clause 1 comprising incubating the collagen solution at greater than about 25 °C to promote polymerization of the collagen in the collagen solution.

3. The method of clause 1 or 2 comprising incubating the collagen solution at about 37 °C to promote polymerization of the collagen in the collagen solution.

4. The method of any one of clauses 1 to 3 wherein the collagen comprises collagen oligomers.

5. The method of any one of clauses 1 to 4 wherein the collagen consists of collagen oligomers.

6. The method of clause 4 wherein the collagen further comprises telocollagen.

7. The method of clause 4 wherein the collagen further comprises atelocollagen.

8. The method of clause 4 wherein the collagen comprising collagen oligomers is obtained from a tissue containing collagen oligomers, from cells producing collagen oligomers, or by chemically crosslinking collagen to obtain the collagen oligomers.

9. The method of any one of clauses 1 to 8 wherein the collagen is derived from porcine skin tissue.

10. The method of any one of clauses 1 to 9 wherein the collagen composition further comprises an acid.

11. The method of clause 10 wherein the acid is selected from the group consisting of hydrochloric acid, acetic acid, lactic acid, formic acid, citric acid, sulfuric acid, and phosphoric acid.

12. The method of clause 11 wherein the acid is hydrochloric acid.

13. The method of clause 12 wherein the hydrochloric acid is about .005 N to about 0.1 N hydrochloric acid.

14. The method of clause 12 or 13 wherein the hydrochloric acid is about .01 N hydrochloric acid.

15. The method of any one of clauses 1 to 14 wherein the collagen is at a concentration of about 0.1 mg/ml to about 40 mg/ml in the collagen solution.

16. The method of any one of clauses 1 to 15 wherein the collagen is at a concentration of about 0.1 mg/ml to about 5 mg/ml in the collagen solution.

17. The method of any one of clauses 1 to 16 wherein the collagen is at a concentration of about 0.5 mg/ml to about 4 mg/ml in the collagen solution.

18. The method of any one of clauses 1 to 13 wherein the collagen composition is sterilized.

19. The method of any one of clauses 1 to 18 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, ultraviolet radiation, gamma irradiation, E-beam, and combinations thereof.

20. The method of any one of clauses 1 to 19 wherein the collagen composition is sterilized by filtration.

21. The method of any one of clauses 1 to 20 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

22. The method of any one of clauses 1 to 20 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

23. The method of any one of clauses 1 to 20 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

24. The method of any one of clauses 1 to 20 wherein the buffer solution does not comprise $MgCl_2$.

25. The method of any one of clauses 1 to 24 wherein the buffer solution further comprises about 0.3 mM to about 3 mM KH_2PO_4.

26. The method of any one of clauses 1 to 25 wherein the buffer solution further comprises about 1 mM to about 10 M Na_2HPO_4.

27. The method of any one of clauses 1 to 26 wherein the buffer solution further comprises about 0.1 mM to about 4 mM KCl.

28. The method of any one of clauses 1 to 27 wherein the buffer solution further comprises about .02 M to about 0.3 M NaCl.

29. The method of any one of clauses 1 to 28 wherein the buffer solution further comprises about .002 N to about .02 N NaOH.

30. The method of any one of clauses 1 to 29 wherein the buffer solution further comprises about 0.5 weight percent to about 5 weight percent of glucose.

31. The method of any one of clauses 1 to 29 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

32. The method of any one of clauses 1 to 31 further comprising adding cells to the collagen solution.

33. The method of any one of clauses 1 to 32 wherein the matrix comprises collagen fibrils.

34. A collagen matrix prepared according to the method of any one of clauses 1 to 33.

35. The collagen matrix of clause 34 wherein the collagen matrix is a medical graft.

36. The collagen matrix of clause 35 wherein the medical graft has a use selected from the group consisting of a tissue graft material, an injectable graft material, a wound dressing, a hemostatic dressing, a delivery vehicle for therapeutic cells, and a delivery vehicle for a therapeutic agent.

37. The collagen matrix of clause 35 wherein the collagen matrix is used for research purposes.

38. The collagen matrix of clause 37 wherein the collagen matrix is used for drug toxicity testing or drug development.

39. A kit comprising a collagen composition and a buffer solution.

40. The kit of clause 39 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

41. The kit of clause 39 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

42. The kit of clause 39 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

43. The kit of clause 39 wherein the buffer solution does not comprise $MgCl_2$.

44. The kit of any one of clauses 39 to 43 wherein the buffer solution further comprises about .003M to about .03 M KH_2PO_4.

45. The kit of any one of clauses 39 to 44 wherein the buffer solution further comprises about .01 M to about 0.1 M Na_2HPO_4.

46. The kit of any one of clauses 39 to 45 wherein the buffer solution further comprises about .001 M to about .04 M KCl.

47. The kit of any one of clauses 39 to 46 wherein the buffer solution further comprises about 0.2 M to about 3.0 M NaCl.

48. The kit of any one of clauses 39 to 47 wherein the buffer solution further comprises about .02 N to about 0.2 N NaOH.

49. The kit of any one of clauses 39 to 48 wherein the buffer solution further comprises about 0.2 weight percent to about 5 weight percent of glucose.

50. The kit of any one of clauses 39 to 48 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

51. The kit of any one of clauses 39 to 50 wherein the collagen in the collagen solution is at a concentration of about 0.1 mg/ml to about 40 mg/ml.

52. The kit of any one of clauses 39 to 51 wherein the collagen in the collagen solution is at a concentration of about 0.1 mg/ml to about 5 mg/ml.

53. The kit of any one of clauses 39 to 52 wherein the collagen solution comprises about .005 N hydrochloric acid to about 0.1 N hydrochloric acid.

54. The kit of any one of clauses 39 to 53 wherein the buffer solution is capable of polymerizing the collagen using a single mixing step comprising mixing the collagen composition with the buffer solution.

55. The kit of any one of clauses 39 to 54 wherein the collagen composition and the buffer solution are in separate containers.

56. The kit of clause 55 wherein the containers are sterilized vials.

57. The kit of clause 55 wherein the containers comprise separate compartments of a dual syringe.

58. The kit of clause 57 wherein the dual syringe comprises a mixing element.

59. The kit of clause 57 wherein the dual syringe is sterilized.

60. The kit of any one of clauses 39 to 59 further comprising instructions for use of components of the kit.

61. A method for preparing a matrix, said method comprising polymerizing collagen by mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix wherein the buffer solution does not contain magnesium ions or manganese ions.

62. The method of clause 61 comprising incubating the collagen solution at greater than about 25 ^{o}C to promote polymerization of the collagen in the collagen solution.

63. The method of clause 61 or 62 comprising incubating the collagen solution at about 37 ^{o}C to promote polymerization of the collagen in the collagen solution.

64. The method of any one of clauses 61 to 63 wherein the collagen comprises collagen oligomers.

65. The method of any one of clauses 61 to 63 wherein the collagen consists of collagen oligomers.

66. The method of clause 64 wherein the collagen further comprises telocollagen.

67. The method of clause 64 wherein the collagen further comprises atelocollagen.

68. The method of clause 64 wherein the collagen comprising collagen oligomers is obtained from a tissue containing collagen oligomers, from cells producing collagen oligomers, or by chemically crosslinking the collagen to obtain the collagen oligomers.

69. The method of any one of clauses 61 to 68 wherein the collagen is derived from porcine skin tissue.

70. The method of any one of clauses 61 to 6 9 wherein the collagen composition further comprises an acid.

71. The method of clause 70 wherein the acid is selected from the group consisting of hydrochloric acid, acetic acid, lactic acid, formic acid, citric acid, sulfuric acid, and phosphoric acid.

72. The method of clause 71 wherein the acid is hydrochloric acid.

73. The method of clause 72 wherein the hydrochloric acid is about .005 N to about 0.1 N hydrochloric acid.

74. The method of clause 72 or 73 wherein the hydrochloric acid is about .01 N hydrochloric acid.

75. The method of any one of clauses 61 to 74 wherein the collagen is at a concentration of about 0.1 mg/ml to about 40 mg/ml in the collagen solution.

76. The method of any one of clauses 61 to 75 wherein the collagen is at a concentration of about 0.1 mg/ml to about 5 mg/ml in the collagen solution.

77. The method of any one of clauses 61 to 76 wherein the collagen is at a concentration of about 0.5 mg/ml to about 4 mg/ml in the collagen solution.

78. The method of any one of clauses 61 to 77 wherein the collagen composition is sterilized.

79. The method of any one of clauses 61 to 78 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, ultraviolet radiation, gamma irradiation, E-beam, and combinations thereof.

80. The method of any one of clauses 61 to 79 wherein the collagen composition is sterilized by filtration.

81. The method of any one of clauses 61 to 80 wherein the buffer solution further comprises about 0.3 mM to about 3 mM KH_2PO_4.

82. The method of any one of clauses 61 to 81 wherein the buffer solution further comprises about 1 mM to about 10 M Na_2HPO_4.

83. The method of any one of clauses 61 to 82 wherein the buffer solution further comprises about 0.1 mM to about 4 mM KCl.

84. The method of any one of clauses 61 to 83 wherein the buffer solution further comprises about .02 M to about 0.3 M NaCl.

85. The method of any one of clauses 61 to 84 wherein the buffer solution further comprises about .002 N to about .02 N NaOH.

86. The method of any one of clauses 61 to 85 wherein the buffer solution further comprises about 0.5 weight percent to about 5 weight percent of glucose.

87. The method of any one of clauses 61 to 85 wherein the buffer solution comprises about 0.5 weight percent of glucose or less.

88. The method of any one of clauses 61 to 87 further comprising adding cells to the collagen solution.

89. The method of any one of clauses 61 to 88 wherein the matrix comprises collagen fibrils.

90. A collagen matrix prepared according to the method of any one of clauses 61 to 89.

91. The collagen matrix of clause 90 wherein the collagen matrix is a medical graft.

92. The collagen matrix of clause 91 wherein the medical graft has a use selected from the group consisting of a tissue graft material, an injectable graft material, a wound dressing, a hemostatic dressing, a delivery vehicle for therapeutic cells, and a delivery vehicle for a therapeutic agent.

93. The collagen matrix of clause 90 wherein the collagen matrix is used for research purposes.

94. The collagen matrix of clause 93 wherein the collagen matrix is used for drug toxicity testing or drug development.

95. A kit comprising lyophilized collagen, a hydrochloric acid solution, and a buffer solution.

96. The kit of clause 95 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

97. The kit of clause 95 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

98. The kit of clause 95 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

99. The kit of clause 95 wherein the buffer solution does not comprise $MgCl_2$.

100. The kit of any one of clauses 95 to 99 wherein the buffer solution further comprises about .003 M to about .03 M KH_2PO_4.

101. The kit of any one of clauses 95 to 100 wherein the buffer solution further comprises about .01 M to about 0.1 M Na_2HPO_4.

