Description

Title of Invention: IRON-OXIDE NANOPARTICLE-LOADED MESENCHYMAL STEM CELLS AND USES THEREOF

BACKGROUND

[0001] Immune imbalance often results in chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease.

[0002] Stem cells hold promise as a treatment for such diseases. In particular, mesenchymal stem cells (MSC) have been reported to exhibit anti-inflammatory and immunomodulatory activities both in vitro and in vivo. MSC have the ability to affect the inflammatory process by reprogramming M1 macrophages (pro-inflammatory) to the M2 phenotype (anti-inflammatory) , inhibiting T-cell proliferation, and attenuating T-cell functionality.

[0003] Despite the properties of MSC discussed above, stem cell therapy for inflammatory disease has drawbacks, among them being poor cell survival after in vivo transplantation, inefficient migration to targeted sites, and reductions in cell quality during in vitro expansion. Further, the responses of MSC to immune cells depend on inflammatory signals in the microenvironment and, in the absence of inflammation, MSC can have a pro-inflammatory phenotype.

[0004] The need exists to improve the effectiveness of stem cells as a therapy for inflammatory disease without the drawbacks discussed above.

SUMMARY

[0005] To meet the need outlined above, a composition is provided that comprises a stem cell and a coated iron oxide nanoparticle. The coated iron oxide nanoparticle is present in a cytoplasm of the stem cell and contains a superparamagnetic iron oxide core that is coated with one or more biocompatible polymers, each of which has a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group.

[0006] Also provided is a method for treating an inflammatory disorder in which stem cells are cultured in the presence of coated iron oxide nanoparticles, whereby the coated iron oxide nanoparticle enters a cytoplasm of the stem cells, and the cultured stem cells are administered to a subject suffering from an inflammatory disorder.

[0007] Further disclosed is a method for tracking stem cells in vivo, the method being carried out by (i) labeling stem cells with coated iron oxide nanoparticles, (ii) administering the labeled stem cell to an individual, and (iii) obtaining one or more T2 weighted magnetic resonance images of the individual, thereby tracking the stem cells. The coated iron oxide nanoparticles include an iron oxide core that is coated with

one or more biocompatible polymers, each of which has a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group, and the labeled stem cells are located at an area showing hypointense spots in the one or more T2 weighted MRI images.

[0008] The details of one or more embodiments are set forth in the description below. Other features, objects, and advantages will be apparent from the detailed description, from the drawings, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Fig. 1 is a set of bar graphs showing the viability of adipocyte-derived stem cells (ADSC) co-cultured for 6, 24, and 48 h with the indicated concentrations of iron calculated from the concentration of coated iron-oxide nanoparticles (IOP) added to the cultures.

[0010] Fig. 2 is a bar graph showing the amount of TGF-βrelease by ADSC loaded with IOP after 1 to 4 passages (P1-P4) in the absence of IOP expressed as a percentage of the initial amount of TGF-β.

[0011] Fig. 3A. shows a plot of Arthritis Score versus days after collagen injection in a Collagen-Induced Arthritis (“CIA”) animal model. Rats were treated with phosphate-buffered saline (PBS) , adipocyte-derived stem cells (ADSC) and ADSC loaded with coated iron-oxide nanoparticles (IOP@ADSC) .

[0012] Fig. 3B shows a plot of paw thickness versus days after collagen injection in the CIA animals. Treatment groups are described above in the legend shown in Fig. 3A.

[0013] Fig. 4 is an exemplary tissue section stained with Prussian blue of bone from the knee joint of CIA rats administered with IOP@ADSC. Arrows indicate the location of IOP@ADSC in the tissue.

[0014] Fig. 5A is a bar graph of the number of $FoxP3^{+}$Treg cells in the lateral posterior femur (LPF) tissue of control (PBS) , ADSC, and IOP@ADSC treated mice. *=significantly different from control at $p<0.05$ by Student’s T-test. **=significantly different from ADSC at $p<0.05$ by Student’s T-test

[0015] Fig. 5B is a bar graph of the number of IL-10^{+}cells in LPF tissue from control and treated rats.

[0016] Fig. 5C is a bar graph of relative staining of tissue for TGF-β^{+}cells for the indicated treatment.

[0017] Fig. 5D is a bar graph of relative staining of tissue for IL-6^{+}cells for the indicated treatment.

[0018] Fig. 5E is a bar graph of relative staining of tissue for TNF-α^{+}cells for the indicated treatment.

[0019] Fig. 6. shows an exemplary MRI scan of the knee of a CIA animal pre-treatment, immediately after treatment with IOP@ADSC (Post) , 3 days, and 7 days after treatment with IOP@ADSC.

[0020] Fig. 7 is a plot of disease activity index (DAI) score versus days after treatment initiation at day 2 (arrow) in a murine model of chronic inflammatory bowel disease. Disease was not induced in control mice (healthy) . Treatments are as shown above in the legend to Fig. 3A.

[0021] Fig. 8A is a bar graph of serum TGF-βconcentration shown for the indicated treatments or control animals. *=significantly different from PBS at $p<0.05$ by Student's T-test

[0022] Fig. 8B is a bar graph of serum IL-6 concentration shown for the indicated treatments or control animals. *=significantly different from PBS at $p<0.05$ by Student's T-test.

DETAILED DESCRIPTION

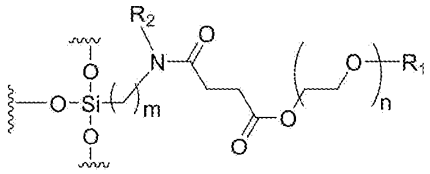
[0023] As summarized above, a composition is disclosed that includes a stem cell and an iron oxide nanoparticle (IOP) coated with a biocompatible polymer. The stem cell can be, but is not limited to, an embryonic stem cell (ESC) , a mesenchymal stem cell (MSC) , an induced pluripotent stem cell (iPSC) , a hematopoietic stem cell (HSC) , aneural stem cell (NSC) , an epithelial stem cell, an endothelial progenitor cell (EPC) , and a pericyte. In certain compositions, the stem cell is an MSC derived from bone marrow, adipose tissue, or umbilical cord tissue. In a particular composition, the stem cell is an adipose-derived stem cell (ADSC) .

