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(71) Applicants: **THE UNIVERSITY OF FLORIDA RE¬ SEARCH FOUNDATION, INC.** [US/US]; 233 Grinter Hall, Gainesville, FL 3261 1 (US). **TUFTS UNIVERSITY** [US/US]; 419 Boston Avenue, Medford, MA 02155 (US).

(72) Inventors: **HARDY, John;** 4 Warding Drive, Little Com- mon Bexhill-on-Sea, East Susse, TN39 4QN (GB). **SCHMIDT, Christine, E.;** 1922 SW 106th Terrace, Gainesville, FL 32607 (US). **KAPLAN, David, L.;** 46 Pond Street, Concord, MA 01742 (US).

(74) Agents: **LINDER, Christopher, B.** et al; Thomas Hor- stemeyer LLP, 400 Interstate North Parkway, SE, Suite 1500, Atlanta, GA 30339 (US).

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[Continued on next page]

(54) Title: ELECTROACTIVE SCAFFOLDS, METHODS OF MAKING ELECTROACTIVE SCAFFOLDS, AND METHODS OF USING ELECTROACTIVE SCAFFOLDS

(57) Abstract: Embodiments of the present disclosure provide for structures including an electroactive scaffold, methods of mak- ing the structure, method of using structure, and the like.

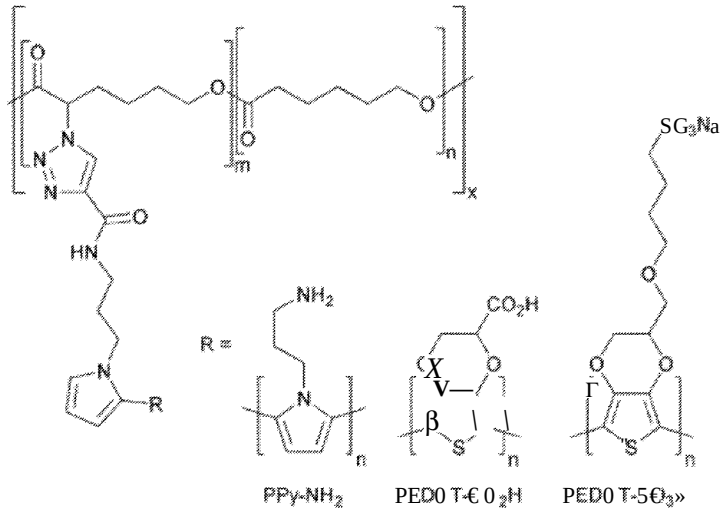

Fig. 1

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ELECTROACTIVE SCAFFOLDS, METHODS OF MAKING ELECTROACTIVE SCAFFOLDS, AND METHODS OF USING ELECTROACTIVE SCAFFOLDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Application Serial No. 62/192,723, having the title "ELECTROACTIVE SCAFFOLDS, METHODS OF MAKING ELECTROACTIVE SCAFFOLDS, AND METHODS OF USING ELECTROACTIVE SCAFFOLDS," filed on July 15, 2015, the disclosure of which is incorporated herein in by reference in its entirety.

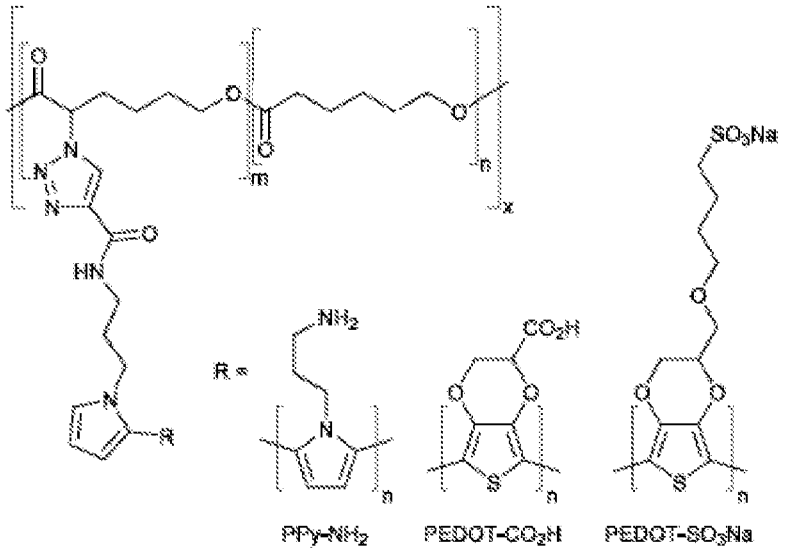
BACKGROUND

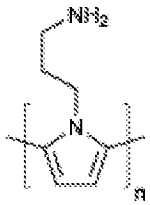
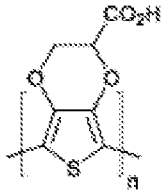
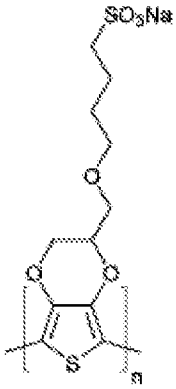
Tissue scaffolds allowing the behavior of the cells that reside on them to be controlled are of particular interest for tissue engineering. Bone conditions requiring surgical intervention are of growing importance in societies with populations in which life expectancies are increasing, motivating the development of pro-regenerative biomaterials. As a result, there is a need to find materials that can be used in this fashion.

SUMMARY

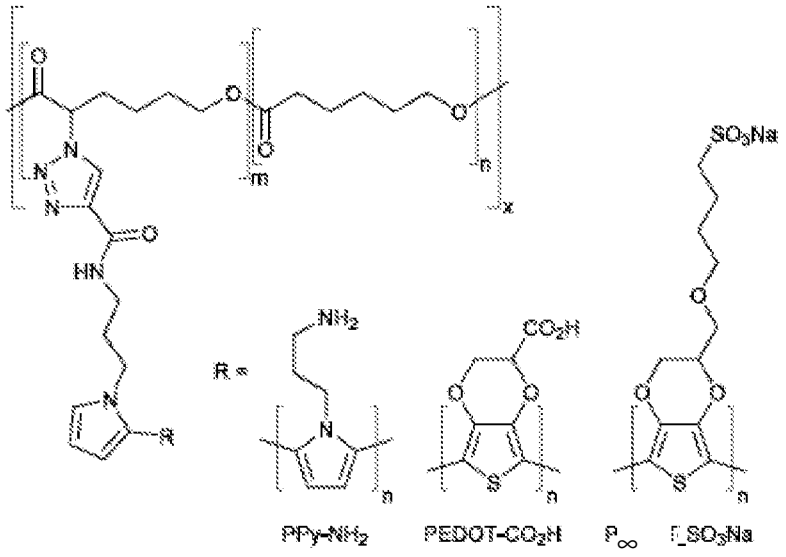
Embodiments of the present disclosure provide for structures including an electroactive scaffold, methods of making the structure, method of using structure, and the like.

An embodiment of the present disclosure includes a structure, among others, having an electroactive scaffold comprising the following polymer:

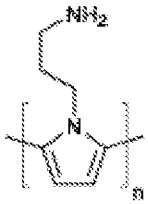


, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and n for each of the R group is 1 to 1,000,000. In an embodiment, human mesenchymal stem cells are disposed on the electroactive scaffold. In a particular embodiment, the electroactive scaffold is a polymer film. In a particular embodiment, the R group is  . In a particular embodiment, the R group is  . In a particular embodiment the R group is  .

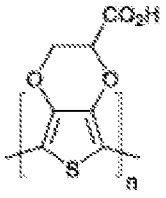
An embodiment of the present disclosure includes a method of differentiation of human mesenchymal stem cells, among others, includes: providing a structure having an electroactive scaffold including the following polymer:



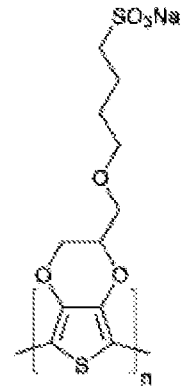
, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and n for each of the R group is 1 to 1,000,000; introducing human mesenchymal stem cells to the structure, wherein the structure and the human mesenchymal stem cells are cultured in an osteogenic medium; and periodically providing electrical stimulation to the human mesenchymal stem cells to cause differentiation of the human mesenchymal stem cells towards osteogenic outcomes. In an embodiment, the method can also include: growing biomineral silica on the structure when the R group is



. In an embodiment, the method can also include: growing biomineral calcium carbonate, calcium phosphate, or a combination thereof on the structure when the R group is



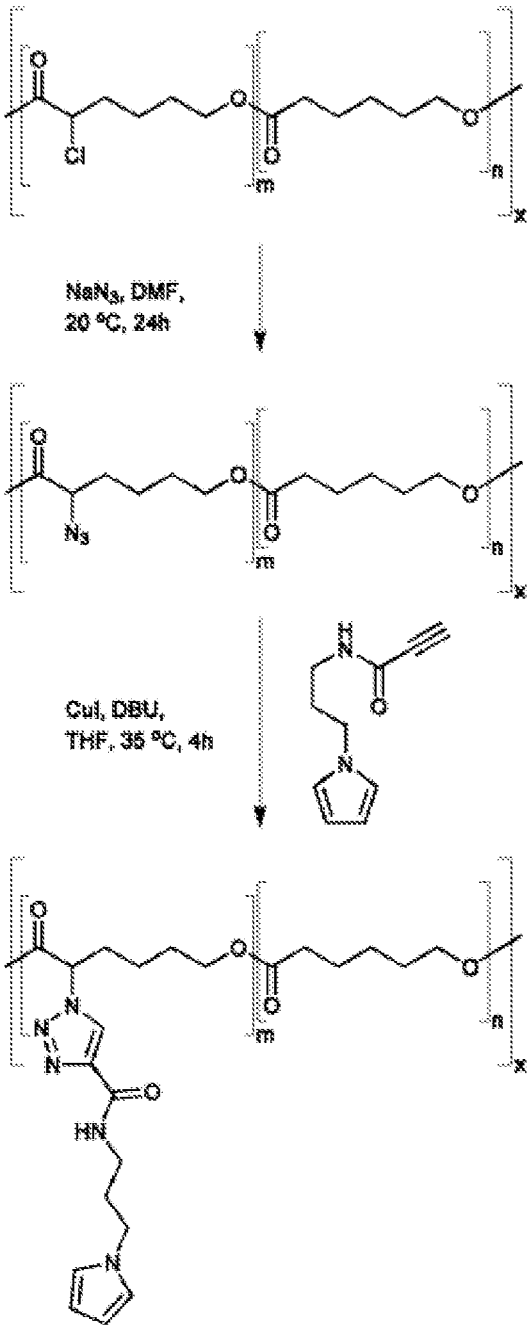
PEDOT-CO_2H . In an embodiment, the method can also include: growing biomineral calcium carbonate, calcium phosphate, or a combination thereof on the structure when the R group is



PEDOT-SO3Na

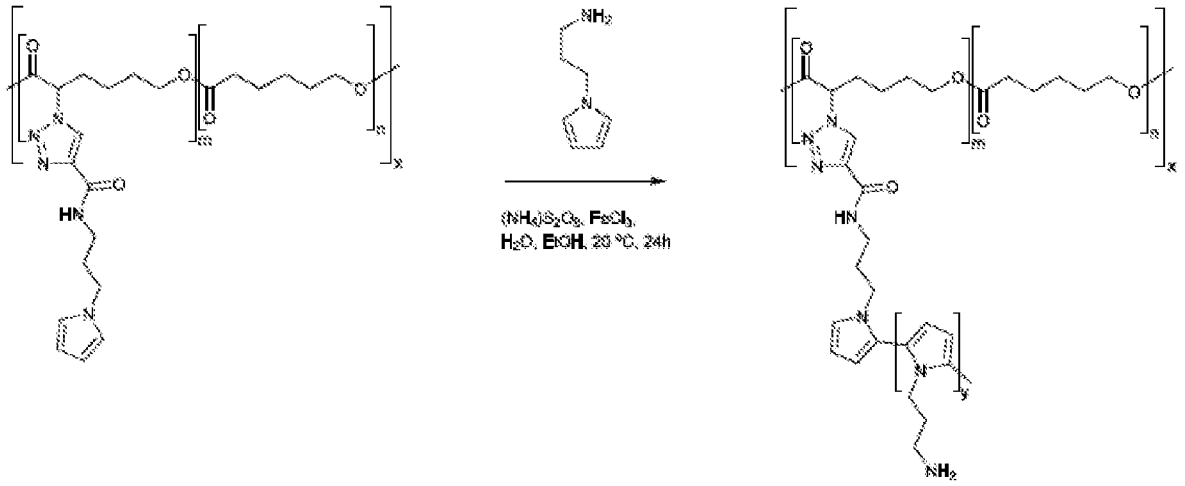
. In an embodiment, the electrical stimulation increases ALP activity in the structure.

An embodiment of the present disclosure includes a method of making a pyrrole-displaying derivative, among others,



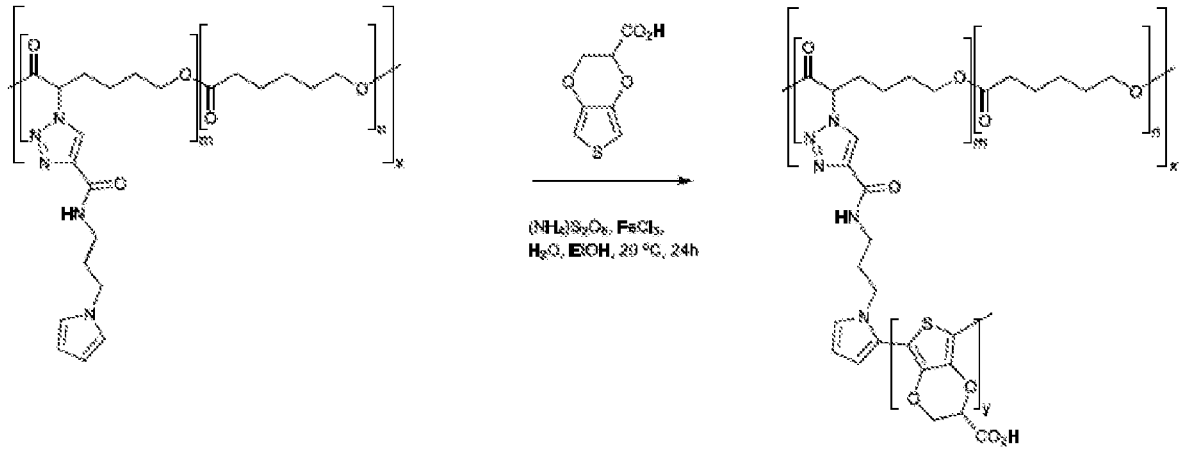
includes: , wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000.

