SUMMARY

[005a] In a first aspect of the present disclosure, there is provided a plurality of extracellular vesicles comprising an exogenous extracellular matrix messenger RNA (mRNA), wherein the extracellular matrix messenger RNA (mRNA) comprises a Col1A1 collagen, and the plurality of extracellular vesicles does not comprise an alpha 2 chain of collagen type I (Col1A2).

[005b] In another aspect of the present disclosure, there is provided a plurality of extracellular vesicles comprising an exogenous extracellular matrix messenger RNA (mRNA), wherein the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 450 EVs.

[006] Described herein, in some examples, is a plurality of extracellular vesicles comprising an exogenous extracellular matrix messenger RNA (mRNA). In some cases, the exogenous extracellular matrix mRNA encodes a collagen. In some cases, the collagen is selected from the group consisting of Collagen type I, Collagen type II, Collagen type III, Collagen type IV, Collagen type V, Collagen type VI, Collagen type VII, Collagen type VIII, Collagen type IX, Collagen type X, Collagen type XI, Collagen type XII, Collagen type XIII, Collagen type XIV, Collagen type XV, Collagen type XVI, Collagen type XVII, Collagen type XVIII, Collagen type XIX, Collagen type XX, Collagen type XXI, Collagen type XXII, Collagen type XXIII, Collagen type XXIV, Collagen type XXV, Collagen type XXVI, Collagen type XXVII, Collagen type XXVIII, or any combination thereof. In some cases, the collagen is collagen type I. In some cases, the collagen is collagen type II. In some cases, the collagen is alpha 1 chain of collagen type I (Col1A1). In some cases, the exogenous extracellular matrix mRNA is the mRNA encoding pro-alpha1(I) chain. In some cases, the collagen is Col1A1, and the plurality of extracellular vesicles do not comprise an alpha 2 chain of collagen type I (Col1A2). In some cases, the collagen is Col1A2.

[007] In some cases, the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 450 EVs. In some cases, the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 200 EVs. In some cases, the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per about 0.1 to 100 EVs. In some cases, the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 2000 EVs, per 1000 EVs, per 500 EVs, per 400 EVs, per 300 EVs, per 200 EVs, per 150 EVs, per 100 EVs, per 90 EVs, per 80 EVs, per

70 EVs, 60 EVs, 50 EVs, per 40 EVs, per 30 EVs, per 20 EVs, per 15 EVs, per 10 EVs, per 5 EVs, per 2 EVs, or per 1 EV. In some cases, the plurality of extracellular vesicles comprise an average of at least 1, 2, 5, 10, 50, 100, 200, 300, 500, or 1000 copies of the exogenous extracellular matrix mRNA per EV. In some cases, the plurality of extracellular vesicles comprise an average of about 1 to 10 copies of the exogenous extracellular matrix mRNA per EV. In some cases, the plurality of extracellular vesicles comprise an average of about 1 copy of the exogenous extracellular matrix mRNA per EV. In some cases, the plurality of extracellular vesicles comprise an average of at least one copy of the exogenous extracellular matrix mRNA per 30 EVs.

fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 50-fold, at least 75-fold, at least 100-fold, at least 500-fold, at least 1000-fold, at least 1500-fold, or at least 2000-fold higher than a level of the exogenous extracellular matrix mRNA in an identical amount of naturally-occurring extracellular vesicles. In some cases, the exogenous extracellular matrix mRNA is present at a level that is about 3000-fold higher than a level of the exogenous extracellular matrix mRNA in an identical amount of naturally-occurring extracellular vesicles. In some cases, the exogenous extracellular matrix mRNA is present at a level that is about 2000-fold higher than a level of the exogenous extracellular matrix mRNA in an identical amount of naturally-occurring extracellular vesicles. In some cases, the plurality of extracellular vesicles expresses higher levels of at last one of the following markers compared to a comparable number of naturally-occurring extracellular vesicles: CD9, CD63, TSG101, or ARF6.

[0015] Described herein, in some examples, is an extracellular vesicle comprising an exogenous extracellular matrix messenger RNA (mRNA). In some cases, the exogenous extracellular matrix mRNA encodes a collagen. In some cases, the collagen is selected from the group consisting of Collagen type I, Collagen type II, Collagen type III, Collagen type IV, Collagen type V, Collagen type VI, Collagen type VII, Collagen type VIII, Collagen type IX, Collagen type X, Collagen type XI, Collagen type XII, Collagen type XIII, Collagen type XIV, Collagen type XV, Collagen type XVI, Collagen type XVII, Collagen type XVIII, Collagen type XIX, Collagen type XX, Collagen type XXI, Collagen type XXII, Collagen type XXIII, Collagen type XXIV, Collagen type XXV, Collagen type XXVI, Collagen type XXVII, Collagen type XXVIII, or any combination thereof. In some cases, the collagen is collagen type I. In some cases, the collagen is collagen type II. In some cases, the collagen is alpha 1 chain of collagen type I (Col1A1). In some cases, the exogenous extracellular matrix mRNA is the mRNA encoding pro-alpha1(I) chain. In some cases, the collagen is Col1A1, and the extracellular vesicle does not comprise an alpha 2 chain of collagen type I (Col1A2). In some cases, the collagen is Col1A2. In some cases, the extracellular vesicle comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, and any combination thereof. In some cases, the extracellular vesicle is an exosome. In some cases, the extracellular vesicle is an apoptotic body. In some cases, the extracellular vesicle is a microvesicle.