[0024] In the above composition, the coated IOP can have a particle size of 10 nm to 1000 nm (e.g., 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 250 nm, 300 nm, 350 nm, 400 nm, 450 nm, 500 nm, 750 nm, and 1000 nm) . The coated IOP can have a transverse magnetic relaxivity rate (R2) measured in saline of 50 $mM^{-1}s^{-1}$to 400 $mM^{-1}s^{-1}$ (e.g., 50 $mM^{-1}s^{-1}$, 75 $mM^{-1}s^{-1}$, 100 $mM^{-1}s^{-1}$, 150 $mM^{-1}s^{-1}$, 200 $mM^{-1}s^{-1}$, 250 $mM^{-1}s^{-1}$, 300 $mM^{-1}s^{-1}$, 350 $mM^{-1}s^{-1}$, and 400 $mM^{-1}s^{-1}$) . The coated IOP after loading into stem cells can have a transverse magnetic relaxivity rate (R2) of 50 $mM^{-1}s^{-1}$to 1000 $mM^{-1}s^{-1}$ (e.g., 50 $mM^{-1}s^{-1}$, 75 $mM^{-1}s^{-1}$, 100 $mM^{-1}s^{-1}$, 150 $mM^{-1}s^{-1}$, 200 $mM^{-1}s^{-1}$, 250 $mM^{-1}s^{-1}$, 300 $mM^{-1}s^{-1}$, 350 $mM^{-1}s^{-1}$, 400 $mM^{-1}s^{-1}$, 450 $mM^{-1}s^{-1}$, 500 $mM^{-1}s^{-1}$, 550 $mM^{-1}s^{-1}$, 600 $mM^{-1}s^{-1}$, 650 $mM^{-1}s^{-1}$, 700 $mM^{-1}s^{-1}$, 750 $mM^{-1}s^{-1}$, 800 $mM^{-1}s^{-1}$, 850 $mM^{-1}s^{-1}$, 900 $mM^{-1}s^{-1}$, 950 $mM^{-1}s^{-1}$, and 1000 $mM^{-1}s^{-1}$) . In a specific embodiment, the particle size is 37 nm and the transverse magnetic relaxivity rate measured in saline is 150 $mM^{-1}s^{-1}$.

[0025] To repeat, the IOP is coated with one or more biocompatible polymer. The biocompatible polymer can be any of those described in Chen et al., US Patent

Application Publication 2016/0008491, the content of which is hereby incorporated herein by reference in its entirety.

[0026] The biocompatible polymer can have the following formula:



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[0027] in which (i) R_1 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, heteroaryl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; (ii) R_2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, or heteroaryl; (iii) m is 1 to 10; and (iv) n is 5 to 1000.

[0028] In a particular biocompatible polymer, R_1 is H; R_2 is H, C_1-C_6 alkyl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; m is 3 to 10; and n is 10 to 200. In an alternative specific biocompatible polymer, R_1 is CH_3; R_2 is H, m is 3; and n is 42 to 45.

[0029] The stem cells in the composition, as compared to the same stem cells in the absence of the coated IOP, have an anti-inflammatory phenotype. For example, stem cells in the composition secrete 1.5-fold to 2-fold more of the anti-inflammatory cytokine TGF-β, as compared to the stem cells not containing the coated IOP. At the same time, stem cells in the composition secrete 4-fold to 7-fold less of the pro-inflammatory cytokine IL-6.

[0030] Also disclosed in the SUMMARY section above is a method for treating an inflammatory disorder. To reiterate, the method is carried out by culturing stem cells in the presence of coated iron oxide nanoparticles (IOP) such that the coated iron oxide nanoparticles enter the cytoplasm of the stem cells and administering the cultured stem cells to a subject suffering from an inflammatory disorder.

[0031] The inflammatory disorders treatable by the method include, but are not limited to, rheumatoid arthritis, inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis) , multiple sclerosis, systemic lupus erythematosus, chronic obstructive pulmonary disease, psoriasis, and osteoarthritis.

[0032] In the above method, the stem cells can be ESCs, MSCs, iPSCs, HSCs, NSCs, epithelial stem cells, EPCs, or pericytes. In a particular method, the stem cells are MSCs derived from bone marrow, adipose tissue, or umbilical cord tissue. In a specific method, the stem cells are ADSCs.

[0033] To repeat from above, this method requires culturing stem cells in the presence of coated IOP. The medium for the culturing step and the culturing conditions are well known in the field of stem cells. The medium and conditions are selected such that the

stem cells remain pluripotent. In other words, differentiation medium is not used in the claimed method. The stem cells are cultured in the presence of coated IOP for a period of time to permit the coated IOP to be taken up by the stem cells. The culturing time can range from 1 h to 96 h (e.g., 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, 36 h, 48 h, 60 h, 72 h, and 96 h) . In a particular method, the culturing time is 24 to 48 h.

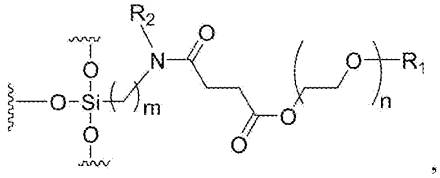
[0034] The method for treating an inflammatory disorder features a coated IOP. The coated IOP contains a superparamagnetic iron oxide core that is coated with one or more biocompatible polymers.