102. The kit of any one of clauses 95 to 101 wherein the buffer solution further comprises about .001 M to about .04 M KCl.

103. The kit of any one of clauses 95 to 102 wherein the buffer solution further comprises about 0.2 M to about 3.0 M NaCl.

104. The kit of any one of clauses 95 to 103 wherein the buffer solution further comprises about .02 N to about 0.2 N NaOH.

105. The kit of any one of clauses 95 to 104 wherein the buffer solution further comprises about 0.2 weight percent to about 5 weight percent of glucose.

106. The kit of any one of clauses 95 to 105 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

107. The kit of any one of clauses 95 to 106 wherein the hydrochloric acid solution comprises about .005 N hydrochloric acid to about 0.1 N hydrochloric acid.

108. The kit of any one of clauses 95 to 107 wherein the buffer solution is capable of polymerizing collagen using a single mixing step comprising mixing the buffer solution with the lyophilized collagen reconstituted in the hydrochloric acid solution.

109. The kit of any one of clauses 95 to 108 wherein the lyophilized collagen, the hydrochloric acid solution, and the buffer solution are in separate containers.

110. The kit of any one of clauses 95 to 109 further comprising instructions for use of components of the kit.

111. A method for preparing a matrix, said method comprising polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, wherein the collagen in the collagen solution polymerizes to form the matrix.

112. The method of any one of clauses 1 to 33, 61 to 89, or 111 wherein the collagen is soluble collagen.

113. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 112 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using ultraviolet radiation.

114. The method of clause 113 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition this is not irradiated, a collagen solution that is not irradiated, or a collagen matrix that is not irradiated, respectively.

115. The method of clause 114 wherein the polymerization property is shear storage modulus.

116. The method of any one of clauses 113 to 115 wherein the radiation dose ranges from about 5 mJ/cm2 to about 800 mJ/cm2.

117. The method of any one of clauses 113 to 115 wherein the radiation dose ranges from about 30 mJ/cm2 to about 300 mJ/cm2.

118. The method of any one of clauses 113 to 117 wherein the sterilization inactivates viruses.

119. The collagen matrix of any one of clauses 34 to 38 or 90 to 94 wherein the collagen matrix is sterilized using ultraviolet radiation.

120. The collagen matrix of clause 119 wherein the collagen matrix maintains a polymerization property relative to a collagen matrix that is not irradiated.

121. The collagen matrix of clause 120 wherein the polymerization property is shear storage modulus.

122. The collagen matrix of any one of clauses 119 to 121 wherein the radiation dose ranges from about 5 mJ/cm2 to about 800 mJ/cm2.

123. The collagen matrix of any one of clauses 119 to 121 wherein the radiation dose ranges from about 30 mJ/cm2 to about 300 mJ/cm2.

124. The collagen matrix of any one of clauses 119 to 123 wherein the sterilization inactivates viruses.

125. The kit of any one of clauses 39 to 60 or 95 to 110 wherein the collagen composition or the lyophilized collagen is sterilized using ultraviolet radiation.

126. The kit of clause 125 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition this is not irradiated or to lyophilized collagen that is not irradiated, respectively.

127. The kit of clause 126 wherein the polymerization property is shear storage modulus.

128. The kit of any one of clauses 125 to 127 wherein the radiation dose ranges from about 5 mJ/cm2 to about 800 mJ/cm2.

129. The kit of any one of clauses 125 to 127 wherein the radiation dose ranges from about 30 mJ/cm2 to about 300 mJ/cm2.

130. The kit of any one of clauses 125 to 129 wherein the sterilization inactivates viruses.

131. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 118 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation.

132. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 118 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation and sterile filtration.

133. The collagen matrix of any one of clauses 34 to 38, 90 to 94, or 119 to 124 wherein the collagen matrix is sterilized using UVC irradiation.

134. The collagen matrix of any one of clauses 34 to 38, 90 to 94, or 119 to 124 wherein the collagen matrix is sterilized using UVC irradiation and sterile filtration.

135. The kit of any one of clauses 39 to 60, 95 to 110, or 125 to 130 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation.

136. The kit of any one of clauses 39 to 60, 95 to 110, or 125 to 130 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation and sterile filtration.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows shear storage modulus for collagen-based matrices polymerized at different oligomer concentrations. The polymerization capacity for a single-step assembly is similar to that obtained with a conventional multi-step procedure.

Fig, 2 shows that self-assembled matrices prepared with a multi-step and single-step procedures show similar biocompatibility and cellular response. Human adipose-derived stem cells were used to create three-dimensional collagen-fibril tissue constructs using a multi-step (fig. 2a) or single-step (fig. 2b) procedures. After 4 days, constructs were fixed, stained with phalloidin, and visualized using confocal microscopy.

Fig. 3 shows a multi-step procedure.

Fig. 4 shows a single-step procedure.

Fig. 5 shows the formulary supporting user customization of self-assembled collagen matrices using a single-step procedure based on starting collagen oligomer concentration.

Fig. 6 shows that research-grade and medical-grade formulations of oligomer show similar non-inflammatory tissue regeneration responses following subcutaneous injection in mice. Research-grade oligomer, medical-grade oligomer, and Integra Flowable were subcutaneously injected (200 ul) in mice. After 4 weeks, implant sites were harvested, fixed, and prepared for histopathological analysis. Images show cross-sections of untreated skin (control; A), Integra Flowable (B), research-grade oligomer (C), and medical-grade oligomer (D).

Fig. 7 shows the shear storage modulus of collagen matrices polymerized from collagen treated with UVC irradiation at doses of 0 (untreated control), 30 mJ/cm^2 (1 minute of irradiation), and 300 mJ/cm^2 (10 minutes of irradiation).

DETAILED DESCRIPTION OF THE ILLUSTRATIVE EMBODIMENTS

As used herein, "sterilized" means removing contaminants including, but not limited to, infectious agents. For example, contaminants (e.g., viruses) can be removed by inactivation, reduction in number or amount, or by inhibition of activity of contaminating agents, whether infectious or not.

As used herein, "purified" means removing contaminants including, but not limited to, cellular contaminants, nucleotide contaminants, and endotoxins.
means inactivating all viruses, whether infectious or not, reducing the number of infectious viruses, or inhibiting the activity of viruses, whether infectious or not.

As used herein "oligomer" or "oligomers" in relation to collagen means collagen monomers (otherwise known as telocollagen) covalently attached to each other (e.g., collagen monomers attached to each other to form dimers, trimers, etc.).

In one embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix.

In another embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen by mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to

form the matrix wherein the buffer solution does not contain magnesium ions or manganese ions.

In yet another embodiment, a method for preparing a matrix is provided. The method comprises polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, wherein the collagen in the collagen solution polymerizes to form the matrix.

In still other embodiments, a collagen matrix prepared according to any of the preceding methods is provided. In yet another embodiment, a kit comprising a collagen composition and a buffer solution is provided. In another embodiment, a kit comprising lyophilized collagen, a hydrochloric acid solution, and a buffer solution is provided.

Several additional embodiments are described by the following enumerated clauses. Any applicable combination of these embodiments is also contemplated, and any applicable combination of these embodiments with the embodiments described in this DETAILED DESCRIPTION OF THE ILLUSTRATIVE EMBODIMENTS section of the application is also contemplated.

1. A method for preparing a matrix, said method comprising polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix.

2. The method of clause 1 comprising incubating the collagen solution at greater than about 25 °C to promote polymerization of the collagen in the collagen solution.

3. The method of clause 1 or 2 comprising incubating the collagen solution at about 37 °C to promote polymerization of the collagen in the collagen solution.

4. The method of any one of clauses 1 to 3 wherein the collagen comprises collagen oligomers.

5. The method of any one of clauses 1 to 4 wherein the collagen consists of collagen oligomers.

6. The method of clause 4 wherein the collagen further comprises telocollagen.

7. The method of clause 4 wherein the collagen further comprises atelocollagen.

8. The method of clause 4 wherein the collagen comprising collagen oligomers is obtained from a tissue containing collagen oligomers, from cells producing collagen oligomers, or by chemically crosslinking collagen to obtain the collagen oligomers.

9. The method of any one of clauses 1 to 8 wherein the collagen is derived from porcine skin tissue.

10. The method of any one of clauses 1 to 9 wherein the collagen composition further comprises an acid.

11. The method of clause 10 wherein the acid is selected from the group consisting of hydrochloric acid, acetic acid, lactic acid, formic acid, citric acid, sulfuric acid, and phosphoric acid.

12. The method of clause 11 wherein the acid is hydrochloric acid.

13. The method of clause 12 wherein the hydrochloric acid is about .005 N to about 0.1 N hydrochloric acid.

14. The method of clause 12 or 13 wherein the hydrochloric acid is about .01 N hydrochloric acid.

15. The method of any one of clauses 1 to 14 wherein the collagen is at a concentration of about 0.1 mg/ml to about 40 mg/ml in the collagen solution.

16. The method of any one of clauses 1 to 15 wherein the collagen is at a concentration of about 0.1 mg/ml to about 5 mg/ml in the collagen solution.

17. The method of any one of clauses 1 to 16 wherein the collagen is at a concentration of about 0.5 mg/ml to about 4 mg/ml in the collagen solution.

18. The method of any one of clauses 1 to 13 wherein the collagen composition is sterilized.

19. The method of any one of clauses 1 to 18 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, gamma irradiation, ultraviolet radiation, E-beam, and combinations thereof.

20. The method of any one of clauses 1 to 19 wherein the collagen composition is sterilized by filtration.

21. The method of any one of clauses 1 to 20 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

22. The method of any one of clauses 1 to 20 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

23. The method of any one of clauses 1 to 20 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

24. The method of any one of clauses 1 to 20 wherein the buffer solution does not comprise $MgCl_2$.

25. The method of any one of clauses 1 to 24 wherein the buffer solution further comprises about 0.3 mM to about 3 mM KH_2PO_4.

26. The method of any one of clauses 1 to 25 wherein the buffer solution further comprises about 1 mM to about 10 M Na_2HPO_4.

27. The method of any one of clauses 1 to 26 wherein the buffer solution further comprises about 0.1 mM to about 4 mM KCl.

28. The method of any one of clauses 1 to 27 wherein the buffer solution further comprises about .02 M to about 0.3 M NaCl.

29. The method of any one of clauses 1 to 28 wherein the buffer solution further comprises about .002 N to about .02 N NaOH.

30. The method of any one of clauses 1 to 29 wherein the buffer solution further comprises about 0.5 weight percent to about 5 weight percent of glucose.

31. The method of any one of clauses 1 to 29 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

32. The method of any one of clauses 1 to 31 further comprising adding cells to the collagen solution.

33. The method of any one of clauses 1 to 32 wherein the matrix comprises collagen fibrils.

34. A collagen matrix prepared according to the method of any one of clauses 1 to 33.

35. The collagen matrix of clause 34 wherein the collagen matrix is a medical graft.

36. The collagen matrix of clause 35 wherein the medical graft has a use selected from the group consisting of a tissue graft material, an injectable graft material, a wound dressing, a hemostatic dressing, a delivery vehicle for therapeutic cells, and a delivery vehicle for a therapeutic agent.