[0016] Described herein, in some example, is a vessel comprising the plurality of extracellular vesicles of any one of the preceding claims, wherein the vessel further comprises a cell. In some cases, the cell comprises a human cell, a human fibroblast cell, a fibroblast cell, a dermal

fibroblast, a human fibroblast, an adult fibroblast, a human adult fibroblast, a neonatal fibroblast, a neonatal human fibroblast, a neonatal human dermal fibroblast, or any combination thereof. In some cases, the plurality of extracellular vesicles are present in the vessel at a ratio of at least 1000, 2000, 5000, 10000, or 12000 extracellular vesicles per cell. In some cases, the plurality of extracellular vesicles are present in the vessel at a ratio of no greater than 1000, 2000, 5000, 10000, 12000, 15000, 20000, 25000, 50000, 75000, 90000, or 100000 extracellular vesicles per cell.

[0017] Described herein, in some examples, is a needle comprising the plurality of extracellular vesicles disclosed herein. In some cases, the needle is a microneedle. In some cases, the needle is solid. In some cases, the needle is a hydrogel needle. In some cases, at least 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the needle comprises hydrogel. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof. In some cases, the hydrogel comprises hyaluronic acid. In some cases, the hydrogel comprises at least 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises at most 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid. In some cases, the hydrogel comprises about 15% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid and at most 30% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 20% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 15% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 18% hyaluronic acid.

[0018] Described herein, in some examples, is a syringe comprising the plurality of extracellular vesicles disclosed herein. In some cases, the plurality of extracellular vesicles are suspended in hyaluronic acid within the syringe.

[0019] Described herein, in some examples, is a hydrogel microneedle, wherein the hydrogel microneedle comprises a plurality of extracellular vesicles. In some cases, the hydrogel comprises at least 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises at least 5%, 7%, 10%, 15%, or 20 % hyaluronic acid. In some cases, the hydrogel comprises no greater than 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid,

polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof. In some cases, the hydrogel comprises hyaluronic acid. In some cases, the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, and any combination thereof. In some cases, the plurality of extracellular vesicles comprise at least one exosome. In some cases, the plurality of extracellular vesicles comprise at least one apoptotic body. In some cases, the plurality of extracellular vesicles comprise at least one microvesicle. In some cases, the plurality of extracellular vesicles comprises at least $1x10^6$, $5x10^6$, $1x10^7$, $5 x10^7$, $1x10^8$, $3x10^8$, $5 x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, $1 x10^{14}$, $1 x10^{15}$, $1 x10^{16}$, $1 x10^{20}$, $1 x10^{25}$, or $1 x10^{30}$ extracellular vesicles. In some cases, the plurality of extracellular vesicles comprises no great than $1x10^6$, $5x10^6$, $1x10^7$, $5 x10^7$, $1x10^8$, $3x10^8$, $5 x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, $1 x10^{14}$, $1 x10^{15}$, $1 x10^{16}$, $1 x10^{20}$, $1 x10^{25}$, $1 x10^{30}$, $1 x10^{30}$, $1 x10^{40}$, $1 x10^{50}$, $1 x10^{60}$, $1 x10^{70}$, $1 x10^{80}$, $1 x10^{90}$, or $1 x10^{100}$ extracellular vesicles. In some cases, the plurality of extracellular vesicles comprises about $1x10^6$ to $1 x10^{30}$ extracellular vesicles. In some cases, the plurality of extracellular vesicles comprises about $1x10^{10}$ to $1 x10^{14}$ extracellular vesicles. In some cases, the plurality of extracellular vesicles comprises about $1x10^{13}$ to $1 x10^{14}$ extracellular vesicles.

[0020] In some cases, the hydrogel microneedle has a length less than 2 mm. In some cases, the hydrogel microneedle has a length less than 1 mm. In some cases, the hydrogel microneedle has a length of at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, at least 1600, at least 1700, at least 1800, at least 1900, at least 2000μm. In some cases, the hydrogel microneedle has a length no greater than 100, no greater than 200, no greater than 300, no greater than 400, no greater than 500, no greater than 600, no greater than 700, no greater than 800, no greater than 900, no greater than 1000, no greater than 1100, no greater than 1200, no greater than 1300, no greater than 1400, no greater than 1500, no greater than 1600, no greater than 1700, no greater than 1800, no greater than 1900, or no greater than 2000μm. In other cases, the hydrogel microneedle has a length from 100μm to 3000μm, from 500μm to 2500μm, from 1000μm to 2500μm, or from 1000μm to 2000μm. In specific examples, the hydrogel microneedle has a length of about 1000μm. In specific examples, the hydrogel microneedle has a length of about 2000μm.

[0021] In some cases, the hydrogel microneedle has a base with a diameter of no greater than 10000 μm, 9000 μm, 8000 μm, 7000 μm, 6000 μm, 5000 μm, 4000 μm, 3000 μm, 2000 μm,

1800 μm, 1500 μm, 1000 μm, 900 μm, 800 μm 700 μm, 600 μm, 500 μm, 400 μm, 300 μm, 200 μm, or 100 μm. In some cases, the hydrogel microneedle has a base with a diameter of more than 10000 μm, 9000 μm, 8000 μm, 7000 μm, 6000 μm, 5000 μm, 4000 μm, 3000 μm, 2000 μm, 1800 μm, 1500 μm, 1000 μm, 900 μm, 800 μm 700 μm, 600 μm, 500 μm, 400 μm, 300 μm, 200 μm, or 100 μm. In some cases, the hydrogel microneedle comprises extracellular vesicles loaded with one or more mRNA cargos.

[0022] Described herein, in some examples, is a needle comprising a plurality of extracellular vesicles wherein the extracellular vesicles comprise at least one extracellular matrix messenger RNA (mRNA). In some cases, the extracellular matrix mRNA encodes a collagen. In some cases, the collagen is selected from the group consisting of collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, collagen type XIV, collagen type XV, collagen type XVI, collagen type XVII, collagen type XVIII, collagen type XIX, collagen type XX, collagen type XXI, collagen type XXII, collagen type XXIII, collagen type XXIV, collagen type XXV, collagen type XXVI, collagen type XXVII, collagen type XXVIII, or any combination thereof. In some cases, the collagen is collagen type I. In some cases, the collagen is collagen type II. In some cases, the collagen is alpha 1 chain of collagen type I (Col1A1). In some cases, the collagen is Col1A1, and the plurality of extracellular vesicles do not comprise an alpha 2 chain of collagen type I (Col1A2). In some cases, the collagen is Col1A2. In some cases, the extracellular matrix mRNA is the mRNA encoding pro-alpha1(I) chain.