[0035] As in the composition described, supra, the coated IOP used in the method can have a particle size of 10 nm to 1000 nm (e.g., 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 250 nm, 300 nm, 350 nm, 400 nm, 450 nm, 500 nm, 750 nm, and 1000 nm) . The coated IOP can have a transverse magnetic relaxivity rate (R2) measured in saline of 50 $mM^{-1}s^{-1}$ to 400 $mM^{-1}s^{-1}$ (e.g., 50 $mM^{-1}s^{-1}$, 75 $mM^{-1}s^{-1}$, 100 $mM^{-1}s^{-1}$, 150 $mM^{-1}s^{-1}$, 200 $mM^{-1}s^{-1}$, 250 $mM^{-1}s^{-1}$, 300 $mM^{-1}s^{-1}$, 350 $mM^{-1}s^{-1}$, and 400 $mM^{-1}s^{-1}$) . The coated IOP after loading into stem cells can have a transverse magnetic relaxivity rate (R2) of 50 $mM^{-1}s^{-1}$ to 1000 $mM^{-1}s^{-1}$ (e.g., 50 $mM^{-1}s^{-1}$, 75 $mM^{-1}s^{-1}$, 100 $mM^{-1}s^{-1}$, 150 $mM^{-1}s^{-1}$, 200 $mM^{-1}s^{-1}$, 250 $mM^{-1}s^{-1}$, 300 $mM^{-1}s^{-1}$, 350 $mM^{-1}s^{-1}$, 400 $mM^{-1}s^{-1}$, 450 $mM^{-1}s^{-1}$, 500 $mM^{-1}s^{-1}$, 550 $mM^{-1}s^{-1}$, 600 $mM^{-1}s^{-1}$, 650 $mM^{-1}s^{-1}$, 700 $mM^{-1}s^{-1}$, 750 $mM^{-1}s^{-1}$, 800 $mM^{-1}s^{-1}$, 850 $mM^{-1}s^{-1}$, 900 $mM^{-1}s^{-1}$, 950 $mM^{-1}s^{-1}$, and 1000 $mM^{-1}s^{-1}$) . In a specific embodiment, the particle size is 37 nm and the transverse magnetic relaxivity rate measured in saline is 150 $mM^{-1}s^{-1}$.

[0036] To repeat, the IOP is coated with one or more biocompatible polymer. Each biocompatible polymer has a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group.

[0037] Like the above-described composition, the biocompatible polymer can be any of those described in Chen et al., US Patent Application Publication 2016/0008491.

[0038] The biocompatible polymer can have the following formula:



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[0039] in which (i) R_1 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, heteroaryl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; (ii) R_2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, or heteroaryl; (iii) m is 1 to 10; and (iv) n is 5 to 1000.

[0040] In a particular biocompatible polymer, R_1 is H; R_2 is H, C_1-C_6 alkyl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; m is 3 to 10; and n is 10 to 200. In an alternative specific biocompatible polymer, R_1 is CH_3; R_2 is H, m is 3; and n is 42 to 45.

[0041] Finally, a method is also mentioned above that is for tracking stem cells in vivo. The method is accomplished by administering to an individual stem cells labeled with a coated IOP and obtaining T2 weighted magnetic resonance images (MRI) of the individual. The labeled stem cells are located at an area showing hypointense spots in the T2 weighted MRI.

[0042] The labeled stem cells can be detected by T2 weighted MRI for a period of 1 h to 7 days (e.g., 1 h, 2 h, 4 h, 8 h, 12 h, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days) after administering them to the individual.

[0043] In this method, the stem cells can be ESCs, MSCs, iPSCs, HSCs, NSCs, epithelial stem cells, EPCs, or pericytes. In some methods, the stem cells are MSCs derived from bone marrow, adipose tissue, or umbilical cord tissue. In a particular method, the stem cells are ADSCs.

[0044] The stem cells are labeled with coated IOP as described above in the method for treating an inflammatory condition by culturing the stem cell in the presence of the coated IOP.

[0045] The coated IOP has the same characteristics as those described above, including an iron oxide core, e.g., a superparamagnetic iron oxide core, that is coated with one or more biocompatible polymers, each of which has a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group. These biocompatible polymers are the same as those described above.

[0046] The coated IOP used in this method has a particle size of 10-1000 nm and a transverse magnetic relaxivity rate (R2) measured in saline of 50 to 400 $mM^{-1}s^{-1}$. The particle size and transverse magnetic relaxivity rate can be any of those set forth above.

[0047] Without further elaboration, it is believed that one skilled in the art can, based on the disclosure herein, utilize the present disclosure to its fullest extent. The following specific examples are, therefore, to be construed as merely descriptive, and not limitative of the remainder of the disclosure in any way whatsoever. All publications and patent documents cited herein are incorporated by reference in their entirety.

[0048] Examples

[0049] Example 1. Iron oxide nanoparticle labeling of BM-MSC and ADSC

[0050] Bone marrow-derived mesenchymal stem cells (“BM-MSCs”) were harvested from a human female iliac crest bone marrow aspirate, and the cells were expanded and tested by the Industrial Technology Research Institute (Hsinchu, Taiwan) .

The BM-MSCs were obtained using standard adherent isolation and growth in Dulbecco's Modified Eagle's Medium (DMEM) +10%FBS. BM-MSCs were grown to 60-80%confluence with a cell viability of at least 90%and subcultured when the growth rate was in the mid-logarithmic phase. The BM-MSCs were subcultured at 37℃, 5%CO_2 in MesenPRO RS™ complete medium (Gibco; cat. no. 12746012, Thermo Fisher, Waltham, MA USA) .

[0051] Adipose-Derived Stem Cells ("ADSCs") isolated from human lipoaspirate tissue and cryopreserved from primary cultures were purchased from Thermo Fisher (cat. no. R778815) . The ADSCs were subcultured in MesenPRO RS™ medium at 37℃, 5%CO_2.

[0052] Stem cells were loaded with PEG-coated iron oxide nanoparticles ("IOP") or with ferumoxytol, which is a commercially available carbohydrate-coated iron oxide nanoparticle. Certain properties of these two tested iron oxide nanoparticles are shown below in Table 1.