An embodiment of the present disclosure includes a method of making an amine-displaying polycaprolactone derivative, among others, includes:



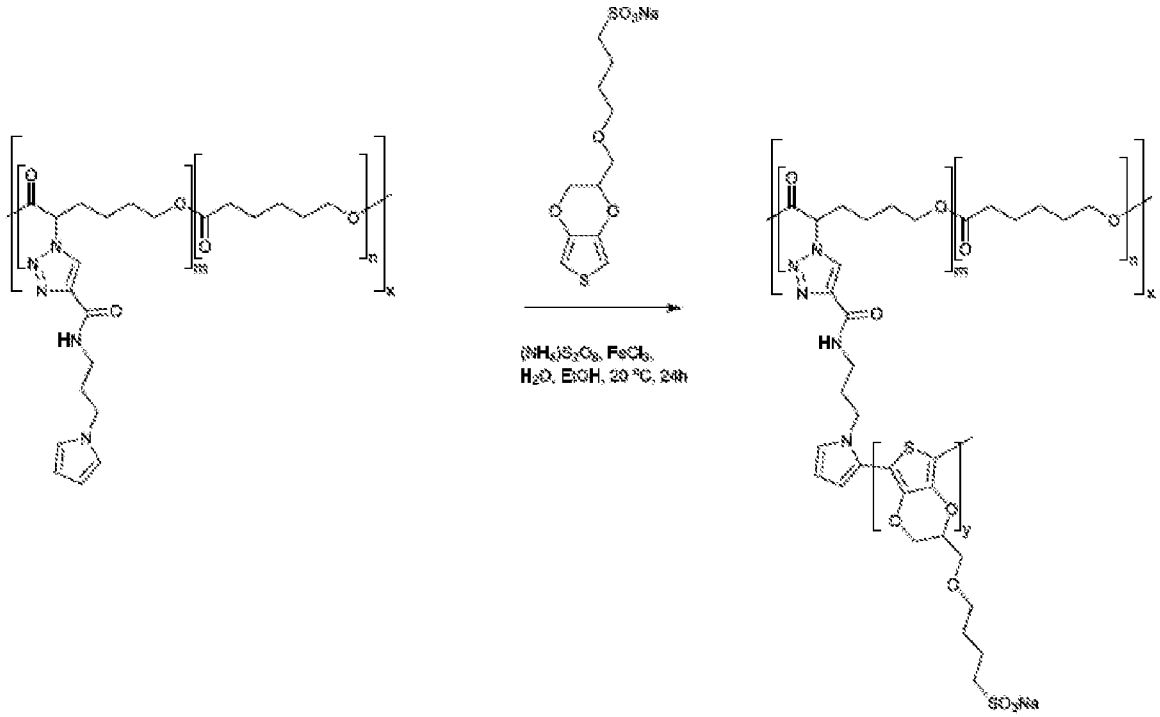
, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

An embodiment of the present disclosure includes a method of making a carboxylic acid-displaying polycaprolactone derivative, among others, includes:



, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

An embodiment of the present disclosure includes a method of making a sulfonate-displaying polycaprolactone derivative, among others, includes:



, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

Other compositions, structure, methods, features, and advantages will be or become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional compositions, structures, methods, features and advantages be included within this description, be within the scope of the present disclosure, and be protected by the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be more readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

Figure 1 shows conducting polymers enabling biomineralization with silica ($R\text{-}NH_2$), or calcium carbonate/phosphate ($R\text{-}CO_2H$ or $R\text{-}SO_3Na$).

Figures 2A-E provide physicochemical analysis of conductive materials. Fig. 2A shows EDX analysis of PCL-triazole-Py functionalized films, inset SEM image. Fig. 2B shows EDX analysis of $PPy\text{-}NH_2$ functionalized films, inset SEM image. Fig. 2C shows EDX analysis of $PPy\text{-}NH_2$ functionalized films biomineralized with silica, inset SEM image. Fig. 2D shows EDX analysis of $PEDOT\text{-}CO_2H$ functionalized films, inset SEM image. Fig. 2E shows EDX analysis of $PEDOT\text{-}CO_2H$ functionalized films biomineralized with calcium phosphate, inset SEM image. Scale bars represent 50 μm.

Figures 3A-F are images of fluorescently stained cells cultured on various substrates. DAPI-stained nuclei are blue and Alexa Fluor® 488-stained actin is green. Fig. 3A shows tissue-culture treated Corning® Costar® tissue culture plate controls; Fig. 3B is PCL control; Fig. 3C shows conducting silica-coated film without electrical stimulation; Fig. 3D shows conducting silica-coated film with electrical stimulation; Fig. 3E shows conducting calcium phosphate-coated film without electrical stimulation; Fig. 3F shows conducting calcium phosphate-coated film with electrical stimulation. Scale bars represent 100 μm.

Figure 4 shows biochemical analysis of in vitro cell culture experiments. ALP activity. TCP, Tissue-culture treated Corning® Costar® tissue culture plate controls. PCL, PCL control. Silica (-), conducting silica-coated film without electrical stimulation. Silica (+), conducting silica-coated film with electrical stimulation. Calcium phosphate (-), conducting calcium phosphate-coated film without electrical stimulation. Calcium phosphate (+), conducting calcium phosphate-coated film with electrical stimulation.

Figure 5 graphs gel permeation chromatograms of pyrrole-displaying polycaprolactone derivative (2) and a commercially available sample of polycaprolactone in DMF at 22 °C. Grey line) commercially available sample of polycaprolactone (GPC: Mn = 40.1 kDa (Mw/Mn of 2.02) from Polysciences Inc., (Warrington, PA, USA). Black line) pyrrole-displaying polycaprolactone derivative (2), GPC: Mn = 5.0 kDa (Mw/Mn of 1.95).

Figure 6 is an EDX spectrum of films of interpenetrating networks of PCL and PEDOT-C02H biomineralized with calcium-carbonate. Peaks in the EDX spectra at 0.277 and 0.525 keV are the characteristic Ka emissions of carbon and oxygen, respectively, and the very weak emission at 0.392 keV is the Ka emission of nitrogen. The peaks in the spectra of the films after the polymerization reactions at 2.621 and 6.398 keV are characteristic Ka emission lines of chlorine and iron, the peak at 0.705 keV is the La emission line of iron, and the peak at 2.307 keV is the Ka emission line of sulphur present in the backbone of the PEDOT-CO_2H. The successful biomineralization of the PEDOT-CO_2H films with calcium carbonate is clear from the appearance of the peak at 3.690 keV that is characteristic of the Ka emission of calcium.

Figure 7 demonstrates that films of interpenetrating networks of PCL and PPy-PSS films (prepared as reported by Hardy and coworkers in Bioengineering, 2015, 2, 15) could be biomineralized with calcium carbonate as shown in the optical micrograph (scale bar represents 200 μm).

DETAILED DESCRIPTION

This disclosure is not limited to particular embodiments described, and as such may, of course, vary. The terminology used herein serves the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

Where a range of values is provided, each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method may be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of organic chemistry, biochemistry, microbiology, molecular biology, pharmacology, medicine, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of microbiology, molecular biology, medicinal chemistry, and/or organic chemistry. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described herein.

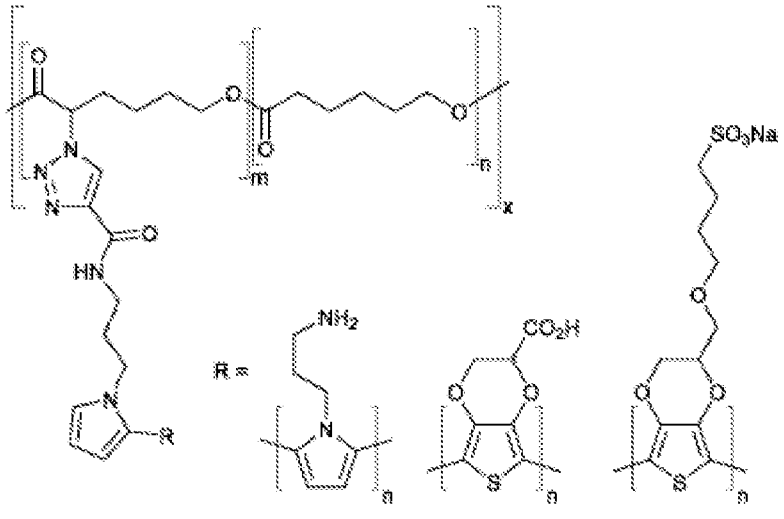
As used in the specification and the appended claims, the singular forms "a," "an," and "the" may include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a support" includes a plurality of supports. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings unless a contrary intention is apparent.

Discussion

Embodiments of the present disclosure provide for structures including an electroactive scaffold, methods of making the structure, method of using structure, and the like. In an embodiment, the structure has electroactive characteristics so that an electrical stimulation can be periodically applied to the electroactive scaffold. In this regard, embodiments of the disclosure provide for a structure that includes the electroactive scaffold that can be used in the differentiation of human mesenchymal stem cells.

Embodiments of the present disclosure can be used as biomineralized conductive bone tissue scaffolds that show that electrical stimulation of human mesenchymal stem cell disposed on the structure can enhance levels of ALP activity. In a particular embodiment, a human mesenchymal stem cell can be incubated with the electroactive scaffold and cultured in an osteogenic medium so that the stem cells differentiate towards osteogenic outcomes. Electrical stimulation can be periodically applied to the human mesenchymal stem cells to cause differentiation of human mesenchymal stem cells towards osteogenic outcomes. Application of electrical stimulation to the electroactive scaffold increases ALP activity, which can lead to formation of calcified bone-like extracellular matrix. As shown in Example 1, electrical stimulation of the electroactive scaffold in the presence of human mesenchymal stem cells in the osteogenic medium shows increased differentiation towards osteogenic outcomes relative to other structures.

In an embodiment, the electroactive scaffold can include a polymer having the following structure:



, where m can be 1 to infinity *(e.g.,* 10,000, 100,000, 1,000,000, or more), n (in the polymer backbone) can be 1 to infinity

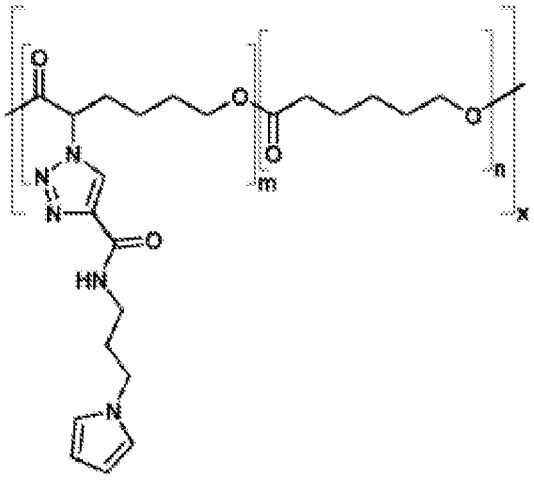
(e.g., 10,000, 100,000, 1,000,000, or more), x can be 1 to infinity *(e.g.,* 10,000, 100,000, 1,000,000, or more), and n for each R group (and referenced as "y" elsewhere) can be 1 to infinity *(e.g.,* 10,000, 100,000, 1,000,000, or more).

In another embodiment, the polymer backbone can be different *(e.g.* a polyester, polyamide, polyphosphazene) and some of the constituent monomers shown above can be incorporated into the backbone of the conducting polymer. For example, the copolymer backbone can be a polyester, for example, and monomer or oligomer groups *(e.g.,* x is 1 to 100) for the represented polymer above can be incorporated into the backbone structure and/or attached to the backbone, where the copolymer has an amount of the monomer or oligomer groups to achieve the goals described herein as it pertains to the polymer described above.

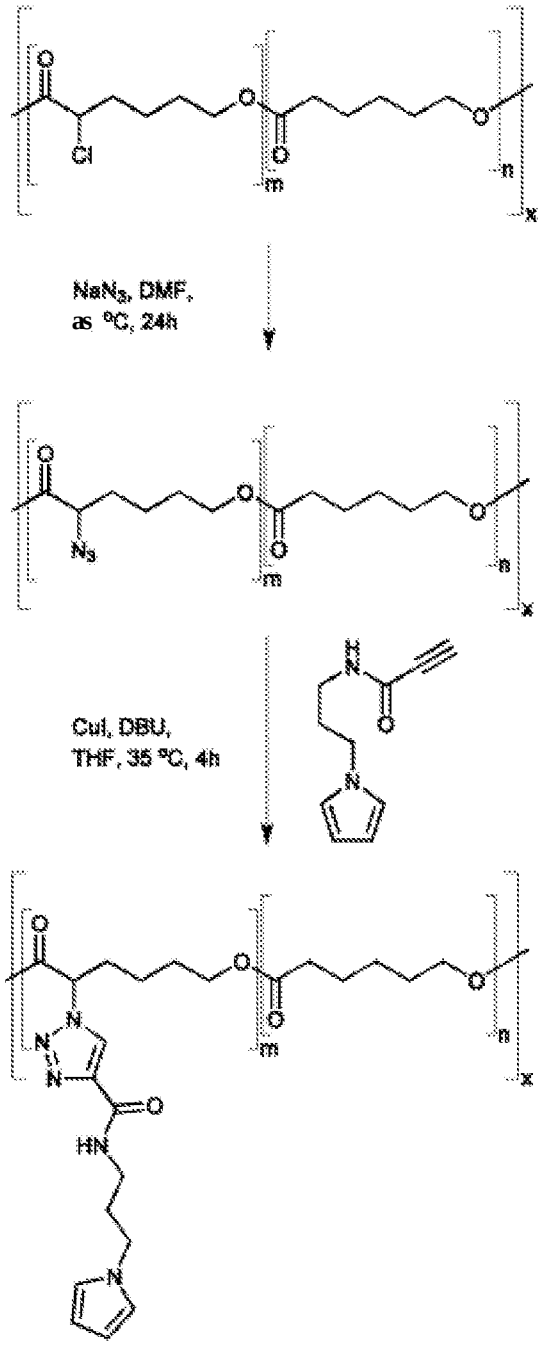
In an embodiment the electroactive scaffold can be a polymer film having a thickness of about 10 nm to 10 cm. The scaffold can be in a materials morphology such as films, foams, fibers, particles, hydrogels, organogels, and the like.

In an embodiment, the electroactive scaffold can be formed using one the following exemplary methods. Additional details regarding the method of synthesis are provided in the Example.