37. The collagen matrix of clause 35 wherein the collagen matrix is used for research purposes.

38. The collagen matrix of clause 37 wherein the collagen matrix is used for drug toxicity testing or drug development.

39. A kit comprising a collagen composition and a buffer solution.

40. The kit of clause 39 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

41. The kit of clause 39 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

42. The kit of clause 39 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

43. The kit of clause 39 wherein the buffer solution does not comprise $MgCl_2$.

44. The kit of any one of clauses 39 to 43 wherein the buffer solution further comprises about .003M to about .03 M KH_2PO_4.

45. The kit of any one of clauses 39 to 44 wherein the buffer solution further comprises about .01 M to about 0.1 M Na_2HPO_4.

46. The kit of any one of clauses 39 to 45 wherein the buffer solution further comprises about .001 M to about .04 M KCl.

47. The kit of any one of clauses 39 to 46 wherein the buffer solution further comprises about 0.2 M to about 3.0 M NaCl.

48. The kit of any one of clauses 39 to 47 wherein the buffer solution further comprises about .02 N to about 0.2 N NaOH.

49. The kit of any one of clauses 39 to 48 wherein the buffer solution further comprises about 0.2 weight percent to about 5 weight percent of glucose.

50. The kit of any one of clauses 39 to 48 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

51. The kit of any one of clauses 39 to 50 wherein the collagen in the collagen solution is at a concentration of about 0.1 mg/ml to about 40 mg/ml.

52. The kit of any one of clauses 39 to 51 wherein the collagen in the collagen solution is at a concentration of about 0.1 mg/ml to about 5 mg/ml.

53. The kit of any one of clauses 39 to 52 wherein the collagen solution comprises about .005 N hydrochloric acid to about 0.1 N hydrochloric acid.

54. The kit of any one of clauses 39 to 53 wherein the buffer solution is capable of polymerizing the collagen using a single mixing step comprising mixing the collagen composition with the buffer solution.

55. The kit of any one of clauses 39 to 54 wherein the collagen composition and the buffer solution are in separate containers.

56. The kit of clause 55 wherein the containers are sterilized vials.

57. The kit of clause 55 wherein the containers comprise separate compartments of a dual syringe.

58. The kit of clause 57 wherein the dual syringe comprises a mixing element.

59. The kit of clause 57 wherein the dual syringe is sterilized.

60. The kit of any one of clauses 39 to 59 further comprising instructions for use of components of the kit.

61. A method for preparing a matrix, said method comprising polymerizing collagen by mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix wherein the buffer solution does not contain magnesium ions or manganese ions.

62. The method of clause 61 comprising incubating the collagen solution at greater than about 25 °C to promote polymerization of the collagen in the collagen solution.

63. The method of clause 61 or 62 comprising incubating the collagen solution at about 37 °C to promote polymerization of the collagen in the collagen solution.

64. The method of any one of clauses 61 to 63 wherein the collagen comprises collagen oligomers.

65. The method of any one of clauses 61 to 63 wherein the collagen consists of collagen oligomers.

66. The method of clause 64 wherein the collagen further comprises telocollagen.

67. The method of clause 64 wherein the collagen further comprises atelocollagen.

68. The method of clause 64 wherein the collagen comprising collagen oligomers is obtained from a tissue containing collagen oligomers, from cells producing collagen oligomers, or by chemically crosslinking the collagen to obtain the collagen oligomers.

69. The method of any one of clauses 61 to 68 wherein the collagen is derived from porcine skin tissue.

70. The method of any one of clauses 61 to 6 9 wherein the collagen composition further comprises an acid.

71. The method of clause 70 wherein the acid is selected from the group consisting of hydrochloric acid, acetic acid, lactic acid, formic acid, citric acid, sulfuric acid, and phosphoric acid.

72. The method of clause 71 wherein the acid is hydrochloric acid.

73. The method of clause 72 wherein the hydrochloric acid is about .005 N to about 0.1 N hydrochloric acid.

74. The method of clause 72 or 73 wherein the hydrochloric acid is about .01 N hydrochloric acid.

75. The method of any one of clauses 61 to 74 wherein the collagen is at a concentration of about 0.1 mg/ml to about 40 mg/ml in the collagen solution.

76. The method of any one of clauses 61 to 75 wherein the collagen is at a concentration of about 0.1 mg/ml to about 5 mg/ml in the collagen solution.

77. The method of any one of clauses 61 to 76 wherein the collagen is at a concentration of about 0.5 mg/ml to about 4 mg/ml in the collagen solution.

78. The method of any one of clauses 61 to 77 wherein the collagen composition is sterilized.

79. The method of any one of clauses 61 to 78 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, gamma irradiation, ultraviolet radiation, E-beam, and combinations thereof.

80. The method of any one of clauses 61 to 79 wherein the collagen composition is sterilized by filtration.

81. The method of any one of clauses 61 to 80 wherein the buffer solution further comprises about 0.3 mM to about 3 mM KH_2PO_4.

82. The method of any one of clauses 61 to 81 wherein the buffer solution further comprises about 1 mM to about 10 M Na_2HPO_4.

83. The method of any one of clauses 61 to 82 wherein the buffer solution further comprises about 0.1 mM to about 4 mM KCl.

84. The method of any one of clauses 61 to 83 wherein the buffer solution further comprises about .02 M to about 0.3 M NaCl.

85. The method of any one of clauses 61 to 84 wherein the buffer solution further comprises about .002 N to about .02 N NaOH.

86. The method of any one of clauses 61 to 85 wherein the buffer solution further comprises about 0.5 weight percent to about 5 weight percent of glucose.

87. The method of any one of clauses 61 to 85 wherein the buffer solution comprises about 0.5 weight percent of glucose or less.

88. The method of any one of clauses 61 to 87 further comprising adding cells to the collagen solution.

89. The method of any one of clauses 61 to 88 wherein the matrix comprises collagen fibrils.

90. A collagen matrix prepared according to the method of any one of clauses 61 to 89.

91. The collagen matrix of clause 90 wherein the collagen matrix is a medical graft.

92. The collagen matrix of clause 91 wherein the medical graft has a use selected from the group consisting of a tissue graft material, an injectable graft material, a wound dressing, a hemostatic dressing, a delivery vehicle for therapeutic cells, and a delivery vehicle for a therapeutic agent.

93. The collagen matrix of clause 90 wherein the collagen matrix is used for research purposes.

94. The collagen matrix of clause 93 wherein the collagen matrix is used for drug toxicity testing or drug development.

95. A kit comprising lyophilized collagen, a hydrochloric acid solution, and a buffer solution.

96. The kit of clause 95 wherein the buffer solution comprises about .03 mM to about 0.2 mM $MgCl_2$.

97. The kit of clause 95 wherein the buffer solution comprises about .002 mM to about .02 mM $MgCl_2$.

98. The kit of clause 95 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

99. The kit of clause 95 wherein the buffer solution does not comprise $MgCl_2$.

100. The kit of any one of clauses 95 to 99 wherein the buffer solution further comprises about .003 M to about .03 M KH_2PO_4.

101. The kit of any one of clauses 95 to 100 wherein the buffer solution further comprises about .01 M to about 0.1 M Na_2HPO_4.

102. The kit of any one of clauses 95 to 101 wherein the buffer solution further comprises about .001 M to about .04 M KCl.

103. The kit of any one of clauses 95 to 102 wherein the buffer solution further comprises about 0.2 M to about 3.0 M NaCl.

104. The kit of any one of clauses 95 to 103 wherein the buffer solution further comprises about .02 N to about 0.2 N NaOH.

105. The kit of any one of clauses 95 to 104 wherein the buffer solution further comprises about 0.2 weight percent to about 5 weight percent of glucose.

106. The kit of any one of clauses 95 to 105 wherein the buffer solution comprises about 0.5 weight percent glucose or less.

107. The kit of any one of clauses 95 to 106 wherein the hydrochloric acid solution comprises about .005 N hydrochloric acid to about 0.1 N hydrochloric acid.

108. The kit of any one of clauses 95 to 107 wherein the buffer solution is capable of polymerizing collagen using a single mixing step comprising mixing the buffer solution with the lyophilized collagen reconstituted in the hydrochloric acid solution.

109. The kit of any one of clauses 95 to 108 wherein the lyophilized collagen, the hydrochloric acid solution, and the buffer solution are in separate containers.

110. The kit of any one of clauses 95 to 109 further comprising instructions for use of components of the kit.

111. A method for preparing a matrix, said method comprising polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, wherein the collagen in the collagen solution polymerizes to form the matrix.

112. The method of any one of clauses 1 to 33, 61 to 89, or 111 wherein the collagen is soluble collagen.

113. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 112 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using ultraviolet radiation.

114. The method of clause 113 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition this is not irradiated, a collagen solution that is not irradiated, or a collagen matrix that is not irradiated, respectively.

115. The method of clause 114 wherein the polymerization property is shear storage modulus.

116. The method of any one of clauses 113 to 115 wherein the radiation dose ranges from about 5 mJ/cm^2 to about 800 mJ/cm^2.

117. The method of any one of clauses 113 to 115 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

118. The method of any one of clauses 113 to 117 wherein the sterilization inactivates viruses.

119. The collagen matrix of any one of clauses 34 to 38 or 90 to 94 wherein the collagen matrix is sterilized using ultraviolet radiation.

120. The collagen matrix of clause 119 wherein the collagen matrix maintains a polymerization property relative to a collagen matrix that is not irradiated.

121. The collagen matrix of clause 120 wherein the polymerization property is shear storage modulus.

122. The collagen matrix of any one of clauses 119 to 121 wherein the radiation dose ranges from about 5 mJ/cm^2 to about 800 mJ/cm^2.

123. The collagen matrix of any one of clauses 119 to 121 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

124. The collagen matrix of any one of clauses 119 to 123 wherein the sterilization inactivates viruses.

125. The kit of any one of clauses 39 to 60 or 95 to 110 wherein the collagen composition or the lyophilized collagen is sterilized using ultraviolet radiation.

126. The kit of clause 125 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition this is not irradiated or to lyophilized collagen that is not irradiated, respectively.

127. The kit of clause 126 wherein the polymerization property is shear storage modulus.

128. The kit of any one of clauses 125 to 127 wherein the radiation dose ranges from about 5 mJ/cm^2 to about 800 mJ/cm^2.

129. The kit of any one of clauses 125 to 127 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

130. The kit of any one of clauses 125 to 129 wherein the sterilization inactivates viruses.

131. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 118 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation.

132. The method of any one of clauses 1 to 19, 21 to 33, 61 to 79, 81 to 89, or 111 to 118 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation and sterile filtration.

