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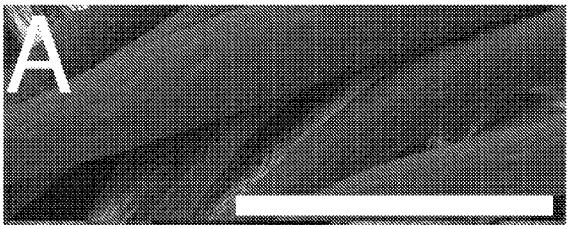

Fig. 1A

(57) Abstract: Embodiments of the present disclosure provide for nonwoven mat of fibers (e.g., polycaprolactone fibers), method of making the nonwoven mat, method of using the nonwoven mat, and the like. In an embodiment, the fibers can be coated with an in-terpenetrating network of a conductive polymer and a dopant on the surface of the fibers.

CONDUCTIVE NONWOVEN MAT AND METHOD OF USING THE CONDUCTIVE NONWOVEN MAT

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Application Serial No. 62/184,324, having the title "CONDUCTIVE NONWOVEN MAT AND METHOD OF USING THE CONDUCTIVE NONWOVEN MAT," filed on June 25, 2015, the disclosure of which is incorporated herein in by reference in its entirety.

BACKGROUND

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. Materials that have been investigated for bone repair and regeneration include non-biodegradable materials or biodegradable materials. However, further research needs to be done to find suitable materials.

SUMMARY

Embodiments of the present disclosure provide for nonwoven mat of fibers *(e.g.,* polycaprolactone fibers), method of making the nonwoven mat, method of using the nonwoven mat, and the like. In an embodiment, the fibers can be coated with an interpenetrating network of a conductive polymer and a dopant on the surface of the fibers.

An embodiment of the present disclosure provides for a method of differentiation of human mesenchymal stem cells, among others, that includes: providing a nonwoven mat of fibers, wherein interpenetrating networks of a conductive polymer and a dopant are on the surface of the fibers; introducing human mesenchymal stem cells to the nonwoven mat, wherein the nonwoven mat 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 human mesenchymal stem cells towards osteogenic outcomes. In an embodiment, the electrical stimulation causes increased ALP activity and increased Ca^{2+} deposition on the fibers relative to not periodically providing electrical stimulation.

An embodiment of the present disclosure includes a structure, among others, that includes: a nonwoven mat of fibers, wherein interpenetrating networks of a conductive

polymer and a dopant are on the surface of the fibers, wherein human mesenchymal stem cells are disposed within the nonwoven mat. In an embodiment, osteoblasts are present within the nonwoven mat after exposure of the nonwoven mat to an osteogenic medium. In an embodiment, the nonwoven mat includes ALP and Ca^{2+} deposition on the fibers.

In an embodiment, the fibers can include one or more of the following: polycaprolactone, polyester, polyamide, PCL, PLLA, PLGA, protein, polysaccharide, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, and chitosan. In an embodiment, the conductive polymer can include one or more of the following: polypyrrole, polyaniline, polythiophene, poly(3,4-ethylenedioxythiophene), polyfluorene, polyphenylene, polypyrene, polyazulene, polynapthalene, polyindole, polyazepine, poly(p-phenylene sulfide), poly(p-phenylene vinylene), and polyfuran.

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.

Figures 1A-D illustrate physicochemical analysis of the electrospun fibers. Figs. 1A and IB are SEM images of mats of electrospun fibers (Fig. 1A is PCL; Fig IB is PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate. Scale bars represent 20 μm). Figs. 1C and ID are XPS spectra: N Is and S 2p, respectively. Grey lines are the spectrum of mats of electrospun PCL, whereas black lines are the spectrum of mats of electrospun PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate.

Figures 2A-2D are images of fluorescently stained cells cultured on various substrates. DAPI-stained nuclei are blue and Alexa Fluor® 488-stained actin is green. Fig. 2A shows tissue-culture treated Corning® Costar® TCP controls. Fig. 2B shows mats of electrospun PCL. Fig. 2C shows mats of electrospun PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate without electrical stimulation. Fig. 2D shows mats of electrospun PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate with electrical stimulation. Scale bars represent 100 μm.

Figures 3A-3C illustrate biochemical analysis of cell culture experiments. Fig. 3A shows DNA content. Fig. 3B shows ALP activity. Fig. 3C shows calcium deposition. TCP, tissue-culture treated Corning® Costar® tissue culture plate controls. PCL, mats of electrospun PCL. PCL-CP (-), mats of electrospun PCL with an interpenetrating network of

polypyrrole and polystyrenesulfonate without electrical stimulation; PCL-CP (+), mats of electrospun PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate with electrical stimulation.