In an embodiment, a pyrrole-displaying derivative

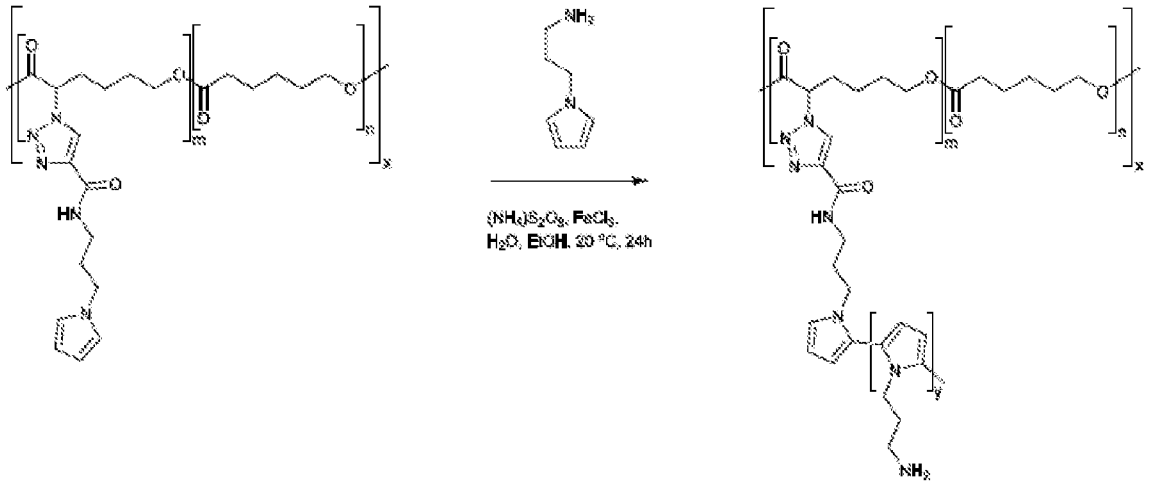


( ) can be prepared using the following reaction sequence:



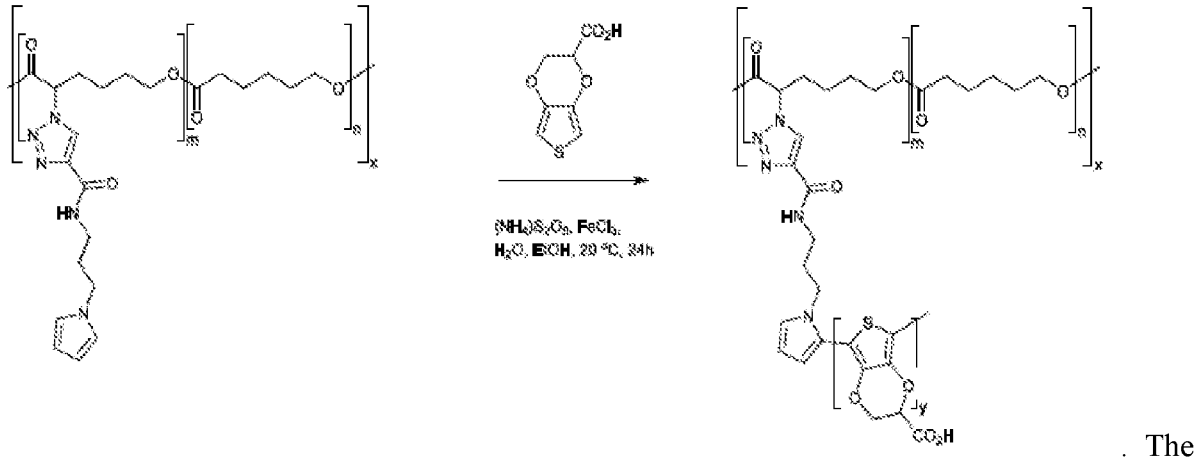
. The solvent, temperature, time, and other reactant variables can be modified accordingly to form the same polymer.

In an embodiment an amine-displaying polycaprolactone derivative can be prepared using the following reaction sequence:



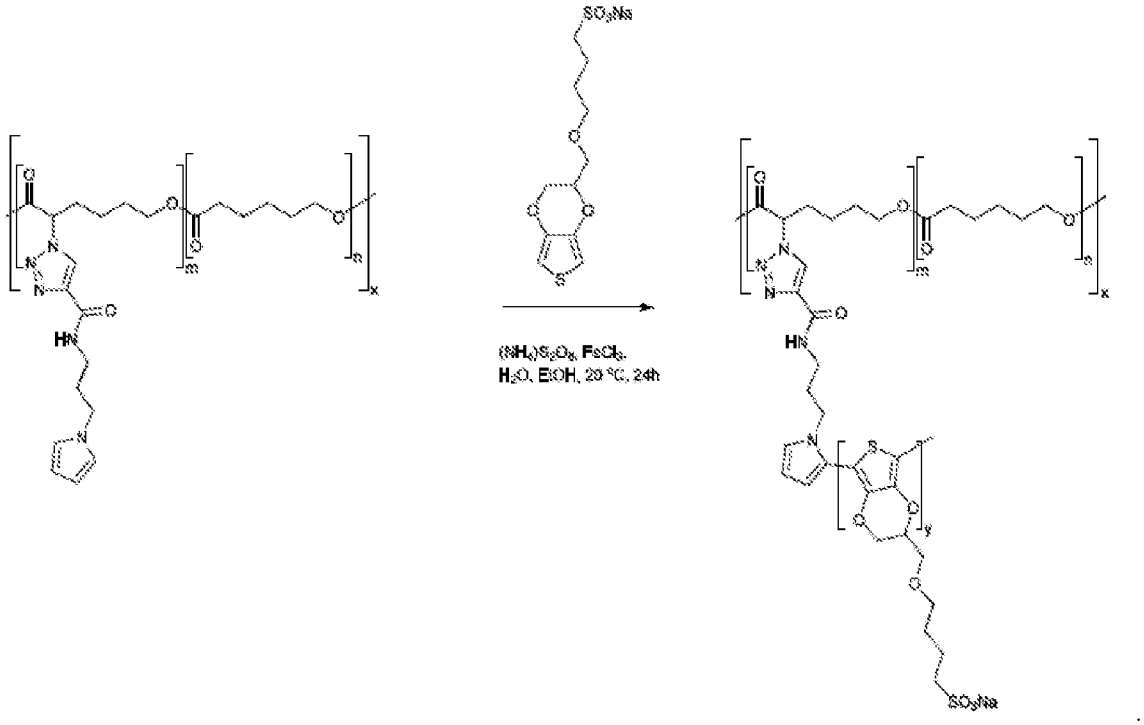
The solvent, temperature, time, and other reactant variables can be modified accordingly to form the same polymer.

In an embodiment a carboxylic acid-displaying polycaprolactone derivative can be prepared using the following reaction sequence:



. The solvent, temperature, time, and other reactant variables can be modified accordingly to form the same polymer.

In an embodiment a sulfonate-displaying polycaprolactone derivative can be prepared using the following reaction sequence:



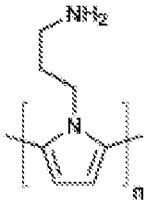
The solvent, temperature, time, and other reactant variables can be modified accordingly to form the same polymer.

As mentioned above, embodiments of the present disclosure provide for differentiation of human mesenchymal stem cells. An embodiment of the method includes introducing human mesenchymal stem cells to the structure including the electroactive scaffold. The structure and the human mesenchymal stem cells are cultured in an osteogenic medium. The human mesenchymal stem cells can be periodically provided electrical stimulation to the human mesenchymal stem cells to cause differentiation of human mesenchymal stem cells towards osteogenic outcomes.

Electrical stimulation can include direct contact of the material with a power source via a wire, wireless energy transfer, magnetic force, and the like. The term "periodically" refers to applying the electrical stimulation at established time frames that may be at regular or irregular time intervals on the time frames of seconds, hours, days, weeks, or months *(e.g.,* about 1 s to 2 months, about 1 hour to 1 day, about 1 day to 1 month, or other the like) depending upon the specific circumstances. In an embodiment, the impulses of the electrical stimulation can last on the time frame of seconds, hours, or days *(e.g.,* about 1 second to 1 day, about 10 seconds to 1 hour, about 1 minute to 12 hours, about 1 hour to 1 day, or the like) depending upon the specific circumstances. In an embodiment, the electrical stimulation can be in the range of millivolts to volts *(e.g.,* about 10 mV to 10 volts, about 1 mV to 100 mV, or the like). The time frame, duration of electrical stimulation, and intensity

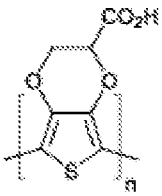
of the electrical stimulation can be designed based on particular circumstances and requirements of a specific situation.

In a particular embodiment, the electroactive scaffold can be designed to enable the growth of biominerals on the surface of the electroactive scaffold. In an embodiment, the biomineral silica (SiO_2) can be grown on the electroactive scaffold when the R group is



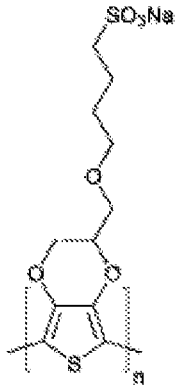
. The amine functional group is conducive to growing the biomineral silica because it is positively charged under physiological pH conditions and interacts with silicic acid ($Si(OH)_4$), which is negatively charged to produce a layer of neutral silica (SiO_2).

In an embodiment, calcium carbonate, calcium phosphate, or a combination thereof can be grown on the electroactive scaffold when the R group displays a negatively charged moiety such as a carboxylic acid or sulfonic acid. Examples of such monomers include



PEDOT-carboxylic acid (PEDOTY-CO_2H): PEDOT-CO_2H . Negatively charged functional groups *(e.g.,* carboxylic acids, CO_2H) are conducive to growing the calcium carbonate, calcium phosphate, or a combination thereof because they form complexes with the positively charged calcium 2+ ions (switching the charge on the surface of the material) enabling the deposition of negatively charged ions *(e.g.,* carbonate or phosphate anions), and this process can be repeated to obtain a layer of the desired thickness (nm to mm scale).

In an embodiment, calcium carbonate, calcium phosphate, or a combination thereof can be grown on the electroactive scaffold when the R group is the negatively charged



PEDOT-SO3Na

sulfonate moiety, *e.g.,* PEDOT-sulfonate (PEDOT-S0 $_3$Na) . The sulfonic acid functional groups are conducive to growing the calcium carbonate, calcium phosphate, or a combination thereof because they form complexes with the positively charged calcium 2+ ions (switching the charge on the surface of the material) enabling the deposition of negatively charged ions *(e.g.,* carbonate or phosphate anions), and this process can be repeated to obtain a layer of the desired thickness (nm to mm scale)

In an embodiment the osteogenic medium is based on standard cell culture medium with the optional addition of other components such as serum, non-essential amino acids, bone morphogenetic protein 2 (BMP-2), dexamethasone, β-glycerophosphate, ascorbic acid, ascorbic acid-2-phosphate, heparin, retinoic acid, and 1,25-dihydroxycholecalciferol (for example: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM)). The volume of medium used should be in line with the recommended guidelines of the manufacturer of the cell culture dishes.

As stated above, embodiments of the present disclosure provide for a structure having an electroactive scaffold. In an embodiment, the electroactive scaffold can include one or more agents *(e.g.,* a chemical or biological agent), where the agent can be disposed indirectly or directly on the electroactive scaffold. As described herein, the agent can include a stem cell such as a human mesenchymal stem cell. In particular, human mesenchymal stem cells *(e.g.,* on collagen-1 coated substrates) and the differentiated products of the stem cells are within the electroactive scaffold. Furthermore, the electroactive scaffold includes ALP, which represents a step towards formation of bone tissue.

In addition, an additional agent that can be disposed on the electroactive scaffold can include, but is not limited to, a drug, a therapeutic agent, a radiological agent, a small molecule drug, a biological agent *(e.g.,* polypeptides *(e.g.,* proteins such as, but not limited to,

antibodies (monoclonal or polyclonal)), antigens, nucleic acids (both monomeric and oligomeric), polysaccharides, haptens, sugars, fatty acids, steroids, purines, pyrimidines, ligands, and aptamers) and combinations thereof, that can be used to image, detect, study, monitor, evaluate, and the like, the differentiation of the stem cells. In an embodiment, the agent is included in an effective amount to accomplish its purpose *(e.g.,* ALP production), where such factors to accomplish the purpose are well known in the medical arts.

In general, the agent can be bound to the structure by a physical, biological, biochemical, and/or chemical association directly or indirectly by a suitable means. The term "bound" can include, but is not limited to, chemically bonded *(e.g.,* covalently or ionically), biologically bonded, biochemically bonded, and/or otherwise associated with the electroactive supramolecular polymeric assembly. In an embodiment, being bound can include, but is not limited to, a covalent bond, a non-covalent bond, an ionic bond, a chelated bond, as well as being bound through interactions such as, but not limited to, hydrophobic interactions, hydrophilic interactions, charge-charge interactions, π-π stacking interactions, combinations thereof, and like interactions. In an embodiment, cell-electroactive scaffold interactions could be controlled through the inclusion of cell-adhesive peptides *(e.g.,* RGD, YIGSR, KQAGDV, KHIFSDDSSE, KRSR), protease-labile domains *(e.g.,* APGL, VRN, or indeed oligoalanines such as those in the backbone of MTTl and MTT2 that are degraded by elastase), osteoinduction can be enhanced *(e.g.,* NSPVNSKIPKACCVPTELSAI), and directing mineralization *(e.g.* FHRRIKA).

While embodiments of the present disclosure are described in connection with the Examples and the corresponding text and figures, there is no intent to limit the disclosure to the embodiments in these descriptions. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

EXAMPLE 1:

Tissue scaffolds allowing the behavior of the cells that reside on them to be controlled are of particular interest for tissue engineering. Herein we describe biomineralized conducting polymer-based bone tissue scaffolds that facilitate the electrical stimulation of human mesenchymal stem cells, resulting in enhancement of their differentiation towards osteogenic outcomes.

Bone conditions requiring surgical intervention are of growing importance in societies with populations in which life expectancies are increasing, motivating the development of pro-regenerative biomaterials.[1] Non-biodegradable materials (e.g. titanium), biodegradable materials (e.g. biopolymers, calcium phosphate cements) and multifunctional materials that combine habitats for the cells with the capability to deliver drugs, have been investigated as potential bone tissue scaffolds.[1] Biomineralized materials are commonly investigated as bone tissue scaffolds, because the presence of the biomineral in the scaffold may promote osteogenesis.[2]

Conducting polymer (CP)-based biomaterials (such as derivatives of polyaniline, polypyrrole or polythiophene), have potential for both long term biomedical applications (e.g. electrodes) and short term biomedical applications (e.g. drug delivery or tissue engineering).[3] CP-based scaffolds have been developed for the regeneration of bone, muscle and nerve tissue.[3] Langer and coworkers first reported the use of CP-based materials for their application as bone tissue scaffolds.[4] The application of a potential difference of 20 mV mm^{-1} over 2-dimensional polypyrrole films encouraged bone marrow-derived stromal cells to differentiate towards osteogenic outcomes (assayed as an increase in alkaline phosphatase (ALP) activity per cell relative to non-stimulated control substrates).[4]

A variety of research groups have reported further developments in conducting polymer-based materials for bone tissue engineering in the absence[5] or presence[6] of an electrical field, commonly finding improved osteogenesis for the electrically stimulated samples. Moreover, the success of inorganic bone substitutes in the clinic has led researchers to develop conducting polymer-based coatings for calcium phosphate-based,[7] steel-based,[8] and titanium-based[9] biomaterials which offer a method of directly electrically stimulating cells residing on the materials, or delivering a drug from such a coating upon the application of an electrical stimulus.[10]

In this Example we describe the preparation of polycaprolactone (PCL derivatives displaying pyrrole moieties from which conducting polymers (such as polypyrrole or polythiophene derivatives) can be grown. Polymers displaying amines, carboxylates or sulfonates (Figure 1) facilitate mineralization of silica or calcium carbonates or phosphates. These conducting bone tissue scaffolds enable electrical stimulation of human mesenchymal stem cells which promotes their differentiation towards osteogenic outcomes.