133. The collagen matrix of any one of clauses 34 to 38, 90 to 94, or 119 to 124 wherein the collagen matrix is sterilized using UVC irradiation.

134. The collagen matrix of any one of clauses 34 to 38, 90 to 94, or 119 to 124 wherein the collagen matrix is sterilized using UVC irradiation and sterile filtration.

135. The kit of any one of clauses 39 to 60, 95 to 110, or 125 to 130 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation.

136. The kit of any one of clauses 39 to 60, 95 to 110, or 125 to 130 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation and sterile filtration.

As would be understood by a skilled artisan, polymerization, self-assembly, self-assembled, fibril formation, and matrix-forming capability have the same meaning, and as would be understood by a skilled artisan the matrix can be in fibrillar form. For preparation of the collagen for use in the methods and compositions described herein, any method known in the art for preparing collagen can be used. In illustrative embodiments, the collagen can be prepared by methods described in Bailey JL, Critser PJ, Whittington C, Kuske JL, Yoder MC, Voytik-Harbin SL; Collagen oligomers modulate physical and biological properties of three-dimensional self-assembled matrices, *Biopolymers* (2011) 95(2):77-93, Kreger ST, Bell BJ, Bailey J, Stites E, Kuske J, Waisner B, Voytik-Harbin SL; Polymerization and matrix physical properties as important design considerations for soluble collagen formulations, *Biopolymers* (2010) 93(8):690-707, U.S. Patent Application Publication Number 20080268052, or U.S. Patent Application Publication Number 20120027732, each of which is incorporated herein by reference.

In various illustrative embodiments, the collagen for use in the methods and compositions described herein can be obtained from any suitable source of collagen known in the art. Exemplary collagen sources include submucosa tissues (U.S. Patents Nos. 4,902,508, 5,281,422, and 5,275,826), pericardial tissue, urinary bladder submucosa tissue, stomach submucosa tissue, liver basement membrane tissue, placental tissue, ovarian tissue, animal tail tissue, skin tissue (e.g., Gallop, et al., Preparation and Properties of Soluble Collagens, *Meth. Enzymol.* 6: 635-641 (1963), incorporated herein by reference), and extracellular matrix tissues generally. In various embodiments, the type of collagen for use in the methods and compositions described herein can be any suitable type of collagen, including, but not limited to, Type I collagen, Type II collagen, Type III collagen, or Type IV collagen, or combinations thereof.

In one embodiment, a tissue enriched in collagen oligomers (e.g., pig skin tissue) can also be used to obtain the collagen for use in the methods and compositions described herein, or the collagen can be obtained from cells producing collagen oligomers (e.g., cells altered by recombinant techniques to express collagen oligomers), or by chemically crosslinking the collagen to obtain collagen oligomers (e.g., using a cross-linking agent known in the art). In various embodiments, the collagen for use in the methods and compositions described herein can comprise oligomers or can consist of oligomers. In another embodiment the collagen can comprise oligomers, and other forms of collagen such as monomers, telocollagen, and/or atelocollagen.

In another embodiment, the collagen can be soluble collagen or solubilized collagen. In the embodiments where the collagen is soluble collagen or solubilized collagen,

the collagen is substantially free of insoluble collagen, but may contain some insoluble collagen. In another embodiment, the collagen consists of soluble collagen or solubilized collagen.

In various illustrative embodiments, the collagen, the collagen composition, the collagen matrix, the collagen solution, the lyophilized collagen, and/or the buffer solution can be sterilized using sterilization techniques known in the art, including but not limited to, propylene oxide or ethylene oxide treatment, gas plasma sterilization, gamma radiation (e.g., 1-4 mrads), ultraviolet radiation (e.g., UVC irradiation), electron beam, viral filtration, sterile filtration (e.g., with a 0.22 μm filter), chloroform exposure, and/or peracetic acid sterilization, and combinations thereof. In this embodiment, the sterilization procedure should not adversely affect the structure of collagen, the polymerization properties of the collagen, or the biological properties of the collagen that is sterilized. In various embodiments, the collagen can be sterilized before or after lyophilization (lyophilization procedures are described below).

In the ultraviolet radiation (e.g., UVC irradiation) embodiment, the collagen matrix that results from collagen polymerization can maintain a polymerization property relative to collagen that is not irradiated, a collagen composition this is not irradiated, a collagen matrix that is not irradiated, a collagen solution that is not irradiated, or lyophilized collagen that is not irradiated, respectively. In this embodiment, the polymerization property can be selected from shear storage modulus, elastic modulus (Young's modulus), tensile modulus, compressive modulus, fibril architecture, proteolytic degradation, cellular signaling, and combinations thereof. In various embodiments, the ultraviolet radiation dose (e.g., UVC irradiation) can range from about 5 mJ/cm^2 to about 800 mJ/cm^2, about 5 mJ/cm^2 to about 700 mJ/cm^2, about 5 mJ/cm^2 to about 600 mJ/cm^2, about 5 mJ/cm^2 to about 500 mJ/cm^2, about 5 mJ/cm^2 to about 400 mJ/cm^2, about 5 mJ/cm^2 to about 300 mJ/cm^2, 5 mJ/cm^2 to about 200 mJ/cm^2, 5 mJ/cm^2 to about 100 mJ/cm^2, 5 mJ/cm^2 to about 50 mJ/cm^2, about 30 mJ/cm^2 to about 800 mJ/cm^2, about 30 mJ/cm^2 to about 700 mJ/cm^2, about 30 mJ/cm^2 to about 600 mJ/cm^2, about 30 mJ/cm^2 to about 500 mJ/cm^2, about 30 mJ/cm^2 to about 400 mJ/cm^2, about 30 mJ/cm^2 to about 300 mJ/cm^2, about 30 mJ/cm^2 to about 200 mJ/cm^2, about 30 mJ/cm^2 to about 100 mJ/cm^2, about 30 mJ/cm^2 to about 50 mJ/cm^2, about 200 mJ/cm^2 to about 800 mJ/cm^2, about 300 mJ/cm^2 to about 800 mJ/cm^2, about 400 mJ/cm^2 to about 800 mJ/cm^2, about 500 mJ/cm^2 to about 800 mJ/cm^2, about 600 mJ/cm^2 to about 800 mJ/cm^2, about 50 mJ/cm^2 to about 300 mJ/cm^2, about 100 mJ/cm^2 to about 300 mJ/cm^2, or about 200 mJ/cm^2 to about 300 mJ/cm^2. In all of the ultraviolet radiation embodiments (e.g., UVC irradiation) described herein, the sterilization inactivates viruses. In this embodiment, "inactivates viruses" means

inactivating all viruses, whether infectious or not, reducing the number of infectious viruses, or inhibiting the activity of viruses, whether infectious or not.

In one aspect, the collagen for use in the methods and compositions described herein can be purified by methods known in the art for purifying collagen. As used herein, "purified" means removing contaminants including, but not limited to, cellular contaminants, nucleotide contaminants, and endotoxins. In various embodiments, the collagen can be purified by removing contaminants so that it is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% pure. In another embodiment the collagen can be isolated. As used herein "isolated" means substantially free of contaminants including, but not limited to, cellular contaminants, nucleotide contaminants, and endotoxins.

In one illustrative embodiment, the collagen for use in the methods and compositions described herein can be lyophilized and then reconstituted to form the collagen composition for mixing with the buffer solution as described herein. In this embodiment, the reconstitution of the lyophilized collagen is not a mixing step for polymerization of the collagen. As used herein, the term "lyophilized" means that water is removed from the protein, compound, or composition, by, for example, freeze-drying under a vacuum. Any lyophilization method known to the skilled artisan can be used. In one aspect, the collagen can be lyophilized in an acid, for example, acetic acid, hydrochloric acid, formic acid, lactic acid, citric acid, sulfuric acid, or phosphoric acid. In another embodiment, the collagen can be lyophilized in water. In other illustrative embodiments, cryoprotectants or lyoprotectants, or combinations thereof, can be used during the lyophilization.

In one illustrative aspect, the lyophilized collagen can be reconstituted to form the collagen composition described herein for mixing with the buffer solution to polymerize the collagen. In various illustrative embodiments, the collagen can be reconstituted in an acidic solution or in water. In various embodiments, the acidic solution can comprise acetic acid, hydrochloric acid, formic acid, lactic acid, citric acid, sulfuric acid, or phosphoric acid. In illustrative embodiments, the acidic solution for reconstitution can have a concentration of the acid of from about .005 N to about 0.1 N, from about .005 N to about .08 N, from about .005 N to about .06 N, from about .005 N to about 0.04 N, from about .005 N to about .02 N, from about .005 N to about .01 N, or about .01 N. In one embodiment, the acid can be hydrochloric acid and the hydrochloric acid can be about .005 N to about 0.1 N hydrochloric acid. In another embodiment, the acid can be hydrochloric acid and the hydrochloric acid can be about .01 N hydrochloric acid.

In one illustrative aspect, the collagen concentration in the collagen composition or in the collagen solution can be from about 0.1 mg/ml to about 40 mg/ml, from about 0.1 mg/ml to about 5 mg/ml, or from about 0.5 mg/ml to about 4 mg/ml. In other embodiments, the collagen concentration in the collagen composition or in the collagen solution can be from about 0.05 to about 5.0 mg/ml, about 1.0 mg/ml to about 3.0 mg/ml, about 0.05 mg/ml to about 10 mg/ml, about 0.05 to about 20 mg/ml, about 0.05 to about 30 mg/ml, about 0.05 to about 40 mg/ml, about 0.05 to about 50 mg/ml, about 0.05 to about 60 mg/ml, about 0.05 to about 80 mg/ml, about 5 mg/ml to 10 mg/ml, about 5 mg/ml to 20 mg/ml, about 5 mg/ml to about 40 mg/ml, about 5 mg/ml to 60 mg/ml, about 5 mg/ml to about 100 mg/ml, about 20 mg/ml to about 40 mg/ml, about 20 mg/ml to 60 mg/ml, or about 20 mg/ml to about 100 mg/ml.

In an illustrative embodiment, the collagen composition is mixed in a single step with the buffer solution to polymerize the collagen. In another embodiment, the collagen composition is mixed with the buffer solution in the absence of magnesium or manganese ions to polymerize the collagen. In one embodiment, the collagen composition is mixed with the buffer solution to form the collagen solution and the collagen solution is incubated at a temperature greater than about 25 °C to promote polymerization of the collagen in the collagen solution. In another embodiment, the collagen solution can be incubated at about 37 °C to promote polymerization of the collagen in the collagen solution. In various other embodiments, the collagen solution can be incubated at about 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, 30 °C, 31 °C, 32 °C, 33 °C, 34 °C, 35 °C, 36 °C, 38 °C, 39 °C, or 40 °C, to promote polymerization of the collagen in the collagen solution. In another embodiment, the collagen solution can be incubated at from about 25 °C to about 40 °C to promote polymerization of the collagen in the collagen solution. In other embodiments, the polymerization can be conducted at temperatures above 20 °C, or at a temperature selected from the range of about 20 °C to about 40 °C. In these embodiments, the collagen can be polymerized to form fibrils similar to those found in the body.