[0053] Table 1. Characterization of IOP and ferumoxytol

Sample	Coating materials	Particle size[a] (nm)	R2 relaxivity[b] ($mM^{-1}s^{-1}$)	Fe^{2+} ratio[c] (%)
IOP	Polyethylene glycol (PEG)	37	150	4.5
Ferumoxytol	carbohydrate	24	66	1.4

[0054] a. Determined by dynamic light scattering (DLS)

[0055] b. Determined by 0.47T relaxometer at 37℃ in saline

[0056] c. Measured by Iron Test kit (Spectroquant 1.00796.0001, Merck) . %of Fe^{2+}= [(Fe^{2+} ion, ppm) / (total iron ion, ppm)] x100

[0057] To load the particles into cells, $2x10^6$ BM-MSCs or ADSCs were incubated for 48 hours with a concentration of IOP or ferumoxytol equivalent to 400μg Fe/mL. After the removal of free iron oxide nanoparticles by centrifugation, the cell pellets were harvested for cellular iron determination.

[0058] Intracellular iron levels were determined by inductively coupled plasma mass spectroscopy (ICP-MS) . The results are shown below in Table 2. The cells incubated with IOP had significantly higher iron content than those incubated with ferumoxytol, ranging from 3.95-fold higher in ADSCs to 20.8-fold higher in BM-MSCs. These in vitro studies demonstrated a significantly higher uptake of IOP into BM-MSCs and ADSCs as compared to ferumoxytol.

[0059] Table 2. Cellular Iron content of iron oxide nanoparticle labeled BM-MSC and ADSCs

Sample	BM-MSC	BM-MSC Fold difference IOP/ferumoxytol	ADSC	ADSC Fold difference IOP/ferumoxytol
IOP	33.3 pg/cell	20.8	9.5 pg/cell	3.95
ferumoxytol	1.6 pg/cell	-	2.4 pg/cell	-

[0060] Example 2. Cell viability of iron oxide labeled ADSC

[0061] To measure the effect of iron oxide nanoparticles on cell viability, triplicate samples of $2x10^6$ ADSCs were incubated for 6, 24, and 48 hours with increasing concentrations of IOP (0-400μg Fe/mL) . The viability of IOP-labeled cells was measured by the 3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide ("MTT") assay using standard protocols and compared to control cells not incubated with the IOP. The viability of ADSCs incubated with ferumoxytol for 48 hours at a concentration of 400μg Fe/mL was also evaluated.

[0062] The results are shown in Fig. 1 and in Table 3 below.

[0063] Unexpectedly, the viability of the IOP-labeled ADSCs was not significantly different from unlabeled cells for all tested iron concentrations and incubation times. See Fig. 1.

[0064] By contrast, ADSCs incubated with ferumoxytol at 400μg Fe/mL for 48 h showed a 10.5%decrease in viability. See Table 3, last column.

[0065] Table 3. Cell viability of ADSCs incubated with IOP and ferumoxytol with 400μg Fe/mL for 48 hours

Sample	IOP			Ferumoxytol
Coculture concentration (Fe equivalent)	100 μg/mL	200 μg/mL	400 μg/mL	400 μg/mL
Viability (48 hours)	98%	97%	99%	89.5%

[0066] This in vitro study demonstrated that IOP was not toxic to ADSCs.

[0067] Example 3: IOP-labeled cells retain stem cell phenotype and trilineage-differentiatio n capacity.

[0068] Triplicate samples of $1x10^6$ BM-MSC or ADSCs were co-incubated with IOP with 400μg Fe/ml for 48 hours to yield IOP-loaded BM-MSCs ("IOP@BM-MSC") and IOP-loaded ADSCs ("IOP@ADSC") . Adherent IOP@BM-MSC or IOP@ADSC were resuspended with 0.25%trypsin. The resuspended cells were fixed for 10 minutes in 1%paraformaldehyde and washed twice with phosphate buffered saline ("PBS") and incubated with primary antibodies at room temperature for 30 minutes. The antibodies used in this evaluation were fluorescein isothiocyanate-conjugated anti-human CD11b, CD34, CD45, CD73, CD90, CD105, and HLA-DR (Thermo Fisher Scientific Life Sciences, Waltham, MA) . Flow cytometry analysis was performed on a fluorescence-activated cell sorter (BD Biosciences, San Jose, CA) . The surface protein

profile of IOP@BM-MSC and IOP@ADSC compared to control (no IOP) are shown in Table 4.

[0069] Table 4. Surface protein profile of IOP@BM-MSC and IOP@ADSC

	BM-MSC		ADSC	
	Control	IOP	Control	IOP
MSC surface markers	CD73≥96%	CD73≥96%	CD73≥96%	CD73≥96%
	CD90≥96%	CD90≥96%	CD90≥96%	CD90≥96%
	CD105≥96%	CD105≥96%	CD105≥96%	CD105≥96%
	CD45, CD34, CD11b, HLA-DR<2%	CD45, CD34, CD11b, HLA-DR<2%	CD45, CD34, CD11b, HLA-DR<2%	CD45, CD34, CD11b, HLA-DR<2%

[0070] The results indicated that IOP@BM-MSC and IOP@ADSC have similar cell surface phenotype as compared to control stem cells without IOP.

[0071] To test the differentiation potential of IOP@BM-MSC and IOP@ADSC, osteoblast, adipocyte, and chondrocyte differentiation assays were performed in vitro. Tested stem cells were incubated with IOP at 400μg Fe/mL to label them as described above. Unlabeled stem cells were tested as a control.

[0072] Osteogenic differentiation of IOP-labeled and unlabeled stem cells was carried out by culturing the cells for 3 weeks in osteogenic induction medium containing 90%low glucose DMEM supplemented with 10%FBS, dexamethasone, 50μg/ml ascorbic acid, andβ-glycerol phosphate. Osteogenic differentiation was detected by staining cells with alizarin red using standard techniques.

[0073] For adipogenic differentiation, BM-MSCs and ADSCs were incubated in adipogenic medium for 3 weeks, which consisted of 90%DMEM supplemented with 10%FBS, insulin, indomethacin, 1-methyl-3-isobutylxanthine, and dexamethasone. Adipogenic differentiation was detected by staining cells with oil red O according to standard procedures.