Propiolic acid was coupled to aminopropylpyrrole[11,12] by carbodiimide-mediated peptide coupling (Scheme SI), and these were coupled to PCL derivatives displaying azide

moiteties[13] by Cu(I)-mediated triazole formation[13] (Scheme S2), after which the copper was removed by incubation in a solution of ethylenediaminetetraacetic acid (EDTA).[14] The material was extensively washed to remove traces of EDTA and vacuum dried yielding pyrrole-displaying PCL derivative (depicted in Figure 1) with M_n = 5.0 kDa and M_w/M_n of 1.95 (Figure 5) in the form of a light brown powder. Films of the resulting polymer were solution cast on either commercially available tissue-culture treated Corning® Costar® tissue culture plates (TCP) or glass. An interpenetrating network of either amine displaying polypyrrole derivative (PPy-NH_2, Figure 1) or carboxylate displaying poly(3,4-ethylenedioxythiophene) derivative (PEDOT-CO_2H, Figure 1) were generated by incubation of the pyrrole-functionalized PCL films in aqueous solutions of the appropriate pyrrole and EDOT derivatives in the presence of the initiators ammonium persulfate and ferric chloride (Scheme S3 and S4, respectively).[15] Films of the amine or carboxylate derivative displaying films were washed thoroughly with water to remove the by-products (e.g. initiators, monomers, oligomers and polymers) and vacuum dried. The brown-black PPy-NH_2 films were biomineralized with silica and those of the blue-grey PEDOT-C0 $_2$H were biomineralized with calcium phosphate. Energy dispersive X-ray (EDX) analysis of the films confirms that their surface chemistry is different. Peaks in the EDX spectra of the PCL derivatives displaying pyrrole moieties have lines at 0.277 and 0.525 keV that are the characteristic Ka emissions of carbon and oxygen, respectively, and the very weak emission at 0.392 keV is the Ka emission of nitrogen (Figure 2A-E). The peaks in the spectra of the films after the polymerization reactions at 2.621 and 6.398 keV are characteristic Ka emission lines of chlorine and iron, the peak at 0.705 keV is the La emission line of iron (Figure 2B-E), and the peak at 2.307 keV is the Ka emission line of sulphur present in the backbone of the PEDOT-C0 $_2$H (Figure 2D and 2E). The successful biomineralization of the PPy-NH_2 films (Figure 2B) with silica is clear from the appearance of the Ka emission peak of silicon at 1.739 keV (Figure 2C). Likewise, the successful biomineralization of the PEDOT-C0 $_2$H films (Figure 2D) with calcium phosphate is clear from the appearance of the peaks at 2.013 and 3.690 keV, that are characteristic of the Ka emissions of phosphorous and calcium, respectively (Figure 2E). The inset SEM images show the surface morphologies of the films (Figure 2A-E), with nanometer to micrometer scale pores present on the surface of the biomineralized films (Figure 2B-E).

The electrical sheet resistance of the biomineralized samples was measured in accordance with the method described by Schmidt[11'16] and Zhang.[17] The PPy-NH_2 films

biomineralized with silica had sheet resistances of 31.6 ± 9.1 kΩ, and those of PEDOT-CO_2H biomineralized with calcium phosphate had sheet resistances of 248.6 ± 71.8 kΩ, which is of a similar order of magnitude to interpenetrating networks of polypyrrole and polystyrenesulfonate in PCL (68.0 ± 18.1 kQ).[16] While the electrochemical stability of the polypyrrole and PEDOT derivatives are known to decrease over long periods of time which may be problematic for biointerfaces intended for long term use,[18] we and others have found them to be acceptable for the short term stimulation of cells residing in tissue scaffolds such as those reported here.[3,4,6,11,16,17]

To investigate the potential of the biomineralized CPs to act as bone tissue scaffolds, we seeded human mesenchymal stem cells (HMSCs) on their surfaces and cultured them in osteogenic medium for 3 weeks. We seeded six different systems: 1) cells seeded on TCP controls; 2) cells seeded on PCL (80 kDa); 3) cells seeded on silica-coated PPy-NH_2 films without electrical stimulation; 4) cells seeded on silica-coated PPy-NH_2 films with electrical stimulation; 5) cells seeded on silica-coated PEDOT-$C0_2H$ films without electrical stimulation; 6) cells seeded on silica-coated PEDOT-$C0_2H$ films with electrical stimulation. Those samples that were electrically stimulated were cultured for 2 days without stimulation, followed by four periods of stimulation at 10 mV mm^{-1} for 8 hours then 40 hours without stimulation, and no stimulation thereafter).

After 3 weeks in culture, cells were fixed with paraformaldehyde and cell nuclei and actin filaments within cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and Alexa Fluor® 488 Phalloidin, respectively. We observed that cells were homogeneously distributed on the TCP and PCL controls, and that cells had infiltrated the biomineral coatings on the biomineralized CP films (Figure 3A-F) which is promising for their integration in the body where infiltration of cells such as macrophages and osteoclasts facilitates remodelling of implanted biomaterials.[19] The differentiation of the cell population towards osteogenic fates was shown using a biochemical assay for alkaline phosphatase (ALP) activity which is a characteristic marker of bone formation. To within experimental error, ALP activity of cells cultured on the TCP and PCL control substrates was the same (Figure 4). ALP activity for cells cultured on the conductive biomineralized scaffolds was reduced relative to the TCP and PCL control substrates, which is likely to be because of subtle differences in cell-matrix interactions as observed for analogous systems.[20] Interestingly, ALP activity of cells cultured on the scaffolds mineralized with calcium phosphate was slightly higher than for cells cultured on the scaffolds mineralized with silica,

which is likely to be because the calcium phosphate acts as a source of calcium and phosphate ions enabling the production of calcified extracellular matrix.[21] Furthermore, the ALP activity of cells cultured on the conductive biomineralized scaffolds was increased after electrical stimulation (four periods during which a potential step of 10 mV mm^{-1} was applied across the conductive substrates for 8 hours), which is in line with reports by Langer[4] and others.[6] Therefore, our biochemical analysis reveals that while the non-conductive scaffolds support differentiation of HMSCs towards osteogenic outcomes, the application of an electrical stimulus to HMSCs residing in a conductive scaffold enhances levels of ALP activity which is a hallmark of bone tissue formation.

Conclusions

Pro-regenerative biomaterials for the treatment of bone conditions and disorders that require surgical intervention are of growing importance in modern societies in which life expectancies are increasing. Bone tissue scaffolds that control the behaviour of cells residing on them are particularly interesting for such applications. We report the first examples of biomineralized conductive bone tissue scaffolds and show that the electrical stimulation of HMSCs residing thereon enhances levels of ALP activity, which represents an important step towards the formation of bone tissue.

Calcium carbonate is increasingly interesting in biomedicine as a novel scaffolds for bone tissue engineering,[22] and it is possible to biomineralize **PEDOT-CO_2H** films with calcium carbonate (Figure 6). While it is possible to biomineralize analogous materials incorporating interpenetrating networks of sulfonate displaying PEDOT-S0 $_3$Na (Figure 1, Scheme S5)[23] with calcium-based biominerals we found them to be mechanically unstable during long term cell culture experiments. PEDOT-S0 $_3$Na is the most hydrophilic/water soluble of the conducting polymers tested, which is likely to increase rates of enzymatic degradation of the PCL matrix as we have observed for interpenetrating networks of PCL with water insoluble polyplexes of polypyrrole/polystyrenesulfonate.[16] Moreover, we know that such PCL/polypyrrole/polystyrenesulfonate-based materials are stable to long term cell culture,[16] and allow the growth of calcium-based biominerals such as calcium carbonate (Figure 7).

We believe it should be possible to prepare a variety of conductive biomineralized tissue scaffolds by chemical modification of the scaffolds with peptides directing the mineralization (e.g. FHRRIKA),[24] and potentially also peptides that control other aspects of

cell behaviour (e.g. RGD, YIGSR or KRSR for cell adhesion, and NSPVNSKIPKACCVPTELSAI for osteoinduction),[24] thereby allowing us to tailor the properties of the scaffold to specific niche applications (and potentially specific patients).

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SUPPLEMENTARY INFORMATION FOR EXAMPLE 1:

Materials

Unless otherwise stated, all chemicals for synthesis and physicochemical analysis were of ACS grade, purchased from Sigma-Aldrich and used as received without further purification. Phosphate buffered saline (PBS) was at pH 7.4. Reagents for cell culture were purchased from Invitrogen (Carlsbad, CA) unless otherwise noted. Human mesenchymal stem cells (HMSCs) from a 24 year old drug- and disease-free male were purchased from Lonza (Gaithersburg, MD).

Experimental methods

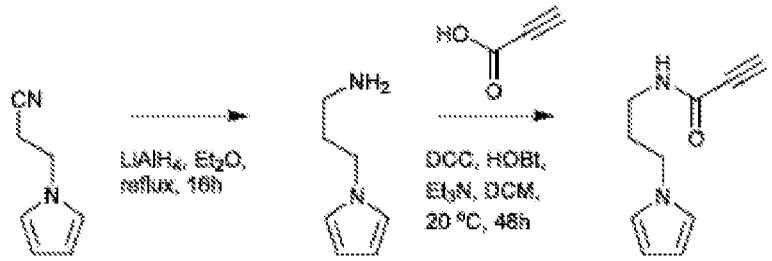
^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz NMR spectrometer, using residual solvent ^{1}H peaks as internal references for the ^{1}H NMR spectra. Mass spectra were recorded on an Agilent 6530 QTOF mass spectrometer in electrospray ionization mode. Infrared spectroscopy was carried out on a Thermo Scientific Nicolet 380 FT-IR Spectrometer (Thermo Fisher Scientific Inc., USA). Spectra were recorded in ATR mode at 21 °C, with a 1 cm^{-1} resolution and 64 scans (corrected for background and atmosphere using OMNIC software provided with the spectrometer). Gel permeation chromatography (GPC) was performed on a Viscotek GPCmax Solvent/Sample Module. Two fluorinated polystyrene columns (FMBHW-3078 and FMBLMW-3078) were used in series and maintained at 22 °C. DMF was used as the eluent at a flow rate of 1 mL min^{-1}. Detection was performed using a Viscotek VE 3580 refractive index detector. Molecular weight and dispersity data are reported relative to polystyrene standards in DMF with 0.01 M LiBr at 40 °C, and a commercially available sample of polycaprolactone (GPC: M_n = 40.1 kDa, M_w/M_n of 2.02) from Polysciences Inc., (Warrington, PA, USA) in DMF at 22 °C. Differential scanning calorimetry (DSC). DSC experiments were carried out with a DSC Q100 (TA Instruments, USA), using airtight aluminum pans. Material was weighed into aluminum pans (TA Instruments, USA), and analyses were carried out under a nitrogen atmosphere (flow rate of 50 mL min^{-1}). The samples were treated as follows: heated from room temperature to 125 °C (10 °C min^{-1}), cooled to 30 °C (10 °C min^{-1}), heated from 30 °C to 125 °C (5 °C min^{-1}) and finally cooled to 30 °C (5 °C min^{-1}). The first temperature ramp removed traces of volatile solvents and the T_m from the second ramp is reported.

Synthesis of alkyne-displaying aminopropylpyrrole derivative

Aminopropylpyrrole (3.6 g, 29 mmol), propiolic acid (1.4 g, 20 mmol), dicyclohexylcarbodiimide (DCC, 6.0 g, 29 mmol), hydroxybenzotriazole (HOBt, 3.9 g, 29 mmol) and triethylamine (3.1 g, 4.4 ml, 29 mmol) were stirred for 48 hours in dichloromethane (DCM, 100 mL), after which the mixture was filtered to remove the precipitated dicyclohexylurea. The filtrate was washed with aqueous solutions of $NaHS0_4$ (160 g L^{-1}), $NaHC0_3$ (saturated), $NaHS0_4$, $NaHC0_3$, water and finally brine. The solution was dried over MgS04 and the volatiles removed with a rotary evaporator. The crude product was purified by silica column chromatography (eluting with a gradient of DCM 100%, to $CHC1_3$ 100%, to DCM:MeOH, 98:2) to give the product, a viscous oil, in a yield of (2.3 g, 13 mmol, 65%). ^{1}H NMR (400 MHz, $CDC1_3$) δ_H 6.65 (2H, $CH_{\beta\text{-pyrrole}}$), 6.16 (2H, $CH_{\alpha\text{-pyrrole}}$),

5.87 (1H, CONH), 3.95 (2H, CH_2), 3.29 (2H, CH_2), 2.75 (2H, CH_2). ESI-MS (m/z) calculated for $\mathbf{C_{10}H_{12}N_2O}$ $[M + H]^+$ requires 177.09; found, 177.10. IR (ATR) ν_{max} cm^{-1} 3344 (CH alkyne), 3224 (NH, amide), 2105 (alkyne), 1636 (amide I), 1530 (amide II). See **Scheme SI.**

Scheme SI. Synthesis of alkyne-displaying aminopropylpyrrole derivative

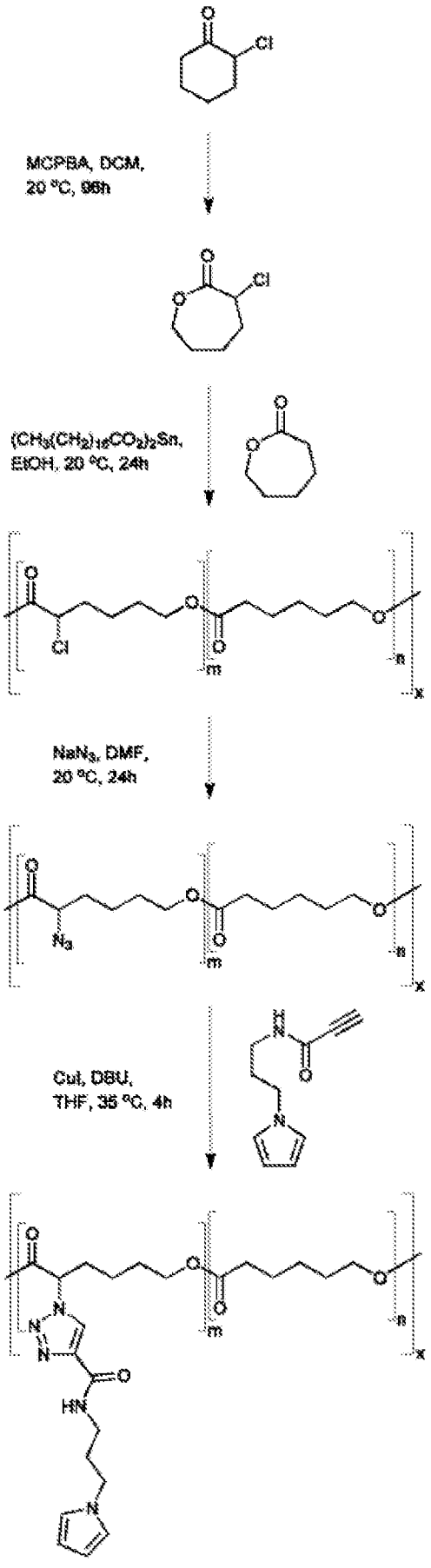


Synthesis of pyrrole-displaying polycaprolactone derivative

To a solution of 2-chlorocyclohexanone (25 g, 189 mmol) in DCM (250 mL) was added 3-chloroperbenzoic acid, (MCPBA, 50 g, 290 mmol) and the reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 96 hours. The reaction mixture was cooled to -20 °C, resulting in the precipitation of residual 3-chlorobenzoic acid. After filtration, the solution was washed three times with a saturated aqueous solution of $NaHS0_3$, three times with an aqueous solution of $NaHC0_3$, and once with water. The organic phase was dried over MgS04, filtered, and the solvent was removed on a rotary evaporator. The residue was distilled under reduced pressure, yielding a-chloro-s-caprolactone (22 g, boiling point 75-77 °C at 0.1 mm Hg). ε-caprolactone (4.0 g), a-chloro-s-caprolactone (1.0 g), stannous stearate (0.212 g) and ethanol (1.8 μL) were dissolved in toluene (15 mL) and stirred under nitrogen for 24 hours at room temperature, after which the product was precipitated in cold hexanes, dissolved in DCM and reprecipitated in cold hexanes and dried under high vacuum for 48 hours, affording poly(s-caprolactone-co-a-chloro-s-caprolactone) as a white powder in a yield of 2.26 g. Poly(s-caprolactone-co-a-chloro-s-caprolactone) (2.26 g) and sodium azide (3.045 g) were dissolved in dimethylformamide (DMF, 15 mL) and stirred under nitrogen for 24 hours at room temperature, after which the product was precipitated in water. The polymer was redissolved in DMF and reprecipitated in water, isolated by filtration under vacuum, and dried under high vacuum for 48 hours, affording poly(s-caprolactone-co-a-N_3-s-caprolactone) as a white powder in a yield of 2.86 g. Poly(s-caprolactone-co-a-N_3-s-caprolactone) (1.14 g), alkyne-displaying aminopropylpyrrole derivative (0.05 g), copper(I) iodide (0.04 g) and l,8-diazabicyclo[5.4.0]undec-7-ene (DBU,