[0023] In some cases, the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, and any combination thereof. In some cases, the plurality of extracellular vesicles comprise at least one exosome. In some cases, the plurality of extracellular vesicles comprise at least one apoptotic body. In some cases, the plurality of extracellular vesicles comprise at least one microvesicle. In some cases, the plurality of extracellular vesicles comprise a mixture of any two of the following: exosome, microvesicle or apoptotic body.

[0024] In some cases, the needle is a microneedle. In some cases, the needle is solid. In some cases, the needle is a hydrogel needle, and optionally wherein at least 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the needle comprises hydrogel. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any

combination thereof. In some cases, the hydrogel comprises hyaluronic acid. In some cases, the hydrogel comprises at least 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid. In some cases, the hydrogel comprises no greater than 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises about 15% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid and at most 30% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 20% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 15% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 18% hyaluronic acid.

[0025] In some cases, the needle has a length less than 2 mm. In some cases, the needle has a length less than 1 mm. In some cases, the needle has a length of at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, at least 1600, at least 1700, at least 1800, at least 1900, at least 2000μm. In some cases, the needle has a length no greater than 100, no greater than 200, no greater than 300, no greater than 400, no greater than 500, no greater than 600, no greater than 700, no greater than 800, no greater than 900, no greater than 1000, no greater than 1100, no greater than 1200, no greater than 1300, no greater than 1400, no greater than 1500, no greater than 1600, no greater than 1700, no greater than 1800, no greater than 1900, or no greater than 2000μm. In other cases, the needle has a length from 100μm to 3000μm, from 500μm to 2500μm, from 1000μm to 2500μm, or from 1000μm to 2000μm. In specific examples, the needle has a length of about 1000μm. In specific examples, the needle has a length of about 2000μm.

[0026] In some cases, the needle has a base with a diameter of no greater than 10000 μm, 9000 μm, 8000 μm, 7000 μm, 6000 μm, 5000 μm, 4000 μm, 3000 μm, 2000 μm, 1800 μm, 1500 μm, 1000 μm, 900 μm, 800 μm, 700 μm, 600 μm, 500 μm, 400 μm, 300 μm, 200 μm, or 100 μm. In some cases, the needle has a base with a diameter of more than 10000 μm, 9000 μm, 8000 μm, 7000 μm, 6000 μm, 5000 μm, 4000 μm, 3000 μm, 2000 μm, 1800 μm, 1500 μm, 1000 μm, 900 μm, 800 μm, 700 μm, 600 μm, 500 μm, 400 μm, 300 μm, 200 μm, or 100 μm.

[0027] Described herein, in some examples, is a method of treating a skin condition in a subject comprising administering at least one extracellular matrix mRNA to a subject in need thereof, thereby treating the skin condition. In some cases, the exogenous extracellular matrix mRNA encodes a collagen. In some cases, the collagen is selected from the group consisting of collagen

days, every 7 days, every 10 days, every 20 days, every 30 days, every 40 days, or every 50 days. In some cases, the administering of the at least one extracellular vesicle comprising the extracellular matrix mRNA to the subject is repeated about every 10 days, every 20 days, every 30 days, every 40 days, or every 50 days. In some cases, the administering of the at least one extracellular vesicle comprising the extracellular matrix mRNA to the subject is repeated about every 30 days. In some cases, the administering of the at least one extracellular vesicle comprising the extracellular matrix mRNA to the subject is repeated about every 60 days. In some cases, the administering of the at least one extracellular vesicle comprising the extracellular matrix mRNA to the subject is repeated about every 90 days.

[0040] In some cases, the method further comprises producing the extracellular vesicles comprising the at least one extracellular matrix mRNA by: (a) introducing a vector or a plasmid correspond to the extracellular matrix mRNA into a donor cell via transfection; (b) culturing the donor cell for a sufficient amount of time in a culture medium for the production of extracellular vesicles encapsulating the at least one extracellular matrix mRNA transcribed from the vector or the plasmid; and (c) collecting the extracellular vesicles from the culture medium. In some cases, in step (c) the collecting the extracellular vesicles occurs about 8-24hr after the transfection. In some cases, the extracellular vesicles are present at a ratio of at least 1000, 2000, 5000, 10000, or 12000 extracellular vesicles per donor cell. In some cases, the extracellular vesicles are present at a ratio of no greater than 1000, 2000, 5000, 10000, 12000, 15000, 17000, 20000, 25000, 30000, 35000, 40000, 45000, or 50000 extracellular vesicles per donor cell. In some cases, the donor cell is a human cell, a human fibroblast cell, a fibroblast cell, a dermal fibroblast, a human fibroblast, an adult fibroblast, a human adult fibroblast, a neonatal fibroblast, a neonatal human fibroblast, a neonatal human dermal fibroblast, or any combination thereof.

[0041] Described herein, in some examples, is a method of producing an extracellular vesicle comprising extracellular matrix mRNA comprising: a) introducing a vector or a plasmid correspond to the extracellular matrix mRNA into a donor cell via transfection; (b) culturing the donor cell for a sufficient amount of time in a culture medium for the production of extracellular vesicles encapsulating the at least one extracellular matrix mRNA transcribed from the vector or the plasmid; and (c) collecting the extracellular vesicles from the culture medium. In some cases, in step (c) the collecting the extracellular vesicles occurs about 8-24hr after the transfection. In some cases, the extracellular vesicles encapsulating the extracellular matrix mRNA transcribed from the vector or plasmid are present at a level of at least 1000, 2000, 5000, 10000, or 12000 extracellular vesicles per donor cell. In some cases, the extracellular vesicles are present at a

ratio of no greater than 1000, 2000, 5000, 10000, 12000, 15000, 17000, 20000, 25000, 30000, 35000, 40000, 45000, or 50000 extracellular vesicles per donor cell. In some cases, the donor cell is a human cell, a human fibroblast cell, a fibroblast cell, a dermal fibroblast, a human fibroblast, an adult fibroblast, a human adult fibroblast, a neonatal fibroblast, a neonatal human fibroblast, a neonatal human dermal fibroblast, or any combination thereof.