In one embodiment, the buffer solution to be mixed with the collagen composition to form the collagen solution can comprise about .03 mM to about 0.2 mM $MgCl_2$, about .002 mM to about .02 mM $MgCl_2$, less than about .02 mM $MgCl_2$, or no $MgCl_2$. In other embodiments, the buffer solution to be mixed with the collagen composition to form the collagen solution can comprise about 0.3 mM to about 3 mM KH_2PO_4, about 1 mM to about 10 M Na_2HPO_4, about 0.1 mM to about 4 mM KCl, about .02 M to about 0.3 M NaCl, and about .002 N to about .02 N NaOH. In another embodiment, the buffer solution to be mixed with the collagen composition to form the collagen solution can comprise about 0.5 weight percent to about 5 weight percent of glucose, about 0.5 weight percent glucose or less, or no glucose.

In one aspect, the buffer solution can be diluted from a 10X, 5X, 2X, or any suitable starting concentration, to make a 1X buffer solution having any of the component concentrations in the preceding paragraph. In one aspect, the kit described herein can contain a buffer solution with a concentration of 10X, 5X, or 2X, or any suitable starting concentration, for dilution to make a 1X buffer solution. In accordance with one embodiment, the 10X buffer solution can comprise the following ingredients at the following concentrations:

1.37 M NaCl
0.027 M KCl
0.081 M Na_2HPO_4
0.015 M KH_2PO_4
0.1 N NaOH
and, optionally, 55.5 mM glucose

In another embodiment, a 1X buffer solution can comprise the following ingredients at the following concentrations:

0.137 M NaCl
0.0027 M KCl
0.0081 M Na_2HPO_4
0.0015 M KH_2PO_4
.01 N NaOH
and, optionally, 5.55 mM glucose

In these embodiments, NaOH is present in the buffer solution. In conventional previously known methods for polymerizing collagen, the NaOH was added separately as an additional mixing step in the methods for polymerization of collagen. In another illustrative embodiment, calcium chloride can be present in the buffer solution at a concentration of about 0.4 mM to about 2.0 mM.

In various embodiments, the buffer in the buffer solution may be selected from the group consisting of phosphate buffer saline (PBS), Tris (hydroxymethyl) aminomethane Hydrochloride (Tris-HCl), 3-(N-Morpholino) Propanesulfonic Acid (MOPS), piperazine-n,n'-bis (2-ethanesulfonic acid) (PIPES), [n-(2-Acetamido)]-2-Aminoethanesulfonic Acid (ACES), N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES), and 1,3-

bis[tris(Hydroxymethyl)methylamino]propane (Bis Tris Propane). In one embodiment the buffer is PBS.

In various illustrative embodiments, the pH of the collagen solution for the polymerization of collagen is selected from the range of about 5.0 to about 11, about 6.0 to about 9.0, about 6.5 to about 8.5, and in another embodiment the pH is about 7.3 to about 7.4.

In various embodiments, nutrients, including minerals, amino acids, sugars, peptides, proteins, vitamins, or glycoproteins that facilitate cellular proliferation, such as laminin and fibronectin, hyaluronic acid, or growth factors such as epidermal growth factor, platelet-derived growth factor, transforming growth factor beta, or fibroblast growth factor, and glucocorticoids such as dexamethasone, can be added to the collagen solution before or after collagen polymerization is complete or during collagen polymerization. In accordance with another embodiment, cells can be added to the collagen solution before or after collagen polymerization is complete or during collagen polymerization. In various embodiments, the cells can be selected from the group consisting of epithelial cells, endothelial cells, mesodermally-derived cells, mesothelial cells, synoviocytes, neural cells, glial cells, osteoblasts, fibroblasts, chondrocytes, tenocytes, smooth muscle cells, skeletal muscle cells, cardiac muscle cells, multi-potential progenitor cells (e.g., stem cells, including bone marrow progenitor cells), adipocytes, and osteogenic cells.

In one embodiment, a collagen matrix prepared according to the any of the methods described herein is provided. In one aspect, the collagen matrix can be a medical graft. In one embodiment, the medical graft has a use selected from the group consisting of a tissue graft material, an injectable graft material, a wound dressing, a hemostatic dressing, a delivery vehicle for therapeutic cells, and a delivery vehicle for a therapeutic agent. In another embodiment, the methods described herein may be used to make a bioink formulation for printing tissues or organs. In another embodiment, the collagen matrix is used for research purposes, such as drug toxicity testing or drug development. In one embodiment, the matrices prepared by the methods described herein can serve as substrates for the regrowth of endogenous tissues at the implantation site (e.g., remodeling) and the matrices can have the characteristics of the damaged or diseased tissues that they replace at the site of implantation or injection.

In one illustrative embodiment, the matrices described herein can contain fibrils with a fibril area fraction (defined as the percent area of the total area occupied by fibrils in a cross-sectional surface of the matrix) or a fibril volume fraction (the percent area of the total area occupied by fibrils in 3 dimensions) of about 0.1% to about 100%, about 0.5% to about 100%, about 0.5% to about 26%, about 1% to about 100%, about 1% to about 26%, about 1%

to about 7%, about 1% to about 15%, of about 7% to about 26%, about 20% to about 30%, about 20% to about 50%, about 20% to about 70%, about 20% to about 100%, about 30% to about 50%, about 30% to about 70%, or about 30% to about 100%, and/or a modulus (e.g., an elastic or linear modulus (defined by the slope of the linear region of the stress-strain curve obtained using conventional mechanical testing protocols; i.e., stiffness), a compressive modulus, or a shear storage modulus) of about 0.5 kPa to about 40 kPa, about 30 kPa to 100 kPa, about 30 kPa to about 1000 kPa, about 30 kPa to about 10000 kPa, about 30 kPa to about 70000 kPa, about 100 kPa to 1000 kPa, about 100 kPa to about 10000 kPa, or about 100 kPa to about 70000 kPa.

In another embodiment, a kit comprising lyophilized collagen, a hydrochloric acid solution, and a buffer solution is described. In yet another embodiment, a kit comprising a collagen composition and a buffer solution is provided. In these kit embodiments, the buffer solution can comprise about .03 mM to about 0.2 mM $MgCl_2$, about .002 mM to about .02 mM $MgCl_2$, less than about .02 mM $MgCl_2$, or the buffer solution does not comprise $MgCl_2$. In various embodiments, the buffer solution further comprises about .003 M to about .03 M KH_2PO_4, about .01 M to about 0.1 M Na_2HPO_4, about .001 M to about .04 M KCl, about 0.2 M to about 3.0 M NaCl, and about .02 N to about 0.2 N NaOH. In another aspect, the buffer solution can comprise about 0.2 weight percent to about 5 weight percent of glucose, about 0.5 weight percent glucose or less, or no glucose.

In one aspect, in the kit embodiment having a hydrochloric acid solution, the hydrochloric acid solution can comprise about .005 N hydrochloric acid to about 0.1 N hydrochloric acid. In one aspect, in the kit embodiment having lyophilized collagen, a hydrochloric acid solution, and a buffer solution the lyophilized collagen, the hydrochloric acid solution, and the buffer solution are in separate containers. In one aspect of the embodiment having the collagen composition and the buffer solution, the collagen in the collagen composition can be at a concentration of about 0.1 mg/ml to about 40 mg/ml or about 0.1 mg/ml to about 5 mg/ml. In this embodiment, the collagen composition and the buffer solution can be in separate containers, such as sterilized vials or separate compartments of a dual syringe comprising a mixing element. In any of the kit embodiments described herein, the kit can comprise instructions for use of components of the kit. In any of the kit embodiments described herein, the buffer solution is capable of polymerizing collagen using a single mixing step comprising mixing the buffer solution with the lyophilized collagen reconstituted in the hydrochloric acid solution or with the collagen composition.

In another embodiment, a kit is provided with collagen in a lyophilized form and the kit further comprises a buffer solution as described herein and a solution of an acid, such as

acetic acid, or another dilute acid including for example, hydrochloric acid, formic acid, lactic acid, citric acid, sulfuric acid, or phosphoric acid for reconstituting the lyophilized collagen.

The following examples illustrate specific embodiments in further detail. These examples are provided for illustrative purposes only and should not be construed as limiting the invention in any way.

EXAMPLE 1

PREPARATION OF COLLAGEN COMPOSITION

Type I collagen oligomers were derived from the dermis of closed herd pigs and prepared as described previously (Bailey JL, Critser PJ, Whittington C, Kuske JL, Yoder MC, Voytik-Harbin SL; Collagen oligomers modulate physical and biological properties of three-dimensional self-assembled matrices, *Biopolymers* (2011) 95(2):77-93 and Kreger ST, Bell BJ, Bailey J, Stites E, Kuske J, Waisner B, Voytik-Harbin SL; Polymerization and matrix physical properties as important design considerations for soluble collagen formulations, *Biopolymers* (2010) 93(8):690-707, both incorporated herein by reference). Prior to use, lyophilized collagen oligomers were dissolved in 0.01 N hydrochloric acid. Research-grade oligomer was rendered aseptic by chloroform exposure at 4°C. Medical-grade oligomer was sterile filtered using a 0.22 μm Millex-GP PES Express syringe filter (Millipore, SLGPO33RS). A Sirius Red (Direct Red 80) assay was used to determine collagen concentration. Oligomer formulations were standardized based upon purity as well as polymerization capacity according to the ASTM international consensus standard F3089-14 (ASTM Standard F3089, 2014, "Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions", ASTM International, West Conshohocken, PA, F3089-14). Polymerization capacity is defined by matrix shear storage modulus (G') as a function of collagen concentration of the polymerization reaction. Multi-step self-assembly was performed and involved 3 reagents—10X Polymerization Buffer PLUS, 0.1N NaOH, and Polymerization Supplement. Single-step self-assembly was performed with 10X self-assembly reagents (diluted 1:10) prepared according to the following recipe in the absence of $MgCl_2$:

2 g KH_2PO_4 (FW 136.09)
11.5 g Na_2HPO_4 (FW 141.96)
2 g KCl (FW 74.55)
10 g glucose
80 g NaCl (FW 58.44)
20 ml 5N NaOH

All reagents were added to Milli-Q filtered water to achieve a finalized volume of 1 liter and sterile filtered (0.22 μm). Integra Flowable was obtained from Integra Life Sciences (Plainsboro, NJ) and handled according to manufacturer's instructions.

EXAMPLE 2

VISCOELASTIC PROPERTIES TESTING

Viscoelastic properties of self-assembled collagen matrices were determined using oscillatory shear mode on an AR2000 rheometer (TA Instruments, New Castle, DE) as previously described (Kreger et al., 2010). Samples were polymerized on the rheometer stage for 30 min followed by a shear-strain sweep from 0.1% to 4% strain at 1 Hz. The shear storage modulus (G') at 1% strain was used as a measure of matrix stiffness.