0.03g) were stirred under nitrogen for 24 hours at 35 °C. The polymer was precipitated in water and the solids were incubated in 50 mL of an aqueous solution of ethylenediaminetetraacetic acid (EDTA, 5g in 100 mL water, pH 8) for 24 hours at 4 °C, after which the EDTA solution was decanted and replaced with fresh EDTA solution and incubated for a further 24 hours at 4 °C, after which the EDTA solution was decanted and replaced with water (50 mL) in which it was incubated for 24 hours at 4 °C, after which the water was decanted and replaced with fresh water (50 mL) in which it was incubated for 24 hours at 4 °C, after which the water was decanted and the polymer dried under high vacuum for 48 hours. This process afforded the pyrrole-displaying polycaprolactone derivative in a yield of 1.1 g in the form of a light brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (C*H* triazole), 7.09 (CON*H*), 6.53 (C$H_{\beta\text{-pyrrole}}$), 6.15 (C$H_{\alpha\text{-pyrrole}}$), 4.21, 4.04, 3.86, 3.65, 3.29, 2.32, 1.92, 1.64, 1.38, 1.10. IR (ATR) ν_{max} cm^{-1} 3326 (ML amide), 2099 (residual azide), 1721 (ester), 1622 (amide I), 1571 (amide II), 1457 (triazole). GPC: M_n = 5.0 kDa (M_w/M_n of 1.95). DSC: T_m = 54.5 °C. See **Scheme S2.**

Scheme S2. Synthesis of pyrrole-displaying polycaprolactone derivative


MCPBA, DCM,
20 °C, 96h
(CH3(CH2)16CO2)2Sn,
EtOH, 20 °C, 24h
NaN3, DMF,
20 °C, 24h
CuI, DBU,
THF, 35 °C, 4h

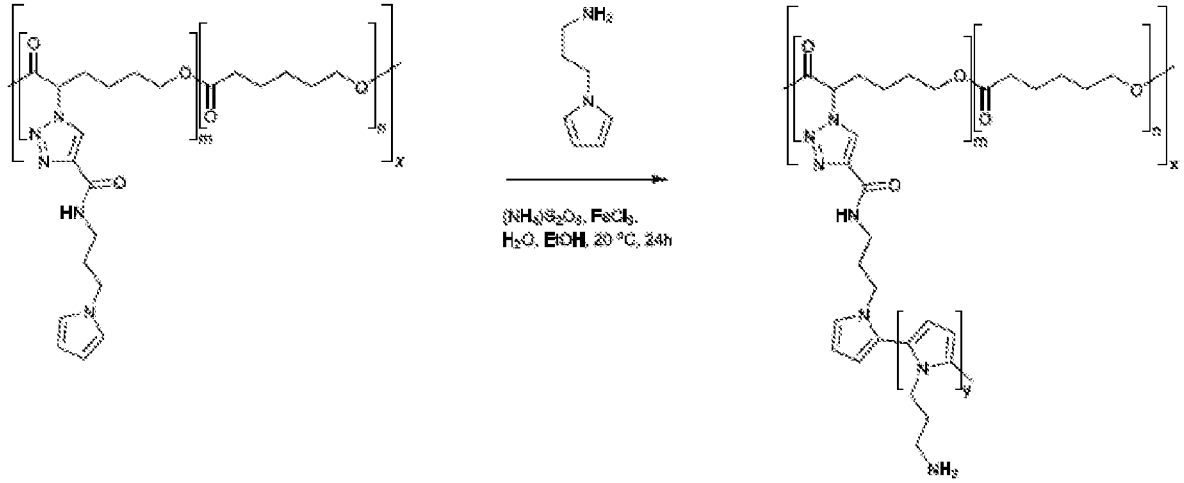
Preparation of films of pyrrole-displaying polycaprolactone derivative

Solutions of the pyrrole-displaying polycaprolactone derivative in chloroform (0.5 g in 20 mL) or hexalfuoroisopropanol (0.5 g in 20 mL) were cast on glass slides or in tissue-culture treated Corning® Costar® tissue culture plates, respectively. The solvent was allowed to evaporate for 24 hours and the films were subsequently dried under high vacuum for 48 hours.

Preparation of conductive films displaying amines

Aminopropylpyrrole (0.2 g) was dissolved in ethanol (2 mL), and added to a solution of ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after which the brown-black films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S3.**

Scheme S3. Synthesis of amine-displaying polycaprolactone derivative

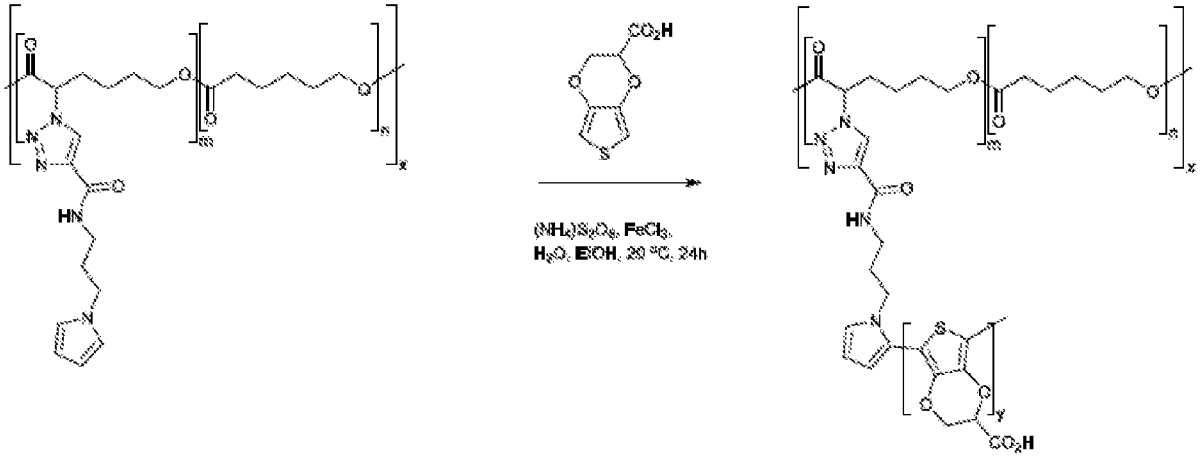


Preparation of conductive films displaying carboxylic acids

EDOT carboxylic acid (0.2 g), ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after

which the blue-grey films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S4.**

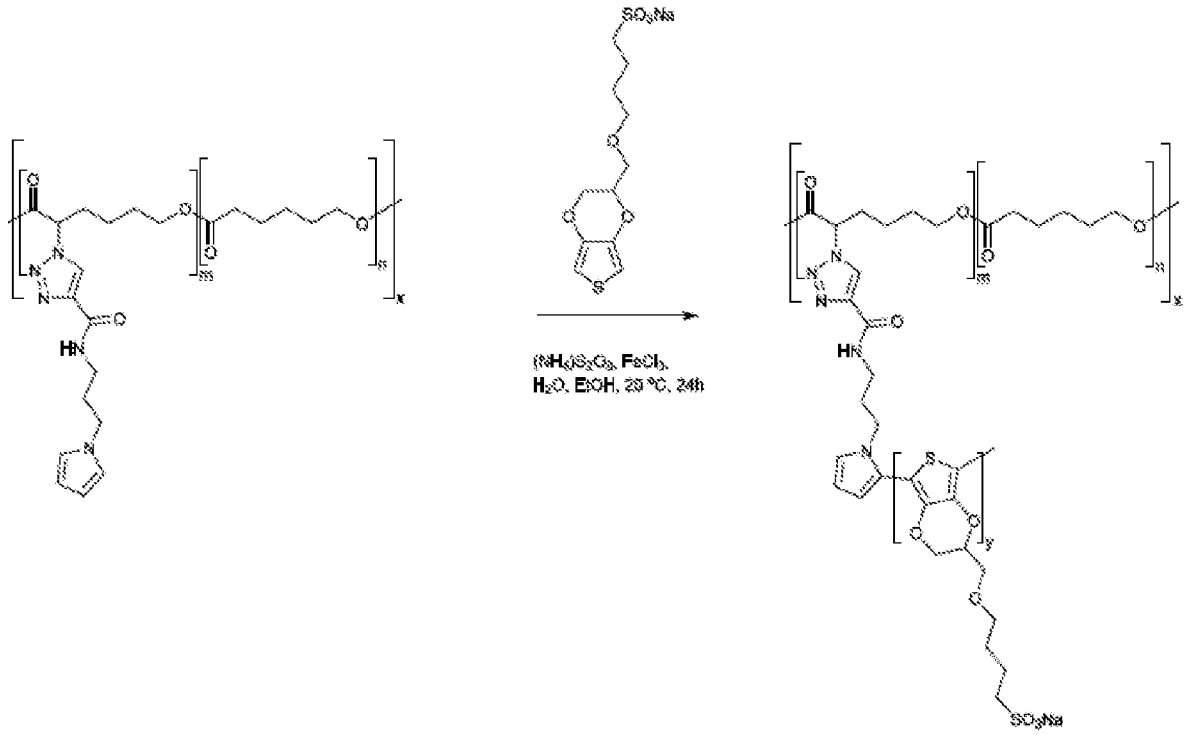
Scheme S4. Synthesis of carboxylic acid-displaying polycaprolactone derivative



Preparation of conductive films displaying sulfonates

Hydroxymethyl EDOT (3 g, 17.8 mmol, Sarchem laboratories, Inc., Farmingdale, NJ, USA) and sodium hydride (0.47 g, 19.6 mmol, 1.1 eq.) and were stirred and heated at reflux in toluene (50 mL) for 2 hours. Butane sultone (2.42 g, 17.8 mmol, 1 eq.) was added and the mixture was heated at reflux for a further 2 hours. After cooling to room temperature, the product was precipitated in acetone, isolated by filtration and subsequently dried under high vacuum for 48 hours. The process yielded a brown powder, EDOT-S (3.71 g, 11.4 mmol). ^{1}H NMR (400 MHz, D_20) δ_H 6.36, 4.70, 4.26, 4.12, 3.94, 3.59, 3.44, 2.78, 1.64, 1.55. EDOT-S (0.2 g), ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after which the blue-black films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S5.**

Scheme S5. Synthesis of sulfonate-displaying polycaprolactone derivative



Preparation of silica-coated conductive films

0.2 mL of phosphate buffer (pH 5.5) was added to conductive films displaying amines. Tetraethylorthosilicate (2.33 mL), water (3.85 mL), ethanol (3.85 mL) and 1N HC1 (0.1 mL) were mixed and incubated for 10 minutes at room temperature, and aliquots of this solution (20 μL) were added to the phosphate buffer covered the amine-displaying conductive films. The reaction mixture was incubated for 1 hour after which it was removed and the films were washed thoroughly with Millipore water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Preparation of calcium phosphate-coated conductive films

Conductive films displaying carboxylic or sulfonic acids were incubated in an aqueous solution (1 mL) of calcium chloride (200 mM) for 20 minutes, after which the solution was removed and the samples were washed with water (3 x 1 mL). Thereafter, samples were incubated in an aqueous solution (1 mL) of sodium phosphate (120 mM) for 20 minutes, after which the solution was removed and the samples were washed with water (3 x 1 mL). The cycle of incubation with calcium chloride and sodium phosphate was repeated a further six times (i.e. a total of 7 cycles), after which the samples were washed with water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Preparation of calcium carbonate-coated conductive films

Conductive films displaying carboxylic or sulfonic acids were incubated in an aqueous solution (1 mL) of calcium chloride (10 mM). The films were placed in an airtight container with a beaker containing ammonium carbonate for 24 hours. The samples were subsequently washed with water until the pH was neutral, after which the samples were washed with water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Electrical properties

Resistance *(R* in Ω) was measured between the two silver electrodes using a digital multimeter (DM-8A, Sperry Instrument, Milwaulkee, WI). Sheet resistance *(R_s)* in Ω/square was calculated as follows:

$$R_s = RW/L$$

where W is the width of the electrode and L is the distance between the two silver electrodes. The electrodes were moved to different positions after each measurement, and the resistance *R* was recorded in at least ten different positions on the materials.

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy

Samples were mounted on a Scanning Electron Microscopy (SEM) stub and sputter coated with carbon. All samples were imaged using a Hitachi S5500 SEM field emission scanning electron microscope equipped with an energy dispersive spectroscopy probe located at the Texas Materials Institute.

In vitro culture of human Mesenchymal stem cells without electrical stimulation

HMSCs were supplied by Lonza (Walkersville, MD). Samples were inserted in untreated polystyrene tissue culture plates and sterilized by incubation in 70% ethanol followed by exposure to UV for 60 min. After sterilization, the samples were incubated for 30 minutes in 24 well plates containing HMSC growth medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng mL^{-1} basic fibroblast growth factor. Medium was aspirated and replaced prior to HMSC seeding. Cell viability before starting the experiment was determined by the Trypan Blue exclusion method, and the measured viability exceeded 95 % in all cases. HMSCs were seeded at 10,000 cells per cm^2, and incubated at 37 °C, 95 % humidity, and a $C0_2$ content of 5 %. After 3 days the medium was aspirated, the materials were washed gently with PBS and transferred to a fresh 24 well plate containing osteogenic medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL);

antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM). Thereafter the osteogenic medium was aspirated and replaced every 2 days until the samples were analysed.