[0042] Described herein, in some example, is a microneedle device, the microneedle device comprising a substrate and a plurality of microneedles, wherein the plurality of microneedles protrude from the substrate, and wherein at least one microneedle of the plurality of microneedles comprises at least one extracellular vesicle (EV).

[0043] In some cases, a density of the plurality of microneedles on the substrate is 0.1 microneedles to 100 microneedles / mm^2 of the substrate. In some cases, a density of the plurality of microneedles on the substrate is at least 0.3 microneedles/ mm^2 of the substrate. In some cases, a density of the plurality of microneedles on the substrate is about 0.59 microneedle/ mm^2 of the substrate. In some cases, the plurality of microneedles comprise about 10-1000 microneedles, and wherein the area of the substrate is 20 - 1000 mm^2. In some cases, the plurality of microneedles comprise about 100 microneedles, and wherein the areas of the substrate is about 169 mm^2.

[0044] In some cases, the plurality of microneedles are arranged in at least 2 rows and at least 2 microneedles in each row. In some cases, the plurality of microneedles are arranged in 10 rows and 10 microneedles in each row.

[0045] In some cases, the at least one microneedle of the plurality of microneedles comprises a hydrogel. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof.

[0046] In some cases, the at least one microneedle of the plurality of microneedles is conical in shape. In some cases, the at least one microneedle of the plurality of microneedles has a tapered shape.

[0047] In some cases, the at least one microneedle of the plurality of microneedles comprises a base with a diameter of less than 1000μm. In some cases, the at least one microneedle of the plurality of microneedles comprises a base with a diameter of from about 100μm to about 800μm. In some cases, the at least one microneedle of the plurality of microneedles comprises a base with a diameter of about 400μm.

[0048] In some cases, a length of the at least one microneedle of the plurality of microneedles is at least 100μm. In some cases, a length of the at least one microneedle of the plurality of microneedles is from 100μm to 3000μm. In some cases, a length of the at least one microneedle of the plurality of microneedles is about 1000μm. In some cases, a length of the microneedle of the plurality of microneedles is about 2000μm.

[0049] In some cases, a center-to-center distance between the microneedle of the plurality of microneedles is at least 100μm. In some cases, a center-to-center distance between the microneedle of the plurality of microneedles is about 100 to 2000μm. In some cases, a center-to-center distance between the microneedle of the plurality of microneedles is about 1000μm.

[0050] In some cases, at least one microneedle of the plurality of microneedles comprises at least $3x10^5$ EVs. In some cases, at least one microneedle of the plurality of microneedles comprises about $3x10^8$ to $3x10^{10}$ EVs.

[0051] In some cases, the EVs are suspended in hydrogel. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof. In some cases, the hydrogel comprises hyaluronic acid.

[0052] In some cases, the EVs comprise an exogenous extracellular matrix mRNA. In some cases, the exogenous extracellular matrix mRNA comprises collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, collagen type XIV, collagen type XV, collagen type XVI, collagen type XVII, collagen type XVIII, collagen type XIX, collagen type XX, collagen type XXI, collagen type XXII, collagen type XXIII, collagen type XXIV, collagen type XXV, collagen type XXVI, collagen type XXVII, collagen type XXVIII, or any combination thereof. In some cases, the exogenous extracellular matrix mRNA is the mRNA encoding pro-alpha1(I) chain.

[0053] In some cases, the EVs comprise exogenous VEGF mRNA. In some cases, the exogenous VEGF mRNA comprises VEGFA, VEGFB, VEGFC, VEGFD, PIGF, or any combination thereof.

[0054] Described herein, in some example, is a method of administering extracellular vesicles (EVs) to a tissue of a subject, the method comprising administering the needle, the syringe, the hydrogel needle, the microneedle, or the microneedle device disclosed herein to the tissue of the subject. In some cases, the method comprises administering the microneedle device disclosed herein to the tissue of the subject. In some cases, the microneedle device is removed after at least

some cases, the method results in more even distribution of EVs in the tissue than administering the EVs in a conventional microneedle or a syringe. In some cases, the conventional microneedle is a solid microneedle or a hollow microneedle.

[0059] Described herein, in some example, is a method of treating a skin condition in a subject in need thereof, the method comprising applying the needle, the syringe, the hydrogel needle, the microneedle, or the microneedle device disclosed herein to the subject. In some cases, the method comprising administering the microneedle disclosed herein to the subject.

[0060] In some cases, the skin condition is skin damage. In some cases, the skin damage is caused by aging or sun damage. In some cases, the skin condition is a wound.

[0061] In some cases, the administering comprises administering at least 0.1 ng, 1 ng, 5 ng, 10 ng, 20 ng, 30 ng, 40 ng, 50 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1μg, or 10 μg of the extracellular matrix mRNA. In some cases, the administering comprises administering no greater 0.1 ng, 1 ng, 5 ng, 10 ng, 20 ng, 30 ng, 40 ng, 50 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1μg, 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, or 900 μg of the extracellular matrix mRNA. In some cases, the administering comprises administering about 0.1 ng-50 μg, 1 ng-50 μg, 1 ng-30 μg, 1 ng-25 μg of the extracellular matrix mRNA. In some cases, the administering comprises administering about 1 ng-20 μg of the extracellular matrix mRNA.