EXAMPLE 3

CELL CULTURE AND PREPARATION OF THREE-DIMENSIONAL COLLAGEN-FIBRIL TISSUE CONSTRUCTS

Low-passage human adipose-derived stem cells (hASC) were obtained from Zen-Bio (Research Triangle Park, NC) and cultured in growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Carlsbad, CA), 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomyosin (Invitrogen, Grand Island, NY). The hASC were grown and maintained in a humidified environment of 5% carbon dioxide in air at 37°C. Cells were kept below 80% confluence and used in experiments at passage 6 to 9.

Cell-encapsulated collagen-fibril tissue constructs were prepared by suspending hASC (300,000 cells/ml) in neutralized oligomer (200 Pa) solutions. Neutralization was achieved using multi-step or single-step procedures and reagents. Tissue constructs were fixed in 3% paraformaldehyde after 3 days of culture and stained with phalloidin for visualization of the actin cytoskeleton. For 3D qualitative analysis, tissue constructs were imaged using an Olympus FluoView FV-1000 confocal system adapted to an inverted microscope (IX81, Olympus Corporation, Tokyo, Japan).

EXAMPLE 4

MOUSE SUBCUTANEOUS INJECTION

C57BL/6 mice (n=5) obtained from Harlan Laboratories (Indianapolis, Indiana) were initially anesthetized in a 1 L induction chamber and then maintained unconscious with a coaxial nose cone non-rebreathing system that administered 1-3% isoflurane in 1.5 L/min

medical grade air. Sterile eye lubricant was applied and animals were positioned on an adjustable heated stage set to 44°C to maintain internal body temperature near 37°C. Heart rate, respiration rate, and body temperature were monitored every 15 minutes with stage electrodes and a rectal probe, respectively. Hair on the dorsal side was removed with clippers and depilatory cream (Nair, Church & Dwight Co., Inc. Ewing, NJ). Experimental groups included 1) research-grade oligomer (500Pa), 2) medical-grade oligomer (500Pa), 3) Integra Flowable, 4) phosphate buffered saline (PBS), or 5) no treatment control. Oligomer, Integra Flowable, and PBS formulations were injected (200 µl) subcutaneously on both sides of the back, parallel to the sagittal plane. Oligomer and PBS formulations were administered using 27G, ½ inch needles, while Integra Flowable required 18 G, ½ inch needles because of material consistency and clogging of smaller needle sizes. After four weeks, animals were euthanized and implant sites harvested along with generous margins of surrounding host tissue. All specimens were fixed in formalin, paraffin-embedded, thin-sectioned, and stained with hematoxylin and eosin (H&E) and Masson's trichrome.

EXAMPLE 5

DEVELOPMENT AND VALIDATION OF SINGLE-STEP SELF-ASSEMBLY PROCEDURE AND REAGENT

The single-step self-assembly procedure and reagent included maintenance of i) physiologically relevant conditions and reagents and ii) polymerization capacity as observed with an established multi-step procedure. Initial attempts to combine all reagents used in a multi-step procedure, including 10X Polymerization Buffer PLUS, Polymerization Supplement, and NaOH, resulted in an unstable solution that formed a flocculent precipitate. However, when $MgCl_2$ was removed, no precipitation was noted, even when stored at 4°C for extended periods of time. Based upon these results, follow-up studies were performed to evaluate the polymerization capacity and *in vitro* cell response. When compared to multi-step procedures, the single-step procedure yielded similar polymerization capacity curves and similar in-vitro cell behavior and morphology as shown in Figures 1 and 2, respectively. Preliminary studies have also been conducted to show utility of single-step self-assembly for three-dimensional bioprinting applications. More specifically, Oligomer, 10X Self-Assembly Reagent, and a diluent have been employed to print tissue constructs containing continuous gradients in collagen-fibril density using a custom-designed single nozzle bioprinting device. The new single-step collagen self-assembly procedure and associated formulary are shown in Figures 3 and 4, respectively.

EXAMPLE 6

DEVELOPMENT AND VALIDATION OF COLLAGEN POLYMER STERILIZATION

Additional studies were performed to evaluate sterilization methods that would support scale-up manufacturing and regulatory approval of medical-grade oligomer products. Sterile filtration was employed using a conventional 0.22 μm syringe-based filter device. Because oligomer represents a soluble molecular solution, it is amenable to sterile filtration. Oligomer formulation (500 Pa) showed statistically similar ($p>0.05$) polymerization kinetics and capacity before (458±55 Pa) and after (434±49) sterile filtration. Additional studies confirmed that similar *in vivo* tissue responses were obtained with research-grade and medical-grade oligomer formulations. Furthermore, the in-vivo tissue response obtained using single-step polymerization of research-grade oligomer was consistent with those obtained using multi-step polymerization procedures (see Fig. 5).

EXAMPLE 7

EFFECTS OF ULTRAVIOLET IRRADIATION OF COLLAGEN ON THE PROPERTIES OF THE COLLAGEN MATRIX

Monochromatic ultraviolet radiation at 254nm (UVC) was produced by a flat-plate collimated beam system, employing a low-pressure Hg lamp source. Thin layers (1 mm) of collagen solution were placed in petri dishes and treated with UVC doses of 0 (untreated control), 30 mJ/cm^2 (1 minute of irradiation) and 300 mJ/cm^2 (10 minutes of irradiation). The polymerization capacity and associated viscoelastic properties of the treated samples were measured as described in Example 2 and compared to untreated controls. Shear storage modulus measurements were statistically similar for oligomer solutions exposed to 0, 30, and 300 mJ/cm^2 doses of UVC irradiation (Fig. 7).

WHAT IS CLAIMED IS:

1. A method for preparing a matrix, said method comprising polymerizing collagen using a single mixing step comprising mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix.

2. The method of claim 1 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, ultraviolet radiation, gamma irradiation, E-beam, and combinations thereof.

3. The method of claim 1 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

4. The method of claim 1 wherein the buffer solution does not comprise $MgCl_2$.

5. A collagen matrix prepared according to the method of claim 1.

6. A kit comprising a collagen composition and a buffer solution wherein the buffer solution is capable of polymerizing the collagen using a single mixing step comprising mixing the collagen composition with the buffer solution.

7. The kit of claim 6 wherein the buffer solution comprises less than about .02 mM $MgCl_2$.

8. The kit of claim 6 wherein the buffer solution does not comprise $MgCl_2$.

9. A method for preparing a matrix, said method comprising polymerizing collagen by mixing a collagen composition with a buffer solution to form a collagen solution, and polymerizing the collagen in the collagen solution to form the matrix wherein the buffer solution does not contain magnesium ions or manganese ions.

10. The method of claim 9 wherein the collagen composition, the collagen solution, or the collagen matrix is sterilized by a method selected from the group consisting of exposure to chloroform, viral filtration, sterile filtration, ultraviolet radiation, gamma irradiation, E-beam, and combinations thereof.

11. A collagen matrix prepared according to the method of claim 9.

12. The method of claim 2 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using ultraviolet radiation.

13. The method of claim 12 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition

this is not irradiated, a collagen solution that is not irradiated, or a collagen matrix that is not irradiated, respectively.

14. The method of claim 13 wherein the polymerization property is shear storage modulus.

15. The method of claim 12 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

16. The method of claim 12 wherein the sterilization inactivates viruses.

17. The collagen matrix of claim 5 wherein the collagen matrix is sterilized using ultraviolet radiation.

18. The collagen matrix of claim 17 wherein the collagen matrix maintains a polymerization property relative to a collagen matrix that is not irradiated.

19. The collagen matrix of claim 18 wherein the polymerization property is shear storage modulus.

20. The collagen matrix of claim 17 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

21. The collagen matrix of claim 17 wherein the sterilization inactivates viruses.

22. The kit of claim 6 wherein the collagen composition or the lyophilized collagen is sterilized using ultraviolet radiation.

23. The kit of claim 22 wherein the collagen matrix that results from collagen polymerization maintains a polymerization property relative to a collagen composition this is not irradiated or to lyophilized collagen that is not irradiated, respectively.

24. The kit of claim 23 wherein the polymerization property is shear storage modulus.

25. The kit of claim 22 wherein the radiation dose ranges from about 30 mJ/cm^2 to about 300 mJ/cm^2.

26. The kit of claim 22 wherein the sterilization inactivates viruses.

27. The method of claim 12 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation.

28. The method of claim 12 wherein the collagen composition, the collagen solution, and/or the collagen matrix is sterilized using UVC irradiation and sterile filtration.

29. The collagen matrix of claim 17 wherein the collagen matrix is sterilized using UVC irradiation.

30. The collagen matrix of claim 17 wherein the collagen matrix is sterilized using UVC irradiation and sterile filtration.

31. The kit of claim 22 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation.

32. The kit of claim 22 wherein the collagen composition or the lyophilized collagen is sterilized using UVC irradiation and sterile filtration.

5

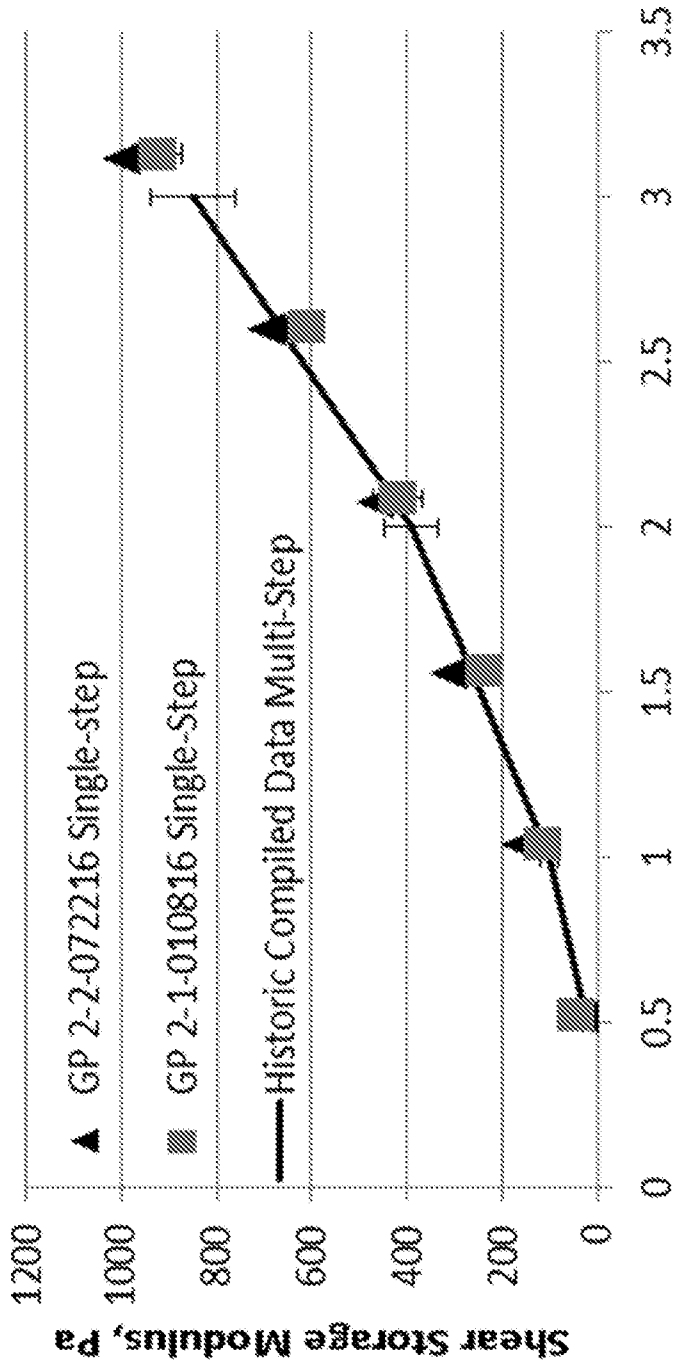
Shear Storage Modulus, Pa
1200
1000
800
600
400
200
0
0
0.5
1
1.5
2
2.5
3
3.5
Oligomer Concentration (mg/ml)
GP 2-2-072216 Single-step
GP 2-1-010816 Single-Step
Historic Compiled Data Multi-Step