In vitro culture of human Mesenchymal stem cells with electrical stimulation

Electrical stimulation of Human Mesenchymal Stem cells was achieved employing a custom built setup. Non-conductive glass slides, polycarbonate wells (square polycarbonate blocks, thickness of 1 cm, sides of 2.5 cm, with square holes with sides of 0.9 cm cut out), Dow Corning® high vacuum grease, and medium binder clips (Staples®, Framingham, MA) were sterilized by autoclaving. Holes were drilled into the sides of 10 cm polystyrene Petri dishes using a Dremel saw (Lowes, Mooresfield, NC, USA), and the plates were sterilized by exposure to UV for 60 min. Adhesive-backed copper tape (5 mm width, Ted Pella, Inc.), waterproof Kapton® tape (1 cm width, Fisher Scientific, Waltham, MA, USA), wires and alligator clips were sterilized by exposure to UV for 60 min.

Electroactive PCL-based tissue scaffolds were placed on glass slides and secured in position with two thin strips of adhesive-backed copper tape that were attached to the scaffolds, parallel to one another and separated by a distance of ca. 4 cm. One face of the polycarbonate wells was coated with vacuum grease and placed on the electroactive tissue scaffolds, greased side down, in contact with the glass slide. A binder clip on either side of the well was used to secure this in position and render it water tight. A strip of copper tape was run between the parallel copper strips attached to the scaffolds and the ends of the slides as points of contact for the alligator clip-terminated wires attached to the multipotentiostat (CH Instruments, Austin, TX, USA). The counter and reference electrodes were connected together and clipped to copper tape on one side of the slide, and the working electrode was clipped to copper tape on the other side of the slide.

The setup was sterilized by exposure to UV (30 minutes), the samples were incubated for 30 minutes in HMSC growth medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng mL^{-1} basic fibroblast growth factor. Medium was aspirated and replaced prior to HMSC seeding. Cell viability before starting the experiment was determined by the Trypan Blue exclusion method, and the measured viability exceeded 95 % in all cases. HMSCs were seeded at 10,000 cells per scaffold, and incubated at 37 °C, 95 % humidity, and a $C0_2$ content of 5 %. After 2 days the medium was aspirated, the materials were washed gently with PBS and transferred to a fresh

24 well plate containing osteogenic medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM).

The tips of the wires attached to the samples were wound around alligator clip-terminated wires attached to the multipotentiostat (CH Instruments, Austin, TX, USA). The counter and reference electrodes were connected together and clipped to the wire protruding from one end of the sample, and the working electrode was clipped to the wire protruding from the other side of the sample. Wires and alligator clips were secured in position with adhesive copper tape (Ted Pella, Inc., Reading, CA, USA) and wrapped in Parafilm® to render them electrically insulating and waterproof (i.e. suitable for use inside an incubator). The electrical stimulation paradigm was as follows: a potential step of 10 mV mm^{-1} was placed across the samples for the duration of 8 hours followed by 40 hours without stimulation, repeated three times (32 hours of stimulation in total), after which the wires were disconnected and the substrates cultured as normal. Throughout the electrical stimulation experiments the osteogenic medium was aspirated and replaced every 2 days. Thereafter the osteogenic medium was aspirated and replaced every 2 days until the samples were analysed.

Biochemical assays

The DNA content and Alkaline Phosphatase (ALP) activity of samples that were broken up in a buffer of 0.2% Triton X-100 were quantified concurrently, using the PicoGreen® assay (Life Technologies, Thermo Fisher Scientific Inc., USA) for DNA quantitation in accordance with the manufacturer's protocol, a SensoLyte® pNPP Alkaline Phosphatase Assay Kit (AnaSpec, Inc., Freemont, CA, USA) for ALP quantitation in accordance with the manufacturer's protocol, and a Synergy HT Multi-Mode Microplate Reader (Bio-tek US, Winooski, VT).

Fluorescence staining and imaging of cells

Cells fixed with paraformaldehyde were permeabilized with 0.1% Triton X-100 (Fluka) and 2% bovine serum albumin (BSA) in PBS buffer for 5 min, followed by blocking with 2% BSA in PBS buffer for 30 min at room temperature. Actin filaments and cell nuclei within cells were stained with Alexa Fluor 488® Phalloidin (Life Technologies, USA) for 30 min and 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, USA) for 5 min, respectively. The cells were thereafter washed three times with PBS and stored at 4 °C until images were acquired. Fluorescence images of cells were obtained using an Olympus 1X70 inverted

microscope equipped with an Olympus DP80 dual color and monochrome digital camera (a 1.4 megapixel Bayer mosaic color CCD camera) that was attached to the microscope with a 0.63 B-mount. Image Analysis was done using Olympus cellSens® imaging software, Version 1.1 1.

It should be noted that ratios, concentrations, amounts, and other numerical data may be expressed herein in a range format. It is to be understood that such a range format is used for convenience and brevity, and thus, should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a concentration range of "about 0.1% to about 5%" should be interpreted to include not only the explicitly recited concentration of about 0.1 wt% to about 5 wt%, but also include individual concentrations *(e.g.,* 1%, 2%, 3%, and 4%) and the sub-ranges *(e.g.,* 0.5%, 1.1%, 2.2%, 3.3%, and 4.4%) within the indicated range. In an embodiment, the term "about" can include traditional rounding according to significant figures of the numerical value. In addition, the phrase "about '**x**' to 'y'" includes "about 'x' to about '**y**"\

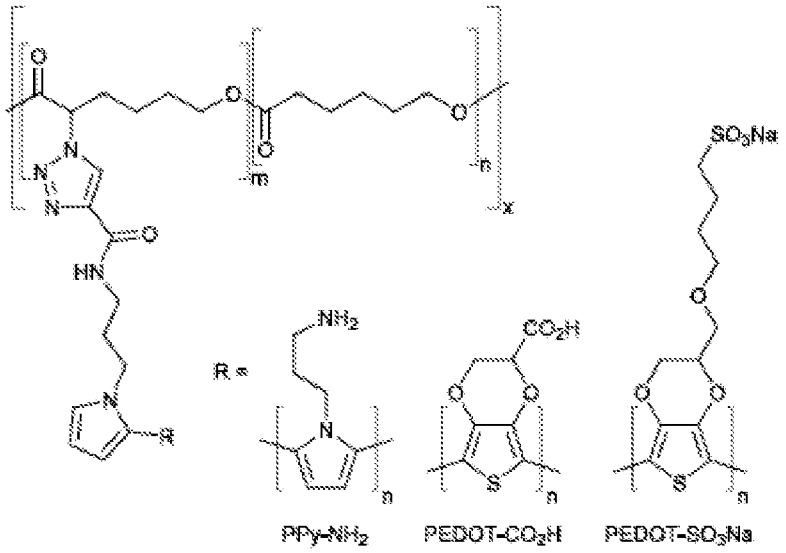
Many variations and modifications may be made to the above-described embodiments. All such modifications and variations are intended to be included herein within the scope of this disclosure and protected by the following claims.

CLAIMS

We claim:

1. A structure, comprising:

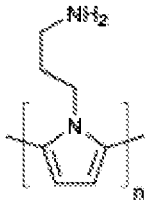
an electroactive scaffold comprising the following polymer:

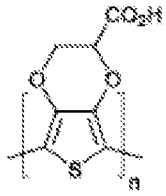


wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and n for each of the R group is 1 to 1,000,000.

2. The structure of claim 1, wherein human mesenchymal stem cells are disposed on the electroactive scaffold.

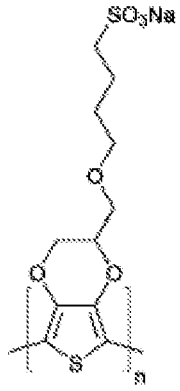
3. The structure of claim 2, wherein the electroactive scaffold is a polymer film.

4. The structure of claim 2, wherein the R group is  .



PEDOT-CO_2H

5. The structure of claim 2, wherein the R group is .

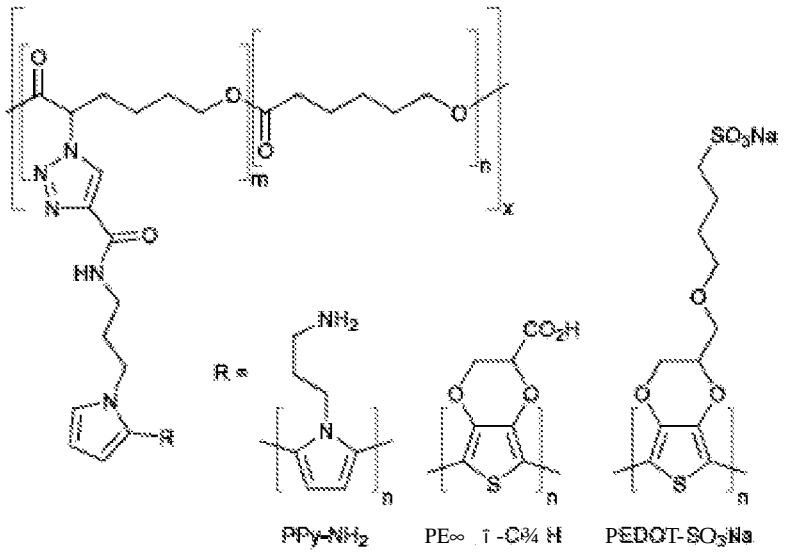


PEDOT-SO_3Na

6. The structure of claim 2, wherein the R group is .

7. A method of differentiation of human mesenchymal stem cells, comprising:

providing a structure having an electroactive scaffold including the following polymer:

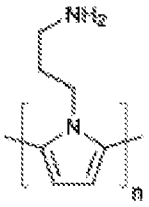


PPy-NH_2 PE∞ ĩ -C¾ H PEDOT-SO_3Na

wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and n for each of the R group is 1 to 1,000,000;

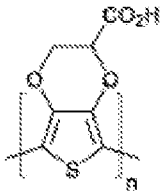
introducing human mesenchymal stem cells to the structure, wherein the structure and the human mesenchymal stem cells are cultured in an osteogenic medium; and

periodically providing electrical stimulation to the human mesenchymal stem cells to cause differentiation of the human mesenchymal stem cells towards osteogenic outcomes.

8. The method of claim 7, further comprising: growing biomineral silica on the structure when the R group is 

.

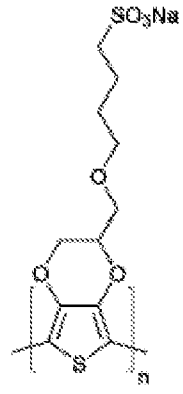
9. The method of claim 7, further comprising: growing biomineral calcium carbonate, calcium phosphate, or a combination thereof on the structure when the R group is



PEDOT-CO_2H

.

10. The method of claim 7, further comprising: growing biomineral calcium carbonate, calcium phosphate, or a combination thereof on the structure when the R group is

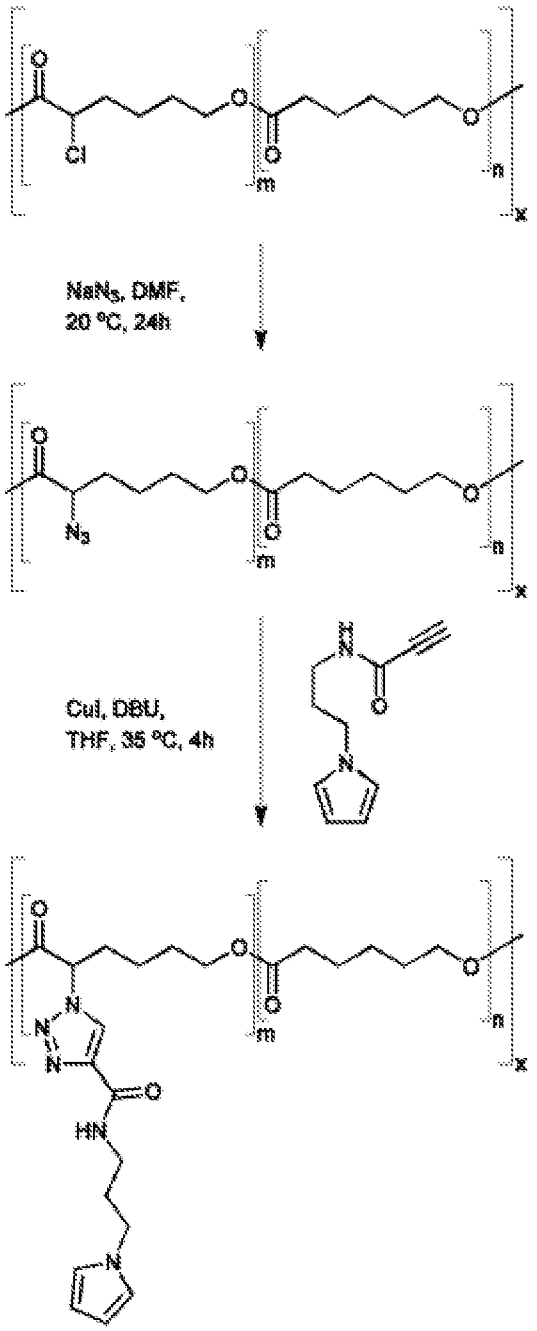


PEDOT-SO_3Na

.

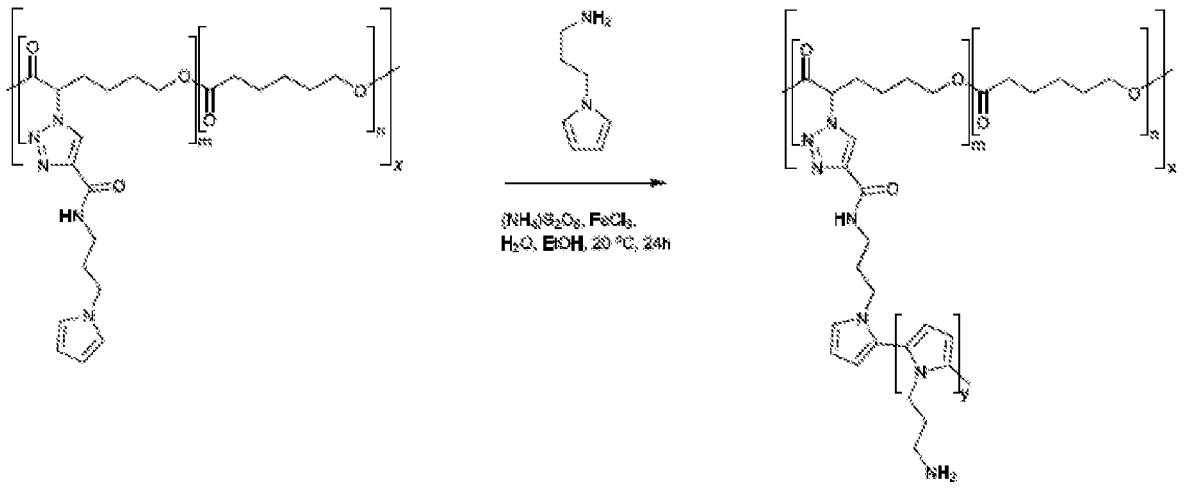
11. The method of claim 7, wherein the electrical stimulation increases ALP activity in the structure.