[0062] In some cases, the method results in at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in appearance of wrinkles. In some cases, the method results in at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more collagen fibers. In some cases, the method results in a higher dermal thickness by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. In some cases, the method results in a prolonged effect for at least 30 days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, or at least 90 days.

[0063] In some cases, at least 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the needle, the microneedle, or the microneedle of the microneedle device comprises hydrogel. In some case, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof. In some cases, the hydrogel comprises hyaluronic acid. In some cases, the hydrogel comprises at least 1%, 5%, 7%, 10%, 12%, 15%, 20%, 25% 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid. In some cases, the hydrogel comprises about 15% hyaluronic acid. In

some cases, the hydrogel comprises at least 5% hyaluronic acid and at most 30% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 20% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 15% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 18% hyaluronic acid.

[0064] In some cases, the administering is performed a single time and administers at least $1x10^6$, $5x10^6$, $1x10^7$, $5 x10^7$, $1x10^8$, $3x10^8$, $5 x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, or $1 x10^{14}$ extracellular vesicles. In some cases, the administering is performed a single time and administers at most $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, $1 x10^{14}$, $1 x10^{15}$, $1 x10^{17}$, $1 x10^{18}$, $1 x10^{19}$, $1 x10^{20}$, or $1 x10^{30}$ extracellular vesicles. In some cases, the administering is performed a single time and administers at most $1x10^{13-14}$ extracellular vesicles. In some cases, the administering is performed multiple times and administers each time at least $1x10^6$, $5x10^6$, $1x10^7$, $5 x10^7$, $1x10^8$, $3x10^8$, $5 x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, or $1 x10^{14}$ extracellular vesicles. In some cases, the administering is performed multiple times and administers each time at most $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, $1 x10^{14}$, $1 x10^{15}$, $1 x10^{17}$, $1 x10^{18}$, $1 x10^{19}$, $1 x10^{20}$, or $1 x10^{30}$ extracellular vesicles. In some cases, the administering is performed multiple times and administers each time at most $1x10^{13-14}$ extracellular vesicles. In some cases, the administering is performed multiple times and administers at most $1x10^{13-14}$ extracellular vesicles within 6-8 weeks. In some cases, the administering is performed multiple times over a period of time and administers at most $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1 x10^{11}$, $1 x10^{12}$, $1 x10^{13}$, $1 x10^{14}$, $1 x10^{15}$, $1 x10^{17}$, $1 x10^{18}$, $1 x10^{19}$, or $1 x10^{20}$, $1 x10^{30}$ extracellular vesicles.

[0065] Described herein, in some example, is a method of manufacturing a microneedle device, wherein the method comprises: (a) mixing extracellular vesicles (EVs) with a first batch of polymerizable solution; and (b) casting the mixture from (a) to a polydimethylsiloxane (PDMS) mold with at least one needle-like shape. In some cases, the mixing in (a) is performed under vacuum.

[0066] In some cases, the method further concentrating the EVs in a tip of the at least one needle-like shape of the PDMS mold. In some cases, the concentrating is by maintaining the PDMS mold at a temperature of at most 10°C. In some cases, the concentrating is by maintaining the PDMS mold at about 4°C. In some cases, the concentrating lasts from about 2 to about 6 hours.

[0067] In some cases, the method further comprises adding a second batch of the polymerizable solution on top of the PDMS mold. In some cases, the polymerizable solution comprises

hydrogel. In some cases, the hydrogel comprises hyaluronic acid, sodium alginate, polylactic acid, polyglycolic acid, and polylactic-glycolic acid, cartilage thioflavin, silk protein, maltose, chitosan, carboxymethyl cellulose, or any combination thereof. In some cases, the hydrogel comprises hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid. In some cases, the hydrogel comprises about 15% hyaluronic acid. In some cases, the hydrogel comprises at least 5% hyaluronic acid and at most 30% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 20% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 15% hyaluronic acid. In some cases, the hydrogel comprises greater than 10% hyaluronic acid and at most 18% hyaluronic acid.

[0068] In some cases, the final ratio between EVs and the polymerizable solution is at least 1:2, 1:3, 1:4, 1:5, 1:10, 1: 15, 1: 20, 1: 25, 1: 30, 1: 35, 1: 40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:80, 1:90, or 1:100. In some cases, the final ratio between EVs and the polymerizable solution is no greater than 1:2, 1:3, 1:4, 1:5, 1:10, 1: 15, 1: 20, 1: 25, 1: 30, 1: 35, 1: 40, 1:45, or 1:50. In some cases, the final ratio between EVs and the polymerizable solution is about 1:5, 1:10, 1: 15, 1: 20, 1: 25, 1: 30, 1: 35, or 1: 40.

[0069] In some cases, the EVs comprise exogenous extracellular matrix mRNA. In some cases, the exogenous extracellular matrix mRNA comprises collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, collagen type XIV, collagen type XV, collagen type XVI, collagen type XVII, collagen type XVIII, collagen type XIX, collagen type XX, collagen type XXI, collagen type XXII, collagen type XXIII, collagen type XXIV, collagen type XXV, collagen type XXVI, collagen type XXVII, collagen type XXVIII, or any combination thereof. In some cases, the EVs comprise exogenous VEGF mRNA. In some cases, the exogenous VEGF mRNA comprises VEGFA, VEGFB, VEGFC, VEGFD, PIGF, or any combination thereof.

[0070] Described herein, in some example, is a method of producing a heterodimer or a heterotrimer of collagen type I in a tissue, wherein the method comprises administering to the tissue the plurality of extracellular vesicles disclosed herein. In some cases, the heterodimer or the heterotrimer comprises at least one alpha chain of collagen type I (Col1A1) and at least one alpha chain of collagen type I (Col1A2), and wherein the Col1A1 is exogenously delivered by the plurality of extracellular vesicles disclosed herein. In some cases, the Col1A2 is endogenous to the tissue.