[0074] Chondrocyte differentiation was carried out as follows. ADSC and IOP@ADSC were each seeded at 2 x 10^5 cells per well in two wells of a 96 well U-bottom suspension culture plate in negative control medium (low glucose DMEM with 2 mM L-glutamine and 10%fetal bovine serum) and cultured for 24 h at 37 ℃, 5%CO_2 to induce spheroids.

[0075] The medium in one well from each pair was replaced with MSCgo™ Chondrogenic Differentiation Medium (Sartorius 05-220-1B) and incubated for 21 days with a change of medium every third day. Cells in the paired wells were maintained with negative control medium.

[0076] After differentiation was complete, the medium was aspirated from the wells and cells were gently washed with Ca^{2+}/Mg^{2+}-free Dulbecco's PBS. The differentiated

spheroids were fixed in Saccomanno Fixation Solution at room temperature for 3 h. The fixative was removed and cells were washed with distilled H_2O.

[0077] Cartilage differentiation was detected by staining with Alcian Blue, which detects aggrecan, under standard conditions. Cells differentiated into chondrocytes stained an intense dark blue, whereas non-differentiated cells or other cell types, at best, stained light blue.

[0078] The results showed that IOP@BM-MSC and IOP@ADSC could each differentiate in vitro into at least osteocytes, adipocytes, and chondrocytes, to the same degree as unlabeled, i.e., IOP-free, stem cells

[0079] In summary, BM-MSC and ADSC after incubation with IOP (400μg/mL) retain the ability to differentiate into the three cell lineages tested.

[0080] Example 4: Augmented TGF-β production and attenuated production of proinflammatory cytokine IL-6 by IOP-labeled MSC.

[0081] Triplicate samples of $1x10^6$ BM-MSC or ADSC were incubated with 400μg Fe/mL of IOP or ferumoxytol for 48 hours to load them with nanoparticles. Unloaded BM-MSC or ADSC were also evaluated as a control group under the same experimental condition. Culture supernatants were collected and the concentrations of anti-inflammatory cytokine TGF-βand pro-inflammatory cytokine IL-6 were measured by an enzyme-linked immunosorbent assay (“ELISA”) using commercial matching monoclonal antibody pairs. Standards were performed in parallel with recombinant cytokines. The results are shown in Table 5 as the relative percentage expression of these cytokines as compared to control cells cultured in the absence of nanoparticles set as 100%. Both IOP@BM-MSC and IOP@ADSC produced significantly higher levels of TGF-βand lower levels of IL-6, as compared to ferumoxytol-labeled BM-MSC and ADSC. Thus, IOP-labeled stem cells have the potential to be used for both therapeutic cells and for their anti-inflammatory effect.

[0082] Table 5. TGF-βand IL-6 production in IOP-and ferumoxitol-loaded MSC

	BM-MSC		ADSC	
	IOP	ferumoxytol	IOP	ferumoxytol
TGF-β (%)	178±6%	97±4%	189±5%	98±3%
IL-6 (%)	16±2%	94±4%	24±5%	88+3%

[0083] Example 5: Continued release TGF-β over four cell generations after IOP labeling.

[0084] Triplicate samples of $1x10^6$ ADSC were incubated with 400μg Fe/mL of IOP. After 48 hours, the IOP-ADSC mixture was washed twice with Phosphate-Buffered Saline (PBS) to remove unlabeled IOP and cell debris. The washed IOP@ADSC were then seeded into a T75 flask in growth medium for further expansion. This was designated as the first passage (P1) of the cell culture. The IOP@ADSC were passaged

every 48 h in the absence of additional IOP. At each passage (P1-P4) , the culture supernatants were collected and the concentration of anti-inflammatory cytokine TGF-βwas measured by a commercial ELISA. Recombinant cytokine and unlabeled ADSC were also tested as controls.

[0085] The results are shown in Fig. 2. IOP@ADSC demonstrated increased TGF-β production throughout 4 cell passages, as compared to the TGF-β production of P1 control cells not exposed to IOP.

[0086] Example 6: Therapeutic efficacy of IOP@ADSC in Collagen-Induced Arthritis animal model

[0087] Animal model and experimental design

[0088] The therapeutic efficacy of unlabeled ADSC and IOP-labeled ADSC was tested in a rat model of arthritis, i.e., Collagen-Induced Arthritis (“CIA”) . Immunization-grade bovine type II collagen was first emulsified with an equal volume of Complete Freund’s adjuvant. A total of 240μL collagen emulsion was then injected intradermally into the backs of 8-week-old male SD rats. Six days after the injections, 100μL of the collagen emulsion was injected subcutaneously into the tail vein of the rats as the booster injection. The successfully established CIA rats were randomly divided into 3 groups: (1) Control (PBS) , (2) unlabeled ADSC, and (3) IOP@ADSC. The ADSC and IOP@ADSC groups received a single intra-articular (IA) injection into both the left and right knee joint of 5×10^6 cells in 50μL PBS. The control group received the same volume of PBS. The time point for the start of the treatment was set as Day 0. Table 6 presents a summary of the experimental design.

[0089] Table 6. Experimental design

Group	Animal number	Treatment	Dose	Dosing route and frequency
1	4	Control (PBS)	no cells for each knee	IA, Once
2	3	ADSC	5×10^6 cells for each knee	IA, Once
3	4	IOP@ADSC	5×10^6 cells for each knee	IA, Once

[0090] Monitoring therapeutic index

[0091] Arthritis Score: Arthritis score was recorded twice weekly for each ankle joint by the same observer. Scoring was performed on a scale of 0-5 (See Hsu et al., 2012; Rheumatology 51: 434-442) . The mean score of both ankle joints was recorded, where score 0 means no redness or swelling; score 1 means slight swelling in the ankle or redness in the foot; score 2 means progressive swelling, inflammation, and redness from the ankle to the midfoot; score 3 means swelling and inflammation of the entire

foot, not including the toes; score 4 means swelling and inflammation of the entire foot, including the toes; and score 5 means swelling and inflammation of the entire foot, with loss of mobility.