12. A method of making a pyrrole-displaying derivative, comprising:



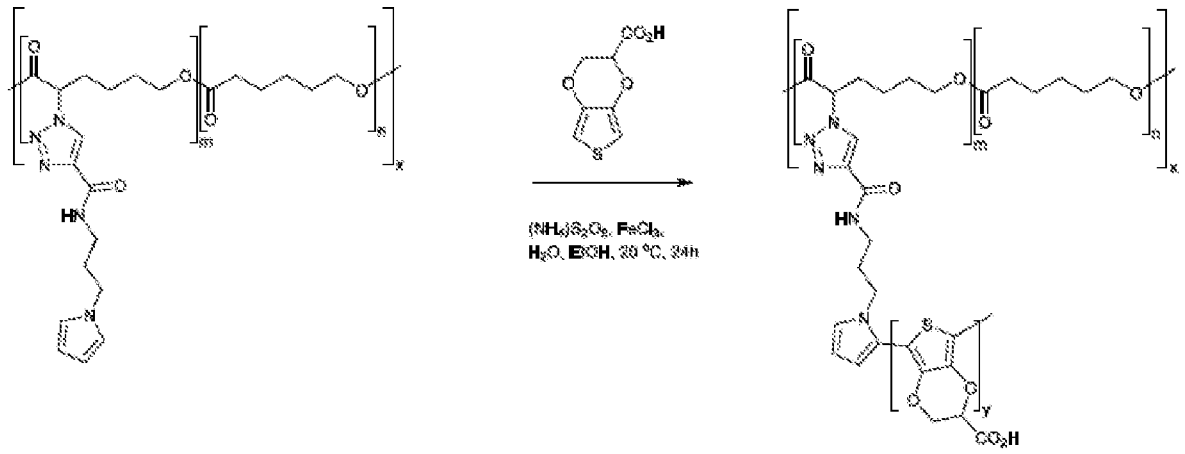
, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000.

13. A method of making an amine-displaying polycaprolactone derivative, comprising:



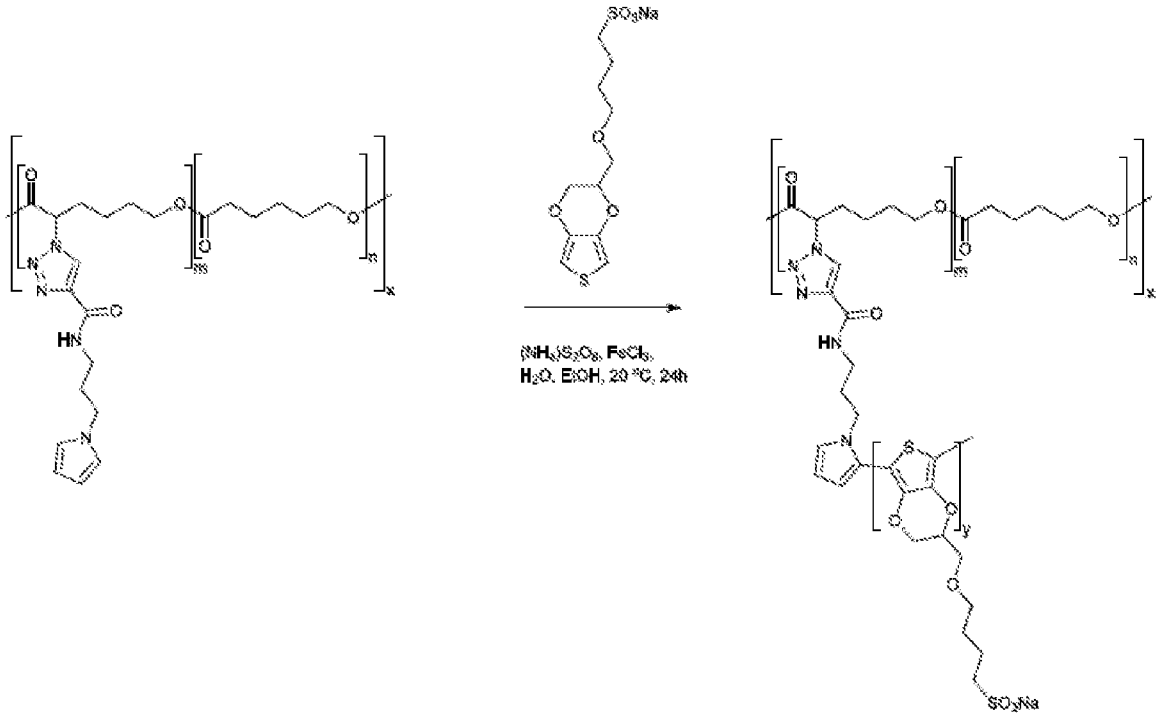
, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

14. A method of making a carboxylic acid-displaying polycaprolactone derivative, comprising:



, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

15. A method of making a sulfonate-displaying polycaprolactone derivative, comprising:



, wherein m is 1 to 1,000,000, n is 1 to 1,000,000, x is 1 to 1,000,000, and y is 1 to 1,000,000.