[0071] Described herein, in some example, is a plurality of extracellular vesicles comprising an average of at least one copy of exogenous VEGF mRNA per 400 extracellular vesicles. In some cases, the exogenous VEGF mRNA is selected from the group consisting of VEGFA, VEGFB, VEGFC, VEGFD, PIGF, and any combination thereof. In some cases, the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, or any combination thereof. In some cases, the plurality of extracellular vesicles comprises an extracellular vesicle comprising a targeting polypeptide.

[0072] In some cases, the plurality of extracellular vesicles comprise at least one copy of exogenous VEGF mRNA per at most 1, 5, 10, 15, 20, 25, 30, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, or 350 extracellular vesicles. In some cases, the plurality of extracellular vesicles comprise at least 1, 2, 5, 10, 20, 25, 30, 35, 30, 50, 60, 70 90 or 100 copies of VEGF mRNA per extracellular vesicle. In some cases, the plurality of extracellular vesicles comprise at least 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 copies of VEGF mRNA per extracellular vesicle. In some cases, the plurality of extracellular vesicles comprise at most 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 copies of VEGF mRNA per extracellular vesicle. In some cases, the plurality of extracellular vesicles comprise at least one copy of exogenous VEGF mRNA per about 0.001 to 100, 0.01 to 100, or 0.01 to 50 extracellular vesicles. In some cases, the plurality of extracellular vesicles comprise at least one copy of exogenous VEGF mRNA per about 0.02 to 50 extracellular vesicles.

[0073] In some cases, the plurality of extracellular vesicles comprise at least $1x10^6$, $5x10^6$, $1x10^7$, $5\ x10^7$, $1x10^8$, $3x10^8$, $5\ x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1\ x10^{11}$, $1\ x10^{12}$, $1\ x10^{13}$, $1\ x10^{14}$, $1\ x10^{15}$, $1\ x10^{16}$, $1\ x10^{20}$, $1\ x10^{25}$, or $1\ x10^{30}$ extracellular vesicles. In some cases, the plurality of extracellular vesicles comprise at most $1x10^6$, $5x10^6$, $1x10^7$, $5\ x10^7$, $1x10^8$, $3x10^8$, $5\ x10^8$, $1x10^9$, $3x10^9$, $1x10^{10}$, $1\ x10^{11}$, $1\ x10^{12}$, $1\ x10^{13}$, $1\ x10^{14}$, $1\ x10^{15}$, $1\ x10^{16}$, $1\ x10^{20}$, $1\ x10^{25}$, or $1\ x10^{30}$ extracellular vesicles.

[0074] In some cases, the plurality of extracellular vesicles are formulated for intravenous injection, intramuscular injection, subcutaneous injection, or injection via a coronary artery catheter.

[0075] In some cases, the mixture comprises at least two extracellular vesicles that comprise between one and six copies of exogenous VEGF mRNA, and wherein the mixture comprises exosomes, microvesicles, and apoptotic bodies. In some cases, said mixture is formulated for injection via an intravenous, intramuscular, or subcutaneous route. In some cases, said mixture is formulated for injection via a coronary artery catheter. In some cases, VEGF mRNA is present at

a level that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 50-fold, at least 75-fold, at least 100-fold, at least 500-fold, at least 1000-fold, at least 1500-fold, or at least 2000-fold higher than a level of VEGF mRNA in an identical amount of naturally-occurring extracellular vesicles. In some cases, the plurality of extracellular vesicles comprise at least 10 pg, 50 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 ng, 100 ng, 500 ng, 1000 ng, 10 μg, 50 μg, 100 μg, 150 μg, or 200 μg of VEGF mRNA. In some cases, the plurality of extracellular vesicles comprise no greater than 10 pg, 50 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 ng, 100 ng, 500 ng, 1000 ng, 10 μg, 50 μg, 100 μg, 150 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, or 1000 μg of VEGF mRNA. In some cases, the plurality of extracellular vesicles comprise about 1 ng to 200 μg VEGF mRNA.

[0076] Described herein, in some example, is a method of treating a blood flow disorder in a subject comprising administering at least one extracellular vesicle comprising VEGF mRNA to the subject, thereby treating the blood flow disorder. In some cases, the treating the blood flow disorder results in at least a 5% increase in revascularization. In some cases, the at least 5% increase in revascularization occurs within 14 days of the administering of the at least one extracellular vesicle comprising VEGF mRNA.

[0077] In some cases, the administering of the at least one extracellular vesicle comprising VEGF mRNA comprises administering the at least one extracellular vesicle comprising VEGF mRNA to the subject via an intravenous, intramuscular or subcutaneous injection or via a coronary artery catheter. In some cases, the intravenous, intramuscular or subcutaneous injection is performed with a needle with a gauge of at least 14 gauge.

[0078] In some cases, the blood flow disorder is ischemia. In some cases, the at least one extracellular vesicle comprising VEGF mRNA is administered in at least one dose. In some cases, the at least one extracellular vesicle comprising VEGF mRNA comprises at least 1,000 extracellular vesicles comprising VEGF mRNA. In some cases, the at least one extracellular vesicle comprising VEGF mRNA is administered in at least two doses.

[0079] In some cases, the administering comprises administering a single time at least $1X10^5$, $1X10^6$, $1X10^7$, $1X10^8$, $1X10^9$, $1X10^{10}$, $1X10^{11}$, $1X10^{12}$, $1X10^{13}$, $1X10^{14}$, $1X10^{15}$, or $1X10^{16}$ EVs. In some cases, the administering comprises administering a single time no greater than $1X10^5$, $1X10^6$, $1X10^7$, $1X10^8$, $1X10^9$, $1X10^{10}$, $1X10^{11}$, $1X10^{12}$, $1X10^{13}$, $1X10^{14}$, $1X10^{15}$, $1X10^{16}$ $1X10^{17}$, $1X10^{18}$, $1X10^{19}$, $1X10^{20}$, $1X10^{21}$, $1X10^{22}$, $1X10^{23}$, or $1X10^{24}$ EVs. In some cases, the administering comprises administering a single time about $1X10^5$ to $1X10^{20}$, $1X10^6$ to $1X10^{19}$,

once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 10 weeks, once every 12 weeks, or once every 16 weeks. In some cases, the extracellular vesicles are administered to the subject at most once a day, once every week, once every 2 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 10 weeks, once every 12 weeks, or once every 16 weeks.