FIG. 1

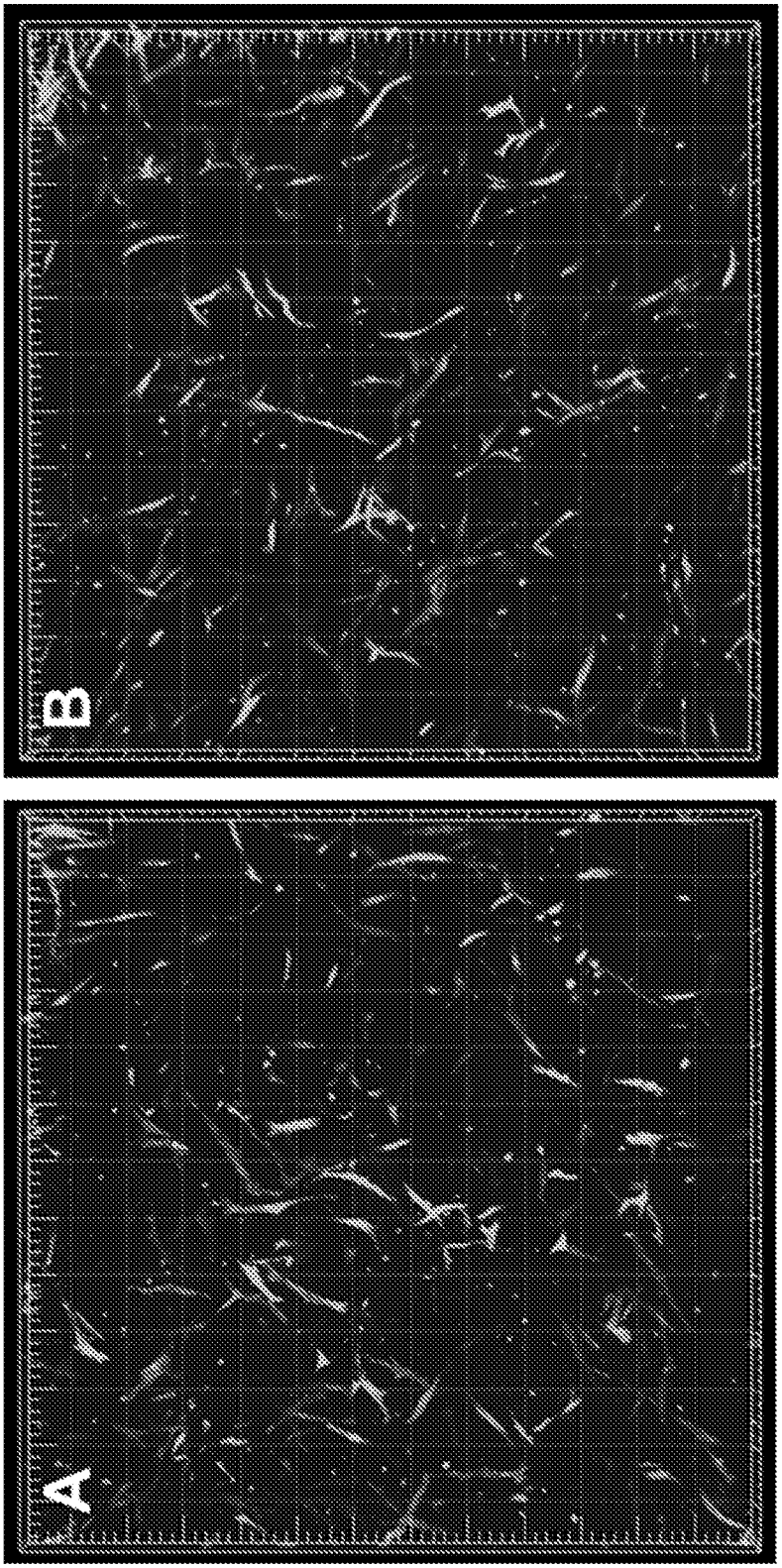

FIG. 2

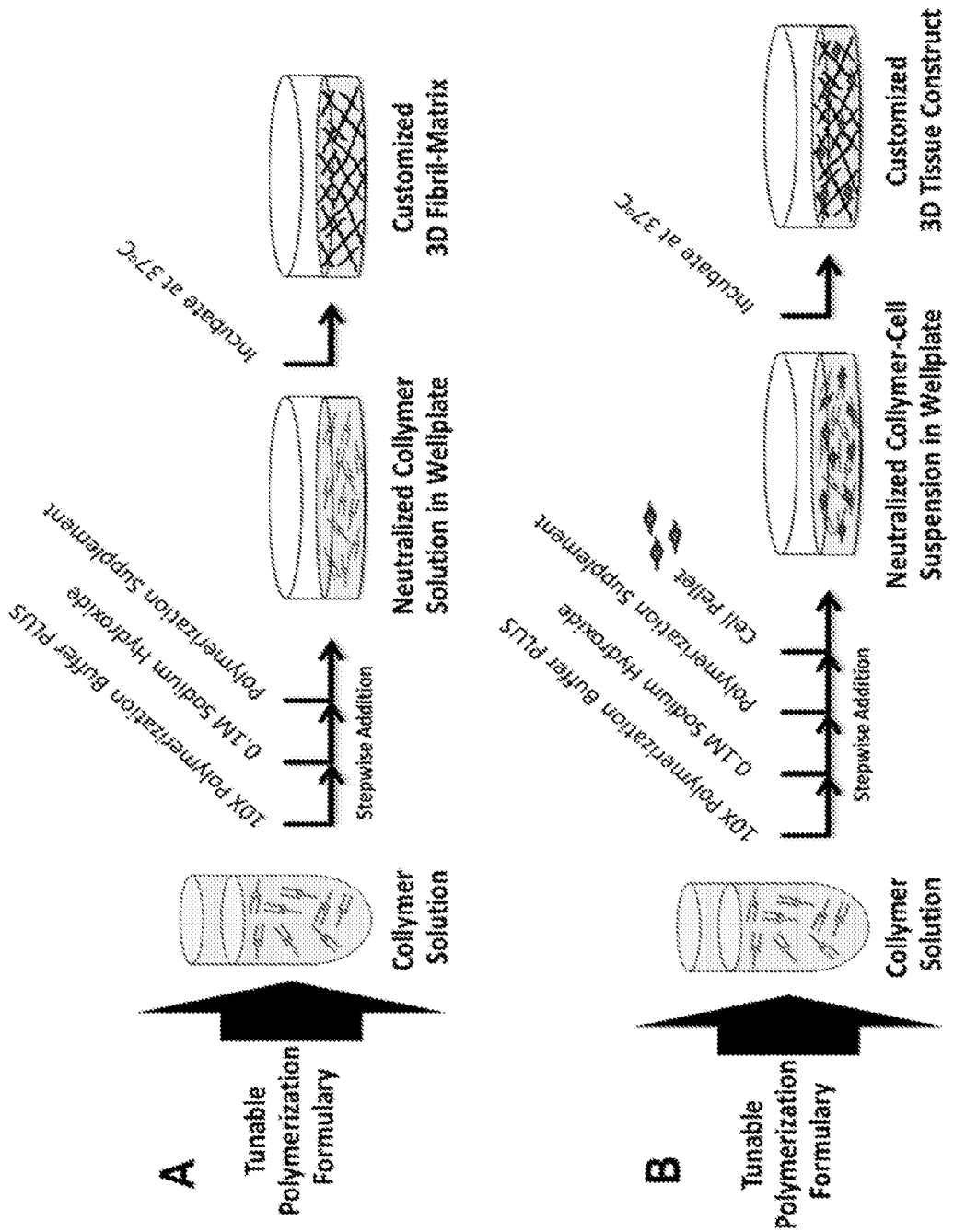
A
Tunable Polymerization Formulary
Collymer Solution
10X Polymerization Buffer PLUS
0.1M Sodium Hydroxide
Polymerization Supplement
Stepwise Addition
Neutralized Collymer Solution in Wellplate
Incubate at 37°C
Customized 3D Fibril-Matrix
B
Tunable Polymerization Formulary
Collymer Solution
10X Polymerization Buffer PLUS
0.1M Sodium Hydroxide
Polymerization Supplement
Cell Pellet
Stepwise Addition
Neutralized Collymer-Cell Suspension in Wellplate
Incubate at 37°C
Customized 3D Tissue Construct