[0092] Footpad thickness: Disease progression was also monitored by measuring footpad thickness and knee thickness using a caliper. Footpad thickness was measured in both hind paws and the mean thickness from both hind paws was recorded.

[0093] Histopathology:

[0094] The entire hind limb tissues were fixed in 10%buffered formalin, decalcified, and embedded in paraffin, and then the samples were sectioned at 4-6μm and stained with hematoxylin and eosin (H&E) . Joints were histologically scored (0=normal; 5 =severe disease) for inflammation, cartilage damage, pannus formation, and bone resorption according to published protocols (See, e.g., Levine et al., PLoS One. 2014; 9(8) : e104530) . Some sections were also stained with Prussian blue to visualize IOP@ADSC in the tissue.

[0095] Immunohistopathology

[0096] Bone tissues in paraffin-embedded sections were incubated with rat anti-mouse monoclonal antibodies against TGFβ, TNFα, IL-10 (Abcam, Cambridge, UK.) , IL-6 (Invitrogen Fisher Scientific, Waltham, MA. United States.) , and FOXP3 (Origene, Maryland, USA) . After washing, all samples were then incubated with HRP-labeled goat anti-rat IgG antibody (Abcam, Cambridge, UK.) and visualized by incubation with diaminobenzidine substrate (Invitrogen Fisher Scientific, Waltham, MA. United States.) . Images were captured with a Nikon Eclipse Ts2 microscope.

[0097] Data was expressed as Mean±SEM. Comparisons between two groups were performed using Student's t test. A P value<0.05 was considered a statistically significant difference.

[0098] Results and discussion

[0099] The mean arthritis score and footpad thickness over time in CIA rats are shown in Figs. 3A and 3B. As shown in Fig. 3A, there were no significant differences in arthritis score between the unlabeled ADSC treatment group and control, i.e., PBS, group for all monitoring periods while the IOP@ADSC treatment group showed significant amelioration of arthritis score as compared to the PBS control group on Day 6 ($P<0.05$) , Day 9 ($P<0.001$) , and Days 11 and 12 ($P<0.01$) . Compared with the PBS control group, paw swelling was significantly lower after treatment with IOP@ADSC from Day 6 ($P<0.05$) to Day 9 ($P<0.01$) post-treatment (See Fig. 3B) .

[0100] Hematoxylin and eosin (H&E) staining was used to analyze the improvement in the knee joint after treatment. The total histopathological lesions (focal inflammation, pannus formation, cartilage damage, and bone resorption) in the knee joint of the

IOP@ADSC group decreased significantly (P<0.05) compared to the PBS group. See Table 7 below.

[0101] Table 7. Semi-quantitative lesion score of the knee joint in rats

	Group		
Measurements	PBS	Unlabeled ADSC	IOP@ADSC
Inflammation	2.13±0.99	3.00±0.63	1.75±0.46
Pannus formation	3.25±1.04	3.17±0.75	2.00±0.53 *
Cartilage damage	3.25±0.89	2.83±0.75	2.00±0.93 *
Bone resorption	2.88±0.64	2.50±0.55	2.00±0.93 *
Total Histological Score	11.50±3.19	11.50±0.87	7.75±2.02 *

[0102] [a] Values represent mean±standard deviation.

[0103] *: P<0.05, significant differences between PBS and other treatment groups by Student t-test.

[0104] Prussian blue staining demonstrated the presence of iron-loaded cells, i.e., IOP@ADSC in the knee tissue. See Fig. 4.

[0105] Immunohistopathology analysis demonstrated that knee joints in IOP@ADSC treated rats showed significantly increased staining for $FoxP3^+$cells, indicative of the presence of Treg cells, as well as for anti-inflammatory cytokine IL-$10^+$and TGF-β^+cells, as compared to PBS-injected knee joints. See Figs. 5A, 5B, and 5D, respectively. At the same time, the number of IL-$6^+$and TNF-α^+cells decreased significantly. See Figs. 5C and 5E, respectively.

[0106] In conclusion, the results indicated that IOP@ADSC could effectively ameliorate the pathological process of CIA in rats by suppressing paw swelling, arthritis score, pannus formation, cartilage damage, and bone resorption. Further, the presence of IOP@ADSC in the joints resulted in the establishment of an anti-inflammatory environment.

[0107] Example 7: In vivo Visualization of IOP@ADSC using MRI in Collagen-Induced Arthritis animal model

[0108] The CIA rat model was also exploited to examine in vivo MRI after injection of unlabeled ADSC and IOP@ADSC. Two rats each were assigned to an ADSC group and an IOP@ADSC group. Each group received a single intra-articular injection of 5×10^6 cells in 50μL PBS into each of the left and right knee joint. The time point for the start of the treatment was set as Day 0. MRI scans of knee joints were performed (i) prior to injection (baseline) , (ii) 1 h after injection, (iii) on Day 3, and(iv) on Day 7.

[0109] MRI scans were obtained with a 7T BioSpec magnetic resonance scanner (Bruker) equipped with an active shielding gradient (18G/cm, 200μs rise time) . Images were acquired using a 72-mm birdcage transmitter coil and a separate quadratic surface coil for signal detection. The animals were initially anesthetized with inhaled isoflurane in

oxygen (5%delivered at an oxygen flow rate of 5 L/min) and placed in a rat restrainer in a supine position. Animals were maintained under anesthesia with 1-2%isoflurane delivered at an oxygen flow rate of 2 L/min throughout the experiments. After each MRI scan, the animals recovered fully from anesthesia. T2*-map images (T2*map) were acquired using Rapid Acquisition with Relaxation Enhancement sequence with a TR of 4,000 ms, an effective TE of 60 ms, an echo train length of 8, a FOV of 3×3 cm, a matrix size of 256×128, a SLTH of 0.5 mm, and a NEX of 10.