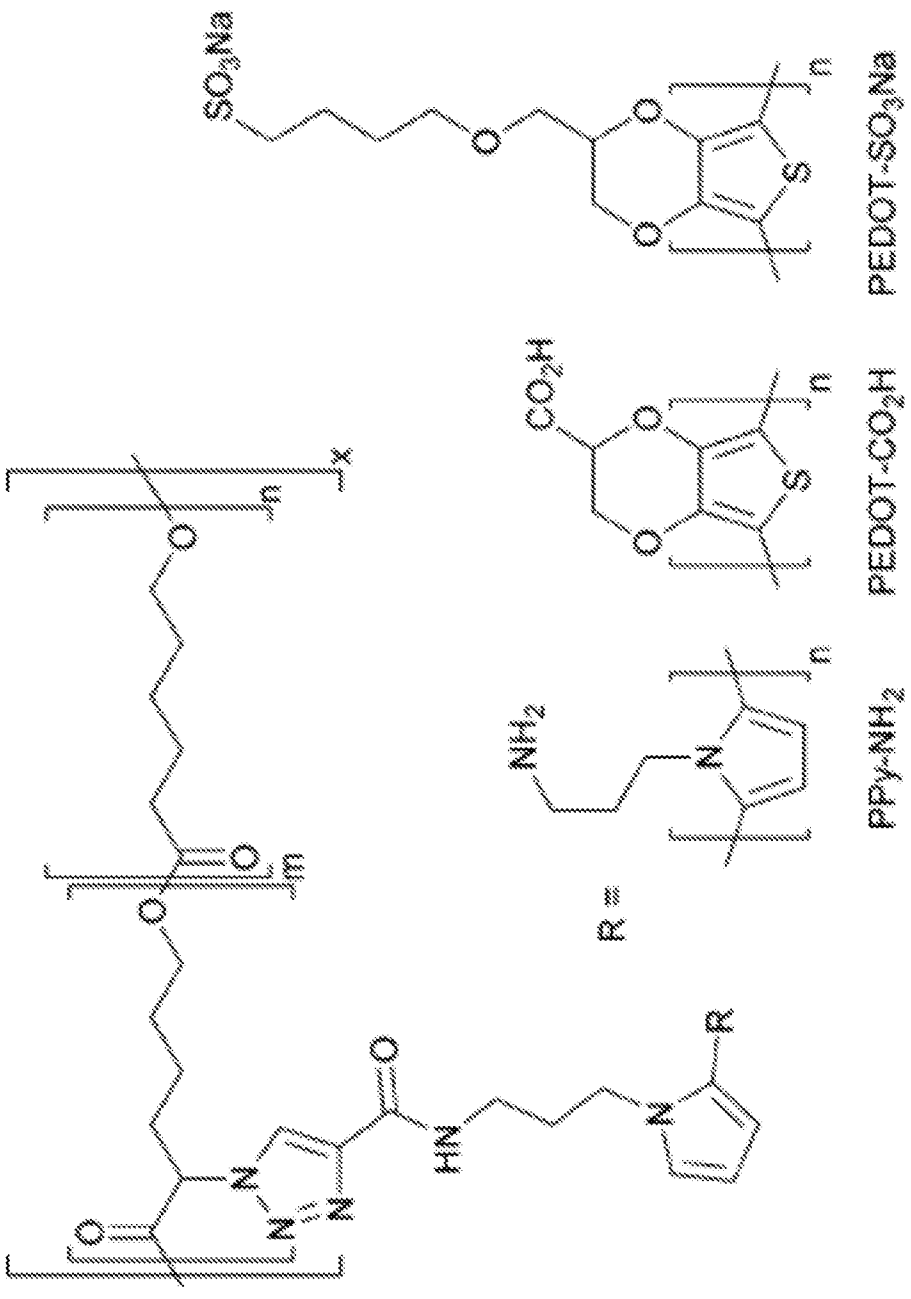


Fig. 1

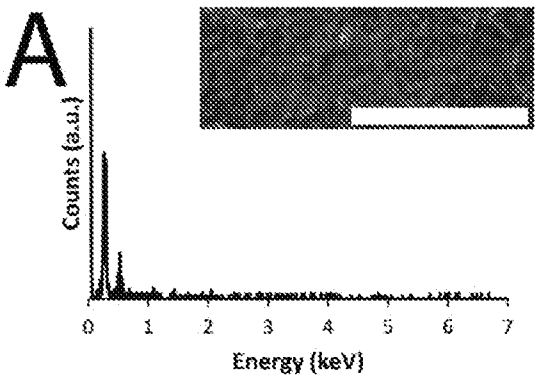


Fig. 2A

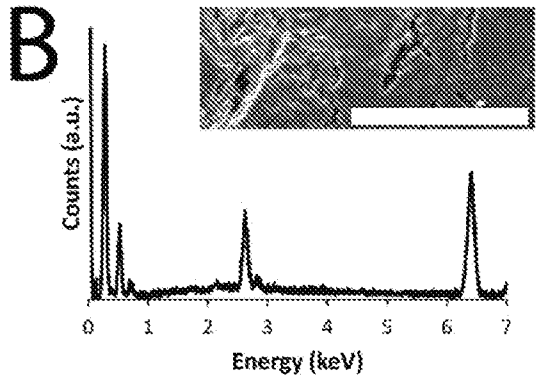


Fig. 2B

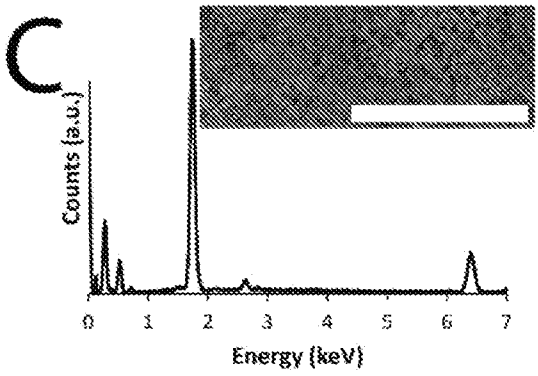


Fig. 2C

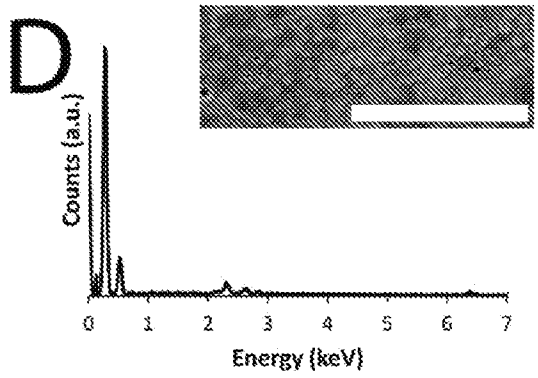


Fig. 2D

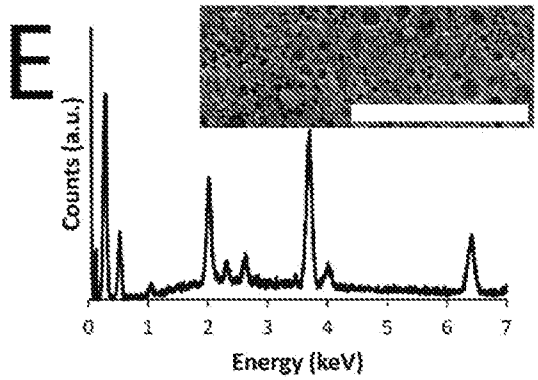


Fig. 2E

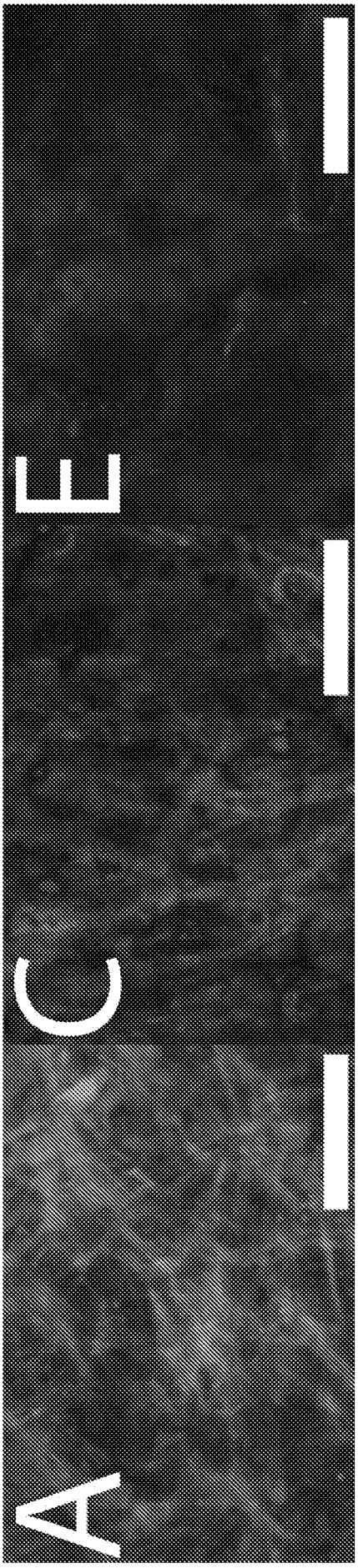


Fig. 3A

Fig. 3C

Fig. 3E

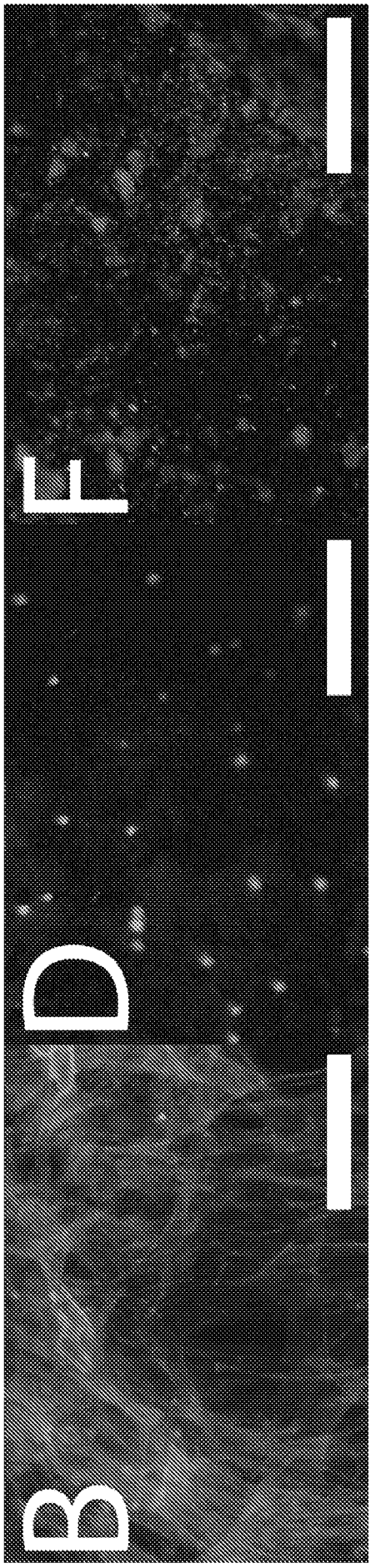


Fig. 3B

Fig. 3D

Fig. 3F

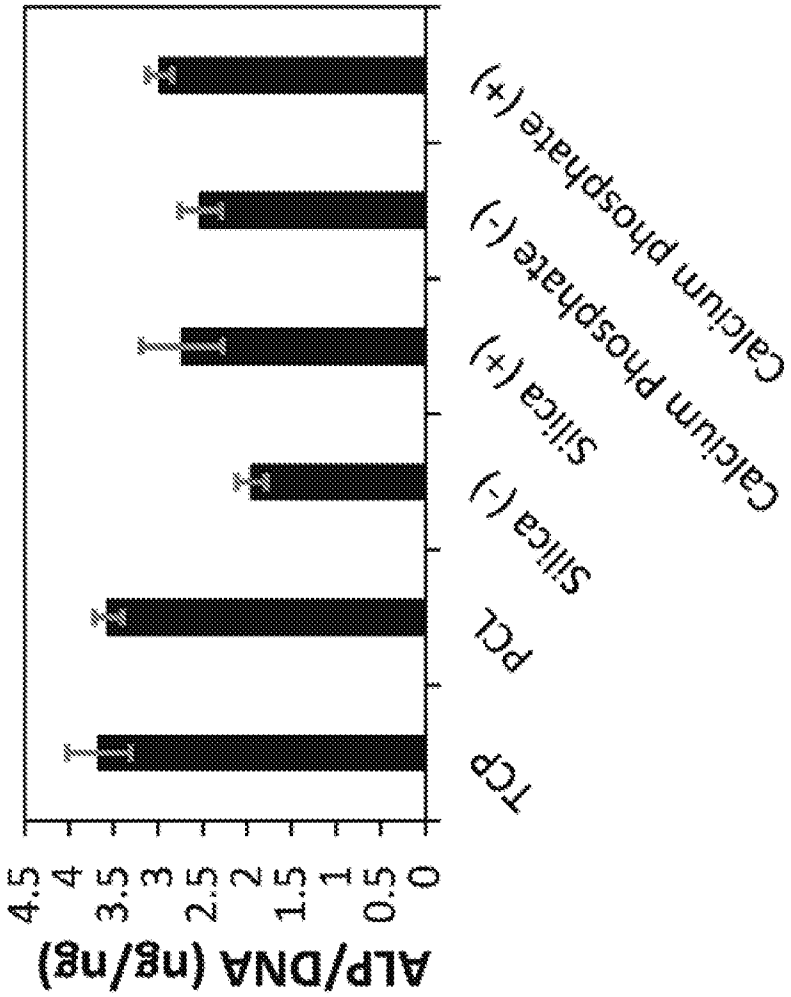

ALP/DNA (ng/ng)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
TCP
PCL
Silica (-)
Silica (+)
Calcium phosphate (-)
Calcium phosphate (+)

Fig. 4

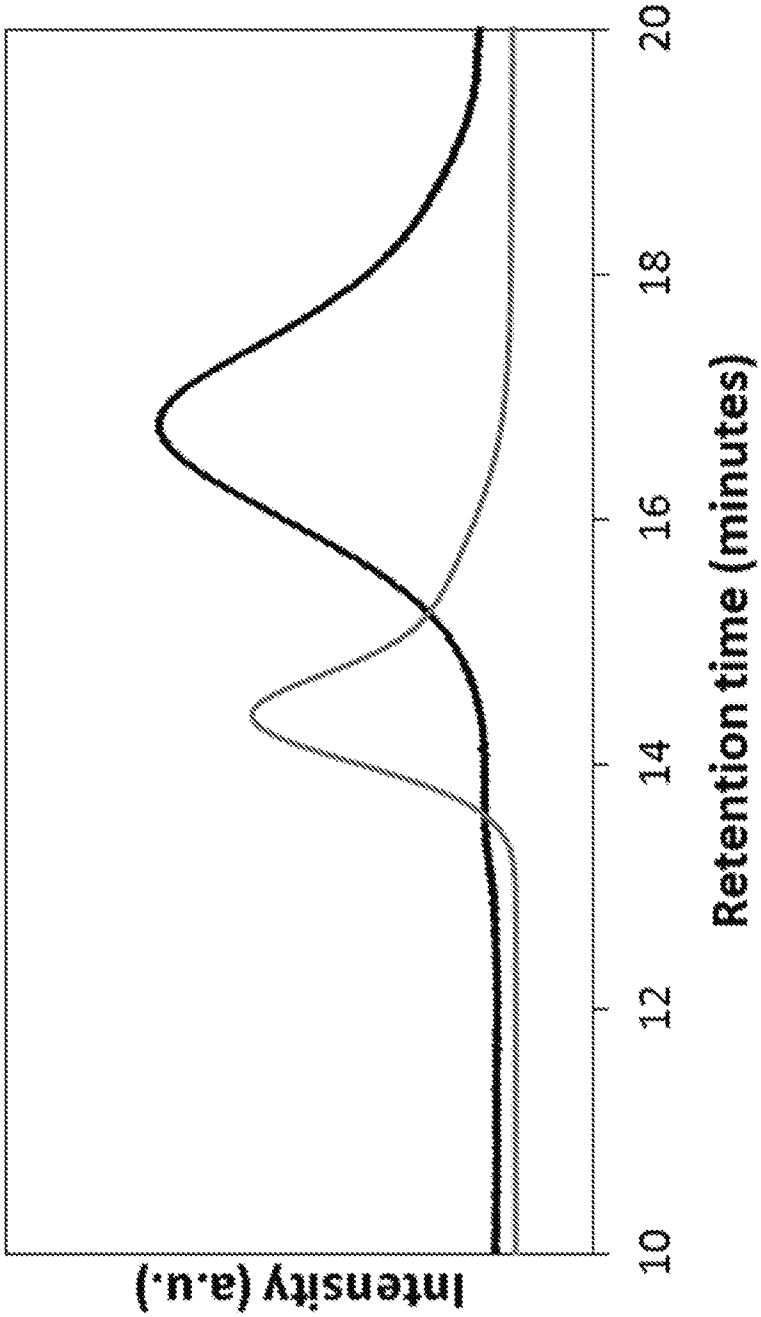


Fig. 5

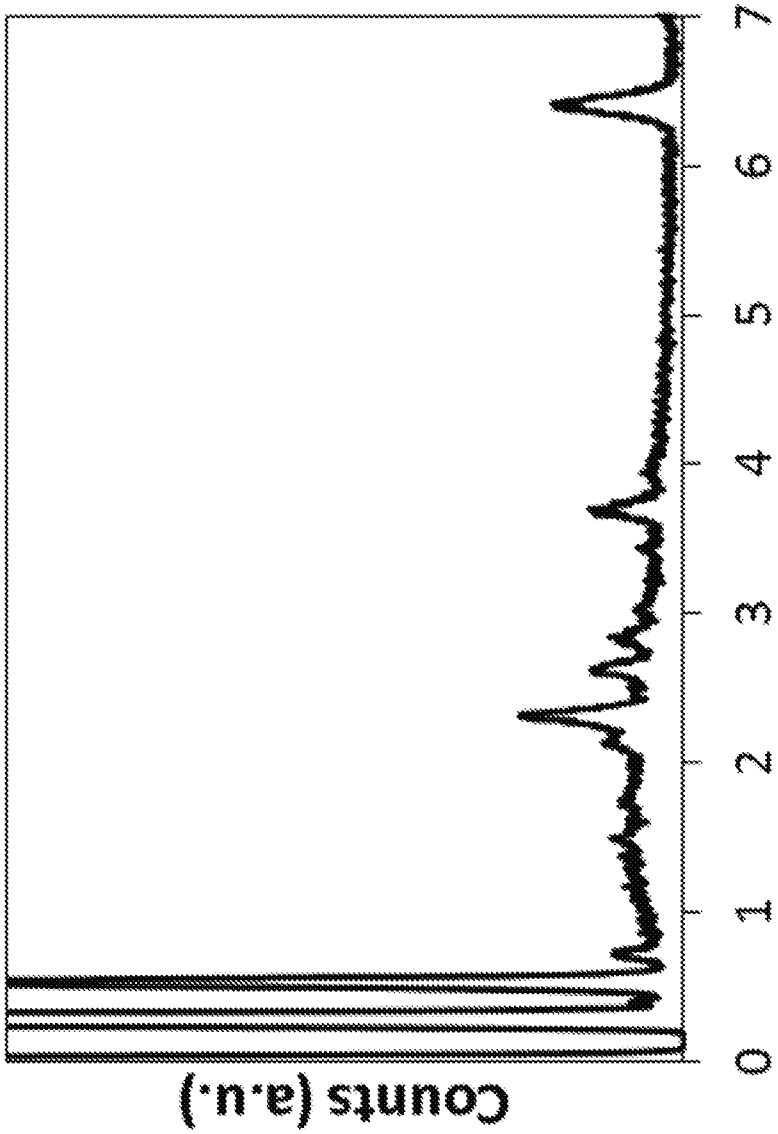


Fig. 6

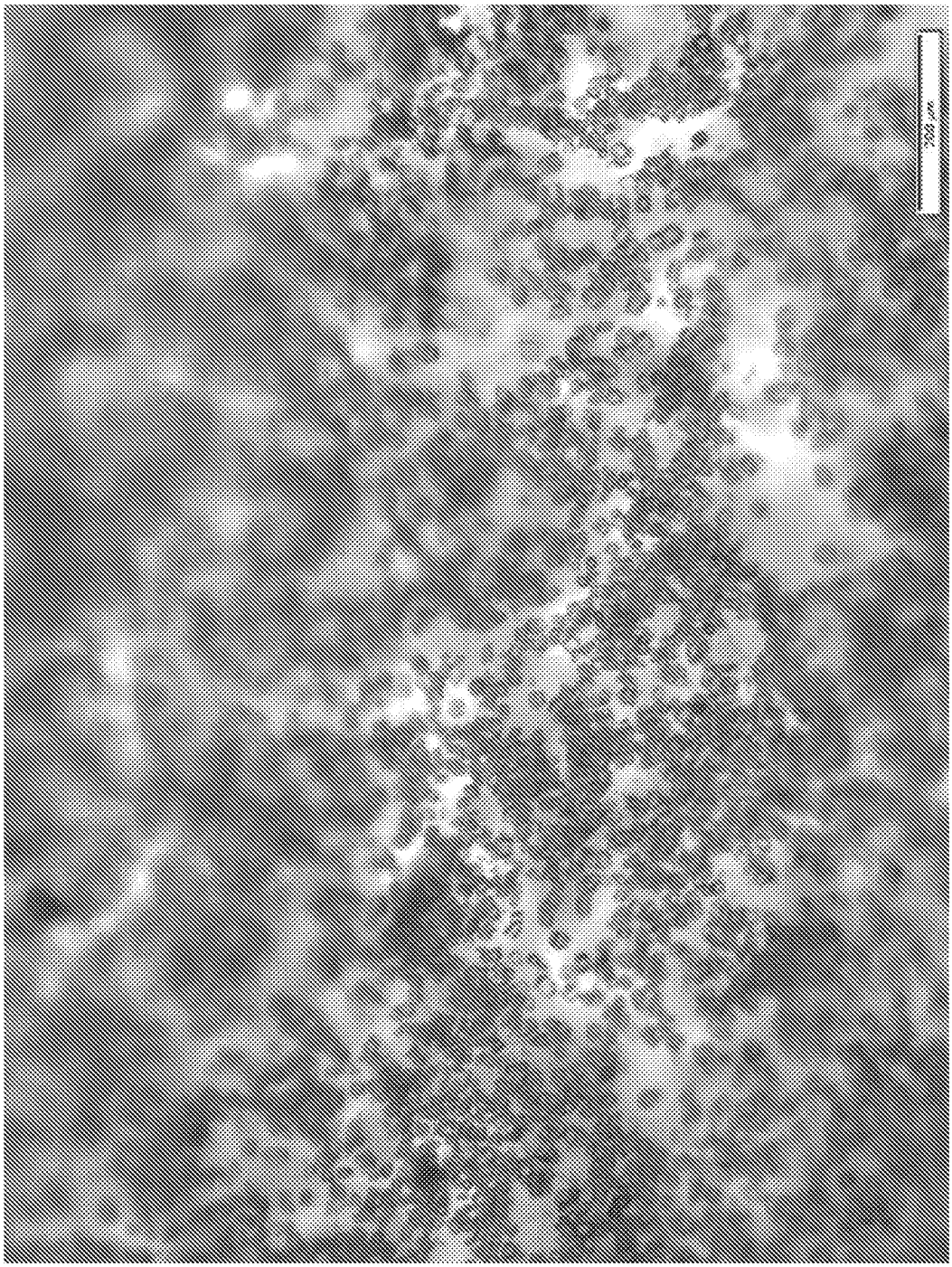


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/41893

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 487/22 (2016.01)
CPC - C07D 487/22, Y10T 428/249953
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07D 487/22 (2016.01)
CPC: C07D 487/22, Y10T 428/249953

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase; Keyword limited: electroactive scaffold; human mesenchymal stem cells; growing biomineral silica; calcium carbonate; ALP activity; pyrrole/amine/ sulfonate/carboxylic acid-displaying; polycaprolactone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0018646 A1 (ZHAO) 15 January 2009 (15.01 .2009), entire document, especially: abstract; para [0004]; para [0007]; para [0041]; Fig 1; Fig. 3; claim 6.	1-15
Y	US 2005/0009986 A1 (GROENENDAAL et al.) 13 January 2005 (13.01 .2005), entire document, especially: abstract; para [0005]-[0006]; para [0009]-[0010]; para [0055], Table, row 1, row 3; para [0089], 4-(2,3-dihydro-thieno[3,4-b)][1,4]dioxin-2-yl-metnoxy)-butane-1-sulfonic acid sodium salt.	1-6,9-10,14-15
Y	HARDY et al. "Electrical Stimulation of Human Mesenchymal Stem Cells on Conductive Nanofibers Enhances their Differentiation toward Osteogenic Outcomes", Macromolecular Rapid Communications, 6 July 2015 (06.07.2015), 7 pages, entire document, especially: pg 2, col 2, para 2; pg 3, Figure 1B; pg 5, col 1, para 1; pg 6, col 1, para 1.	7-8,9-10,1 1-13

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2016	29 SEP 2016

Name and mailing address of the ISA/US	Authorized officer.
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774