[0084] Described herein, in some example, is a method of producing an extracellular vesicle comprising VEGF mRNA comprising: (a) introducing a vector or plasmid encoding VEGF into a donor cell via transfection; (b) incubating the donor cell for a sufficient time for the production of extracellular vesicles encapsulating VEGF mRNA transcribed from the vector or plasmid; and (c) collecting the extracellular vesicles encapsulating the VEGF mRNA transcribed from the vector or plasmid.

[0085] Described herein, in some example, is a method of treating a blood flow disorder a subject in need thereof, the method comprising administering the extracellular vesicles disclosed herein. In some cases, the blood flow disorder is ischemia.

[0086] Described herein, in some examples, is a plurality of extracellular vesicles comprising an average of at least one copy of exogenous VEGF mRNA. In some examples, the exogenous VEGF mRNA is selected from the group consisting of VEGFA, VEGFB, VEGFC, VEGFD, PIGF, and any combination thereof. In some examples, the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, or any combination thereof. In some examples, the plurality of extracellular vesicles comprises an extracellular vesicle comprising a targeting polypeptide.

[0087] In some examples, described herein are mixtures of extracellular vesicles, wherein the mixture comprises at least two extracellular vesicles that comprise between one and six copies of exogenous VEGF mRNA, and wherein the mixture comprises exosomes, microvesicles, and apoptotic bodies. In some examples, the mixture is formulated for injection via an intravenous, intramuscular, or subcutaneous route. In some examples, the mixture is formulated for injection via a coronary artery catheter.

[0088] In some examples, described herein are methods of treating a blood flow disorder in a subject comprising administering at least one extracellular vesicle comprising VEGF mRNA to the subject, thereby treating the blood flow disorder. In some examples, treating the blood flow disorder results in at least a 5% increase in revascularization. In some examples, the at least 5% increase in revascularization occurs within 14 days of the administering of the at least one extracellular vesicle comprising VEGF mRNA. In some examples, administering of the

at least one extracellular vesicle comprising VEGF mRNA comprises administering the at least one extracellular vesicle comprising VEGF mRNA to the subject via an intravenous, intramuscular or subcutaneous injection or via a coronary artery catheter. In some examples, the intravenous, intramuscular or subcutaneous injection is performed with a needle with a gauge of at least 14 gauge. In some examples, the blood flow disorder is ischemia. In some examples, the at least one extracellular vesicle comprising VEGF mRNA is administered in at least one dose. In some examples, the dose comprises at least 1,000 extracellular vesicles comprising VEGF mRNA. In some examples, the at least one extracellular vesicle comprising VEGF mRNA is administered in at least two doses. In some examples, administering of the at least one extracellular vesicle comprising VEGF mRNA to the subject comprises administering of the at least one extracellular vesicle comprising VEGF mRNA to a tissue of the subject.

[0089] In some examples, described herein is a plurality of extracellular vesicles comprising an average of at least one copy of exogenous extracellular matrix mRNA. In some examples, the exogenous extracellular matrix mRNA is selected from the group consisting of collagen, COL1, collagen type I, collagen type II, collagen type III, collagen type V, collagen type XI, collagen type IX, collagen type XII, collagen type XIV, collagen type VIII, collagen type X, collagen type IV, collagen type VI, collagen type VII, collagen type XIII, collagen type XV, collagen type XVII, and collagen type XVIIII. In some examples, the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, or any combination thereof. In some examples, the plurality of extracellular vesicles comprises an extracellular vesicle comprising a targeting polypeptide.

[0090] In some examples, described herein are mixtures of extracellular vesicles, wherein the mixture comprises at least two extracellular vesicles that comprise between one and two copies of exogenous extracellular matrix mRNA, and wherein the mixture comprises exosomes, microvesicles, and apoptotic bodies. In some examples, the mixture is formulated for injection via an intravenous, intramuscular, or subcutaneous route.

[0091] In some examples, described herein are methods of treating a skin damage in a subject comprising administering at least one extracellular vesicle comprising extracellular matrix mRNA to the subject, thereby treating the skin damage in the subject. In some examples, treating the skin damage results in at least a 10% reduction in appearance of wrinkles. In some examples, the at least 10% reduction in appearance of wrinkles occurs within 14 days of the

administering of the at least one extracellular vesicle comprising extracellular matrix mRNA. In some examples, the administering of the at least one extracellular vesicle comprising extracellular matrix mRNA comprises administering the at least one extracellular vesicle comprising extracellular matrix mRNA to the subject via a subcutaneous injection. In some examples, subcutaneous injection is performed with a needle with a gauge of at least 14. In some examples, the skin damage is caused by aging or sun damage. In some examples, the at least one extracellular vesicle comprising extracellular matrix mRNA is administered in at least one dose. In some examples, the dose comprises at least 1,000 extracellular vesicles comprising extracellular matrix mRNA. In some examples, the at least one extracellular vesicle comprising extracellular matrix mRNA is administered in at least two doses. In some examples, administering of the at least one extracellular vesicle comprising extracellular matrix mRNA to the subject comprises administering of the at least one extracellular vesicle comprising VEGF mRNA to a tissue of the subject. In some examples, within 72 hours of the administering of the at least one extracellular vesicle comprising extracellular matrix mRNA to the subject, the tissue has a concentration of extracellular matrix protein of at least 6000 pg/ml.