[0110] The signal intensities were measured from a region of interest (ROI) covering a substantial part of the bone muscle parenchyma. Care was taken to avoid the inclusion of large vessels in the ROIs. Representative MRI images are shown in Fig. 6. Quantitative analysis is shown below in Table 8, expressed as the T2*relaxation time of the synovial fluid (synovial capacity) as a function of time. Similar T2*values were measured for unlabeled ADSCs and IOP@ADSCs groups before administration of cells. CIA rat knees treated with IOP@ADSC, as compared to the baseline value, demonstrated significantly shorter T2*relaxation time within 1 hour ($P<0.05$) after injection of cells, which was maintained at Day 3 ($p<0.05$) , and Day 7 ($P<0.05$) . Conversely, CIA rat knees treated with unlabeled ADSCs did not show significant changes in T2*relaxation time over time.

[0111] Table 8. MRI T2*relaxation time in CIA rat knee

Group	Baseline (Pre-scan)	Post injection		
		1h	Day 3	Day 7
Unlabeled ADSC	7.02±0.08[a]	6.87±0.32	6.65±0.51	6.98±0.68
IOP@ADSC	6.98±0.05	2.43±0.25*	3.14±0.37*	5.87±0.53*

[0112] [a] T2*relaxation time in ms

[0113] *: $P<0.05$, significant difference by Student's t-test as compared with baseline.

[0114] The results showed that, in vivo, synovial T2*relaxation times were significantly lower after treatment with IOP@ADSC compared to unlabeled ADSC. The decrease in T2*signal remained significantly lower than control up to at least 7 days post injection.

[0115] Clearly, IOP can be used for in vivo tracking of injected ADSC and can be applied to track MSCs in cells therapy trials in the near future.

[0116] Example 8: Treatment of Inflammatory Bowel Disease with IOP@ADSC Induction of inflammatory bowel disease ("IBD")

[0117] Experimental colitis was induced in mice by administration of 3%dextran sulfate sodium ("DSS") in drinking water ad libitum for 7 consecutive days (Days 1 to 7) followed by administration of normal drinking water for two days (Days 8 and 9) . Injection of cells or PBS was performed on Day 2.

[0118] Animal grouping and treatment

[0119] Mice in the ADSC group and IOP@ADSC group were injected intraperitoneally with 5×10^6 cells in 100μL PBS on Day 2, and the PBS group mice were injected with the same volume of PBS also on Day 2. Mice in the healthy control arm were not injected with DSS, cells, or PBS. The experimental design is summarized in Table 9 below.

[0120] Table 9. Experimental design for IBD treatment

Group	Animal number	Treatment	Dose	Dosing volume (mL/kg)	Dosing route and frequency
G1	7	Vehicle (PBS)	-	5	IP, Once on Day 2
G2	7	ADSC alone	5×10^6 cells	5	IP, Once on Day 2
G3	7	IOP@ADSC	5×10^6 cells	5	IP, Once on Day 2
G4	3	Healthy control	-	-	-

[0121] Disease Activity Index Score ("DAI")

[0122] During the study period, animals were inspected daily and body weight, stool consistency, and presence or absence of bleeding were recorded. Criteria for the different scores are shown in Table 10 below, adapted from Wirtz et al., Nature Protocols, 2017, 12: 1295-1309. The sum of the scores for stool consistency, bleeding, and body weight loss is the DAI.

[0123] Table 10. DAI scoring criteria

Score	Weight loss	Stool consistency	Blood
0	None	Normal	No blood traces observed in stool
1	1-5%	Soft but still formed	No blood traces observed in stool
2	6-10%	Soft	No blood traces observed in stool
3	11-18%	Very soft; wet	Blood traces in stool visible
4	>18%	Watery diarrhea	Gross rectal bleeding

[0124] The results are shown in Fig. 7. As expected, mice injected with PBS showed a time-dependent increase in DAI throughout the experiment. A similar result was obtained in animals injected with ADSC. By contrast, experimental colitis in mice injected with IOP@ADSC showed significantly lower DAI scores ($p<0.01$) , particularly at experimental days 8 to11, corresponding to 6 to 9 days after injection of the cells.

[0125] Serum cytokine analysis

[0126] On Day 10, all mice were sacrificed and blood samples were taken by cardiac puncture. The serum levels of IL-10, IL-6, and TGF-βin mice were detected with commercially available ELISA kits (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. IL-10 levels were below the detection level of

the ELISA assay. The results for TGF-βand IL-6 are shown in Figs. 8A and 8B, respectively.

[0127] The serum levels of anti-inflammatory cytokine TGF-βwere similar in healthy mice, experimental mice injected with PBS, and experimental mice injected with ADSC. Only experimental mice injected with IOP@ADSC showed a statistically significant increase in serum TGF-βlevels. See Fig. 8A.

[0128] As expected, serum levels of the pro-inflammatory cytokine IL-6 were significantly lower in healthy animals as compared to experimental animals injected with PBS. See Fig. 8B. Animals injected with ADSC showed an approximately 35%reduction in IL-6 levels compared to PBS-injected mice. See id. Mice injected with IOP@ADSC had even lower levels of serum IL-6, approximately 65%lower than PBS-injected animals.

[0129] The results of the inflammatory bowel disease study again indicate that IOP@ADSC can be employed as a treatment for inflammatory conditions by simultaneously increasing anti-inflammatory processes and decreasing the pro-inflammatory environment in diseased tissues.

[0130] OTHER EMBODIMENTS

[0131] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

[0132] From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the following claims.

Claims

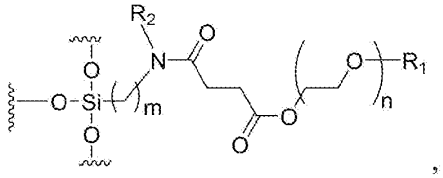
[Claim 1] A composition comprising a stem cell and a coated iron oxide nanoparticle (IOP) , wherein the coated IOP is present in a cytoplasm of the stem cell and contains a superparamagnetic iron oxide core that is coated with one or more biocompatible polymers, each of which has a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group.