Figure 4 is a schematic of an experimental setup for electrical stimulation of cells on conducting PCL-based nanofibers (Not to scale). (CE) counter electrode. (CT) copper tape. (PCW) polycarbonate well. (RE) reference electrode. (WE) working electrode.

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.

Prior to describing the various embodiments, the following definitions are provided and should be used unless otherwise indicated.

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 nonwoven mat of fibers *(e.g.,* polycaprolactone fibers), method of making the nonwoven mat, method of using the nonwoven mat, and the like. In an embodiment, the fibers can be coated with an interpenetrating network of a conductive polymer and a dopant on the surface of the fibers.

In an embodiment, the nonwoven mat has electroactive characteristics so that an electrical stimulation can be periodically applied to the nonwoven mat. In a particular embodiment, a cell such as a human mesenchymal stem cell can be incubated with the nonwoven mat and cultured in an osteogenic medium so that the stem cells differentiate towards osteogenic outcomes *(e.g.,* osteoblasts). As shown in Example 1, electrical stimulation of the nonwoven mat in the presence of human mesenchymal stem cells in the osteogenic medium shows increased differentiation towards osteogenic outcomes as compared to nonwoven mats without electrical stimulation and other types of mat.

In this regard, embodiments of the disclosure provide for methods of differentiation of human mesenchymal stem cells. An embodiment of the present disclosure includes introducing human mesenchymal stem cells to the nonwoven mat, where the nonwoven mat and the human mesenchymal stem cells are cultured in an osteogenic medium. Subsequently, electrical stimulation can be periodically applied to the human mesenchymal stem cells to enhance differentiation of human mesenchymal stem cells towards osteogenic outcomes. Application of electrical stimulation to the nonwoven mat increases ALP activity and increased Ca^{2+} deposition on the fibers of the nonwoven mat, which can lead to formation of calcified bone-like extracellular matrix.

Electrical stimulation can include direct or indirect 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 an embodiment, the nonwoven mat of fibers includes an interpenetrating network of a conductive polymer and a dopant on the surface of the fibers. In an embodiment, the fiber of the nonwoven mat can be polycaprolactone fiber, where the fibers include a conductive polymer and a dopant on the surface of the fiber. In a particular embodiment, an interpenetrating network of polypyrrole and polystyrenesulfonate is on the surface of the polycaprolactone fibers.

In an embodiment, the conductive polymer and the dopant on the surface forms a layer *(e.g.,* having a thickness of about 1 to 5000 nm) of these materials, where the layer may or may not be continuous. In an embodiment, the amount of the conductive polymer and the dopant disposed on the fibers can be about 1 to 100% by mass, where the range includes each 1% increment *(e.g.,* 1 to 10%, about 50 to 80%, about 20 to 60%, and the like).

In an embodiment, the fibers can be synthetic polymers *(e.g.,* polyesters, polyamides, polycaprolactone, PCL, PLLA, PLGA, etc.) and natural polymers *(e.g.* proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, and the like). In an embodiment, the nonwoven mat can include a mixture of different types of fibers *(e.g.,* a portion can be polycaprolactone fibers and another portion can be polyester fibers).

In an embodiment, the conducting polymer can include polyaniline, polythiophene, poly(3,4-ethylenedioxythiophene), polyfluorenes, polyphenylenes, polypyrenes, polyazulenes, polynapthalenes, polyindoles, polyazepines, poly(p-phenylene sulfide)s, poly(p-phenylene vinylene)s, and polyfurans, as well as copolymers and derivatives thereof. In an embodiment, there are biodegradable versions, in which there are block(s) of conducting units within a polymer chain containing biodegradable bonds *(e.g.* esters and amides), that can also be used as the conducting polymer.

In an embodiment, the dopant can be a polymer that has the opposite charge to the conducting polymer, and can be low molecular weight *(e.g.,* chlorine ions, tosylate ions, and the like) or high molecular weight *(e.g.,* collagen, hyaluronic acid, and the like).

In an embodiment, the fibers can have a diameter of about 10 nm to 500 micrometers and a length of about 1 micrometer to 1 meter. In an embodiment, the nonwoven mat can include 1 to 1 million fibers. In an embodiment, the nonwoven mat can have a length of about 500 micrometers to 2 meters and a width of about 500 micrometers to 2 meters.

In an embodiment, the fibers *(e.g.,* polycaprolactone fibers) can be formed using techniques such as electrospinning, wet spinning, or dry spinning. In a particular embodiment, the fibers are formed by disposing a polycaprolactone fluid (or other fiber material) in a syringe with a solvent *(e.g.,* chloroform and methanol) and dispensing the fiber of polycaprolactone, which is subsequently dried. The length and the diameter of the fiber can be controlled so that the desired dimensions of the fiber can be selected.