FIG. 3

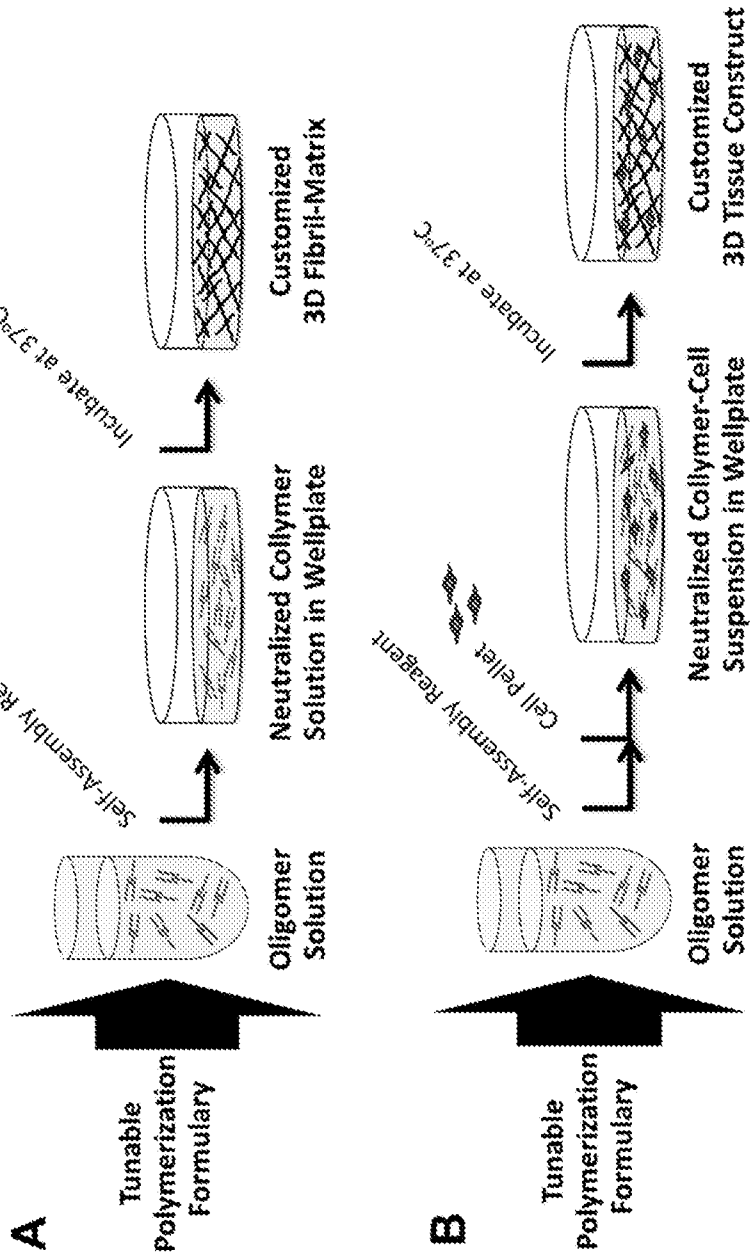
A
Tunable Polymerization Formulary
Oligomer Solution
Self-Assembly Reagent
Neutralized Collymer Solution in Wellplate
Incubate at 37°C
Customized 3D Fibril-Matrix
B
Tunable Polymerization Formulary
Oligomer Solution
Self-Assembly Reagent
Cell Pellet
Neutralized Collymer-Cell Suspension in Wellplate
Incubate at 37°C
Customized 3D Tissue Construct

FIG. 4

FIG. 4

CUSTOMIZABLE COLLYMER POLYMERIZATION FORMULARY-SINGLE STEP POLYMERIZATION

Product Name: Standardized Oligomer Polymerization Kit

Product Number: OPK1001

Lot Number: 16001BA

Customize 3D Collagen-Fibril Matrix Stiffness (G', Pa)

Oligomer Concentration (mg/ml)	Matrix Stiffness (G', Pa)	Final Volume (ml)	0.01M HCl (ml)	Stock Oligomer (ml)	10X Self Assembly Reagent (ml)
0.58	50	1.000	0.789	0.111	0.100
0.97	100	1.000	0.715	0.185	0.100
1.61	250	1.000	0.591	0.309	0.100
2.30	500	1.000	0.459	0.441	0.100
2.82	750	1.000	0.359	0.541	0.100
3.28	1000	1.000	0.276	0.624	0.100
3.64	1250	1.000	0.203	0.697	0.100

	Desired Stiffness	Desired Final Volume			
Customized Formulation	2.30	500	1.000	0.459	0.441	0.100

Customize 3D Collagen-Fibril Matrix Concentration (mg/ml)

Oligomer Concentration (mg/ml)	Matrix Stiffness (G', Pa)	Final Volume (ml)	0.01M HCl (ml)	Stock Oligomer (ml)	10X Self Assembly Reagent (ml)
0.50	43	1.000	0.804	0.096	0.100
1.00	196	1.000	0.708	0.192	0.100
1.50	218	1.000	0.613	0.287	0.100
2.00	378	1.000	0.517	0.383	0.100
2.50	586	1.000	0.421	0.479	0.100
3.00	847	1.000	0.325	0.575	0.100
4.00	1513	1.000	0.134	0.766	0.100

	Desired Concentration	Desired Final Volume			
Customized Formulation	2.00	378	1.000	0.517	0.383	0.100

FIG. 5

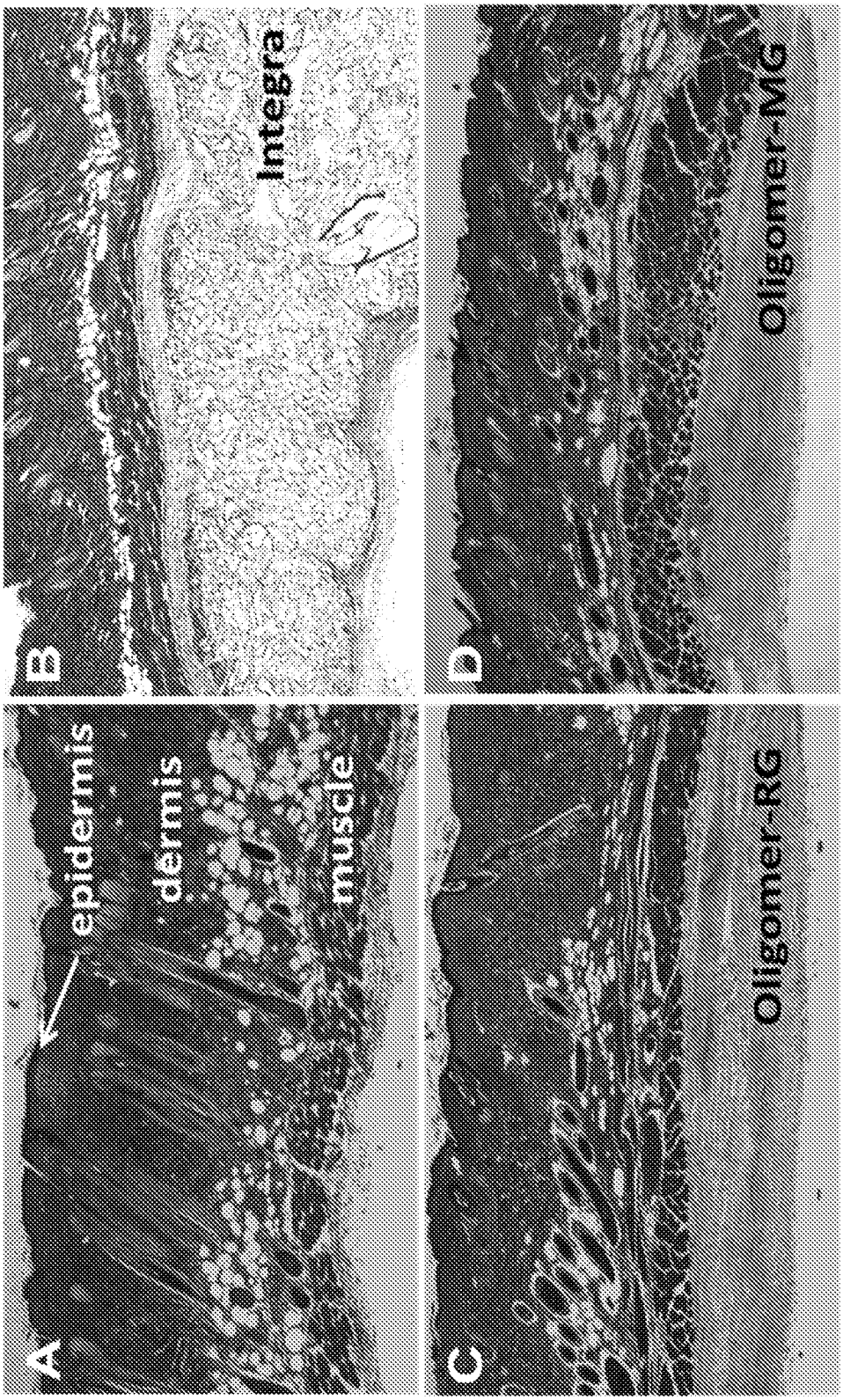
A
epidermis
dermis
muscle
B
Integra
C
Oligomer-RG
D
Oligomer-MG

FIG. 6

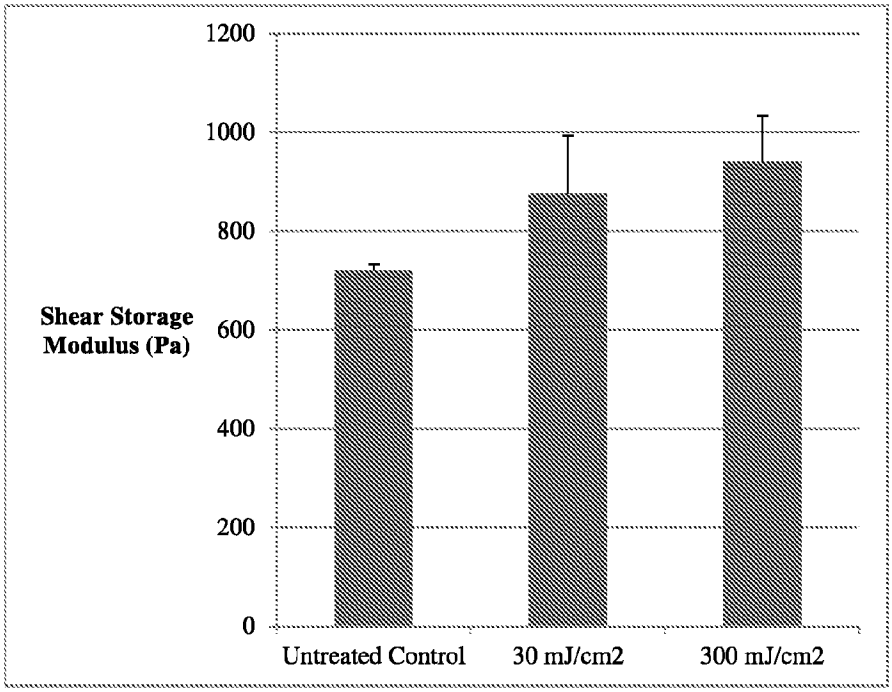
1200
1000
800
600
400
200
0
Shear Storage Modulus (Pa)
Untreated Control
30 mJ/cm2
300 mJ/cm2

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/016069

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/39; A61L 27/24; C07K 14/78 (2018.01)
CPC - A61L 27/24; A61L 27/34; C08H 1/06; C08L 89/06 (2018.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2015/0367030 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) 24 December 2015 (24.12.2015) entire document	1-11 --- 12-32
Y	US 2012/0297550 A1 (NGO et al) 29 November 2012 (29.11.2012) entire document	12-32
Y	US 2009/0280180 A1 (VOYTIK-HARBIN et al) 12 November 2009 (12.11.2009) entire document	13, 14, 18, 19, 23, 24, 28, 30, 32
A	US 2008/0268052 A1 (VOYTIK-HARBIN et al) 30 October 2008 (30.10.2008) entire document	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 March 2018	23 MAR 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774