[Claim 2] The composition of claim 1, wherein the stem cell is an embryonic stem cell (ESC) , a mesenchymal stem cell (MSC) , an induced pluripotent stem cell (iPSC) , a hematopoietic stem cell (HSC) , a neural stem cell (NSC) , an epithelial stem cell, an endothelial progenitor cell (EPC) , or a pericyte.

[Claim 3] The composition of claim 2, wherein the stem cell is an MSC derived from bone marrow, adipose tissue, or umbilical cord tissue.

[Claim 4] The composition of any one of claims 1 to 3, wherein the coated IOP has a particle size of 10-1000 nm and a transverse magnetic relaxivity rate of 50 $mM^{-1}s^{-1}$ to 1000 $mM^{-1}s^{-1}$ in the cytoplasm of the stem cell

[Claim 5] The composition of claim 4, wherein the one or more biocompatible polymers have the following formula:



,

in which

R_1 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, heteroaryl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group;

R_2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, or heteroaryl;

m is 1 to 10; and

n is 5 to 1000.

[Claim 6] The composition of claim 5, wherein R_1 is H; R_2 is H, C_1-C_6 alkyl, aC_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; m is 3 to 10; and n is 10 to 200.

[Claim 7] A method for treating an inflammatory disorder, the method comprising culturing stem cells in the presence of coated iron oxide nanoparticles

(IOP) , whereby the coated IOP enter a cytoplasm of each of the stem cells, and administering the cultured stem cells to a subject suffering from an inflammatory disorder.

[Claim 8] The method of claim 7, wherein the inflammatory disorder is rheumatoid arthritis Crohn's disease, multiple sclerosis, systemic lupus erythematosus, chronic obstructive pulmonary disease, psoriasis, or osteoarthritis.

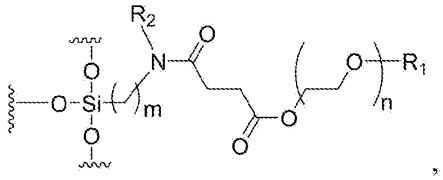
[Claim 9] The method of claim 8, wherein the stem cells are embryonic stem cells (ESC) , mesenchymal stem cells (MSC) , induced pluripotent stem cells (iPSC) , hematopoietic stem cells (HSC) , neural stem cells (NSC) , epithelial stem cells, endothelial progenitor cells (EPC) , or pericytes.

[Claim 10] The method of claim 9, wherein the stem cells are MSC derived from bone marrow, adipose tissue, or umbilical cord tissue.

[Claim 11] The method of any one of claims 7 to 9, wherein the coated IOP each contain a superparamagnetic iron oxide core that is coated with one or more biocompatible polymers, each of the one or more biocompatible polymers including a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group.

[Claim 12] The method of claim 11, wherein the coated IOP each have a particle size of 10-1000 nm and a transverse magnetic relaxivity rate measured in saline of 50 $mM^{-1}s^{-1}$ to 400 $mM^{-1}s^{-1}$.

[Claim 13] The method of claim 12, wherein the one or more biocompatible polymers have the following formula:



in which

R_1 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, heteroaryl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group;

R_2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, or heteroaryl;

m is 1 to 10; and

n is 5 to 1000.

[Claim 14] The method of claim 13, wherein R_1 is H; R_2 is H, C_1-C_6 alkyl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group; m is 3 to 10; and n is 10 to 200.

[Claim 15] A method for tracking stem cells in vivo, the method comprising labeling stem cells with coated iron oxide nanoparticles (IOP) , administering the labeled stem cells to an individual, and obtaining one or more T2 weighted magnetic resonance images of the individual, thereby tracking the stem cells, wherein the coated IOP each include an iron oxide core that is coated with one or more biocompatible polymers, each of the one or more biocompatible polymers including a polyethylene glycol group, a silane group, and a linker covalently linking the polyethylene glycol group and the silane group, and the labeled stem cells are located at an area showing hypointense spots in the one or more T2 weighted MRI images.

[Claim 16] The method of claim 15, wherein the stem cells are embryonic stem cells (ESC) , mesenchymal stem cells (MSC) , induced pluripotent stem cells (iPSC) , hematopoietic stem cells (HSC) , neural stem cells (NSC) , epithelial stem cells, endothelial progenitor cells (EPC) , or pericytes.

[Claim 17] The method of claim 16, wherein the stem cells are MSC derived from bone marrow, adipose tissue, or umbilical cord tissue.

[Claim 18] The method of any one of claims 15 to 17, wherein the iron oxide core is a superparamagnetic iron oxide core.

[Claim 19] The method of claim 18, wherein the coated IOP has a particle size of 10-1000 nm and a transverse magnetic relaxivity rate measured in saline of 50 $mM^{-1}s^{-1}$ to 400 $mM^{-1}s^{-1}$.

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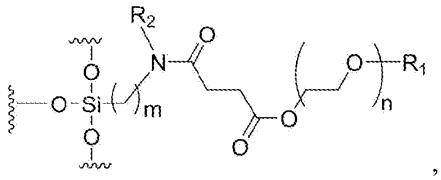
[Claim 20] The method of claim 19, wherein the one or more biocompatible polymers have the following formula:



,

in which

R_1 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, heteroaryl, a C_1-C_{10} carbonyl group, or a C_1-C_{10} amine group;

R_2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_1-C_{10} heterocycloalkyl, aryl, or heteroaryl;

m is 1 to 10; and

n is 5 to 1000.

[Claim 21] The method of claim 20, wherein R_1 is H; R_2 is H, C_1-C_6 alkyl, a C_1-C_{10} carbonyl group,or a C_1-C_{10} amine group;m is 3 to 10;and n is 10 to 200.