[0092] In some examples, described herein are methods of producing an extracellular vesicle comprising VEGF mRNA comprising: introducing a vector or plasmid encoding VEGF into a donor cell via transfection; incubating the donor cell for a sufficient time for the production of extracellular vesicles encapsulating VEGF mRNA transcribed from the vector or plasmid; and collecting the extracellular vesicles encapsulating the VEGF mRNA transcribed from the vector or plasmid.

[0093] In some examples, described herein are methods of producing an extracellular vesicle comprising extracellular matrix mRNA comprising: introducing a vector or plasmid encoding an extracellular matrix protein into a donor cell via transfection; incubating the donor cell for a sufficient time for the production of extracellular vesicles encapsulating extracellular matrix mRNA transcribed from the vector or plasmid; and collecting the extracellular vesicles encapsulating the extracellular matrix mRNA transcribed from the vector or plasmid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0094] This patent application contains at least one drawing executed in color. Copies of this patent or patent application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

CLAIMS

WHAT IS CLAIMED IS:

1. A plurality of extracellular vesicles comprising an exogenous extracellular matrix messenger RNA (mRNA), wherein the extracellular matrix messenger RNA (mRNA) comprises a Col1A1 collagen, and the plurality of extracellular vesicles does not comprise an alpha 2 chain of collagen type I (Col1A2).

2. The plurality of extracellular vesicles of claim 1, wherein the collagen is selected from the group consisting of collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, collagen type XIV, collagen type XV, collagen type XVI, collagen type XVII, collagen type XVIII, collagen type XIX, collagen type XX, collagen type XXI, collagen type XXII, collagen type XXIII, collagen type XXIV, collagen type XXV, collagen type XXVI, collagen type XXVII, collagen type XXVIII, or any combination thereof.

3. The plurality of extracellular vesicles of claim 1, wherein the collagen is collagen type I.

4. The plurality of extracellular vesicles of claim 1, wherein the collagen is collagen type II.

5. The plurality of extracellular vesicles of claim 1, wherein the collagen is alpha 1 chain of collagen type I (Col1A1) or pro-alpha1(I) chain.

6. The plurality of extracellular vesicles of claim 1, wherein the collagen is Col1A2.

7. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 450 EVs.

8. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 2000 EVs, per 1000 EVs, per 500 EVs, per 400 EVs, per 300 EVs, per 200 EVs, per 150 EVs, per 100 EVs, per 90 EVs, per 80 EVs, per 70 EVs, 60 EVs, 50 EVs, per 40 EVs, per 30 EVs, per 20 EVs, per 15 EVs, per 10 EVs, per 5 EVs, per 2 EVs, or per 1 EV.

9. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles are produced by transfecting a cell with a plasmid encoding an extracellular matrix protein.

10. The plurality of extracellular vesicles of claim 9, wherein the transfecting of the cell is performed by cellular nanoporation.

11. The plurality of extracellular vesicles of any one of the previous two claims, wherein the cell is a human cell.

12. The plurality of extracellular vesicles of any one of the previous three claims, wherein the cell is a fibroblast selected from the group consisting of dermal fibroblast, human fibroblast, adult fibroblast, human adult fibroblast, neonatal fibroblast, neonatal human fibroblast, and neonatal human dermal fibroblast.

13. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises an extracellular vesicle selected from the group consisting of exosome, microvesicle, apoptotic body, and any combination thereof.

14. The plurality of extracellular vesicles of claim 13, wherein the plurality of extracellular vesicles comprises at least one exosome.

15. The plurality of extracellular vesicles of claim 13, wherein the plurality of extracellular vesicles comprises a mixture of at least any two of the following: exosome, microvesicle or apoptotic body.

16. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles are formulated for injection via an intravenous route, an intramuscular route, a subcutaneous route, or any combination thereof.

17. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises at least $1x10^{6}$, $5x10^{6}$, $1x10^{7}$, $5\ x10^{7}$, $1x10^{8}$, $3x10^{8}$, $5\ x10^{8}$, $1x10^{9}$, $3x10^{9}$, $1x10^{10}$, $1\ x10^{11}$, $1\ x10^{12}$, $1\ x10^{13}$, $1\ x10^{14}$, $1\ x10^{15}$, $1\ x10^{16}$, $1\ x10^{20}$, $1\ x10^{25}$, or $1\ x10^{30}$ extracellular vesicles.

18. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises at least 1 ng, 10 ng, 20 ng, 40 ng, 50 ng, 100 ng, 500 ng, 1 μg, 10 μg, 20 μg, 30 μg, 40 μg, or 50 μg of the extracellular matrix mRNA.

19. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles comprises a size distribution with a peak at 50 nm to 200 nm in diameter.

20. The plurality of extracellular vesicles of any one of the preceding claims, wherein the extracellular vesicles are greater than 20 nm, 30 nm, 50 nm, 75 nm, or 100 nm in diameter.

21. The plurality of extracellular vesicles of any one of the preceding claims, wherein the exogenous extracellular matrix mRNA is present at a level that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 50-fold, at least 75-fold, at least 100-fold, at least 500-fold, at least 1000-fold, at least 1500-fold, or at least 2000-fold higher than a level of the exogenous extracellular matrix mRNA in an identical amount of naturally-occurring extracellular vesicles.

22. The plurality of extracellular vesicles of any one of the preceding claims, wherein the plurality of extracellular vesicles expresses higher levels of at last one of the following markers compared to a comparable number of naturally-occurring extracellular vesicle: CD9, CD63, TSG101, or ARF6.

23. A plurality of extracellular vesicles comprising an exogenous extracellular matrix messenger RNA (mRNA), wherein the plurality of extracellular vesicles comprises an average of at least one copy of the exogenous extracellular matrix mRNA per 450 EVs.