In an embodiment, the polypyrrole (or other conducting polymer) and polystyrenesulfonate (or other dopant) can be disposed on the polycaprolactone nanofibers by incubating the fibers *(e.g.,* polycaprolactone fibers) with a monomer *(e.g.* pyrrole, thiophene, aniline, 3,4-ethylenedioxythiophene), polystyrenesulfonate, and an agent capable of polymerizing the monomer *(e.g.,* an oxidizing agent such as ferric chloride, a light source or suitable electrochemistry apparatus) for an appropriate time period *(e.g.,* about 1 to 36 hours or about 24 hours). After incubation, the residual materials can be washed away and the fibers *(e.g.,* polycaprolactone fibers) having interpenetrating networks of conducting polymer and dopant *(e.g.,* polypyrrole and polystyrenesulfonate) are formed. Additional details are provided in Example 1.

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 nonwoven mat of fibers *(e.g.,* polycaprolactone fibers), where the fibers include interpenetrating networks of

the conducting polymer and the dopant on the surface of the fibers *(e.g.,* polypyrrole and polystyrenesulfonate). In addition, human mesenchymal stem cells *(e.g.,* on collagen-1 coated substrates) and the differentiated products of the stem cells are disposed within the nonwoven mat. Furthermore, the nonwoven mat includes ALP and Ca^{2+} can be deposited on the fibers. In an embodiment, the nonwoven mat can include one or more agents *(e.g.,* a chemical or biological agent), where the agent can be disposed indirectly or directly on the nonwoven mat. As described herein, the agent can include a stem cell such as a human mesenchymal stem cell.

In addition, an additional agent that can be disposed on the nonwoven mat 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 and/or Ca^{2+} production), where such factors to accomplish the purpose are well known in the medical arts.

In general, the agent can be bound to the nonwoven mat 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-nonwoven mat interactions could be controlled through the inclusion of cell-adhesive peptides *(e.g.,* RGD, YIGSR, KQAGDV, KHIFSDDSSE, KRSR), and 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).

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:

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. Materials that have been investigated for bone repair and regeneration include non-biodegradable materials (e.g. ceramic-, glass-, polymethylmethacrylate-, and titanium-based materials) or biodegradable materials (e.g. autografts, allografts, and polycaprolactone (PCL)), and those that simultaneously deliver drugs.[1] Electrospun polymer tissue scaffolds are popular because of their fibrous morphologies that mimic the fibrous nature of the extracellular matrix of a variety of tissues, and their readily tuneable chemical compositions and mechanical properties. Such materials have been investigated for their application as tissue scaffolds for a variety of tissues including bone.[2]

Conducting polymer (CP) biointerfaces (e.g. based on derivatives of aniline, pyrrole or thiophene), are of interest for both long term applications (e.g. electrodes) and short term applications (e.g. drug delivery devices or tissue scaffolds).[3] CP-based scaffolds have been developed for the regeneration of bone, muscle and nerve tissue,[3] and conductive electrospun fiber-based scaffolds for each of these tissues have been reported.[4] Early studies focused on methodologies for obtaining conductive nanofibers and characterization of their physichochemical properties,[5] and subsequent studies characterized their application as tissue scaffolds,[6] with exciting results showing that conductive nanofibers promoted the formation of muscle-like tissue, with altered gene expession from C2C12 cells and enhanced myotube maturation.[7] A number of interesting studies have been carried out, including those demonstrating that electrical stimulation of human dermal fibroblasts on conductive nanofibers promoted their proliferation;[8] those revealing that electrical stimulation of cells from the central nervous system (e.g. rat-derived PC12 cells[9] and stem cells[10]) or peripheral nervous system (explanted dorsal root ganglia from chicken embryos/ [11] increased the length and number of neurites protruding from the cells.

CP-based materials were first investigated for their potential application as bone tissue scaffolds by Langer and coworkers.[12] Bone marrow-derived stromal cells were encouraged to differentiate towards osteogenic outcomes when cultured on 2-dimensional polypyrrole films across which a potential difference of 20 mV mm^{-1} was applied, with an increase in

alkaline phosphatase (ALP) activity per cell relative to non-stimulated control substrates.[12] Bones are inherently porous (the porosity of cortical canals is approximately 3.5%, whereas that of trabecular bones is approximately 80%),[13] and electrospun materials are popular bone tissue scaffolds in part because their inherent porosity facilitates the ingrowth of cells and potentially vascularization, [14] consequently, research groups have built on the report from Langer and coworkers[12] to develop conductive nanofiber-based bone tissue scaffolds.[15] Indeed, Haimi and coworkers reported such scaffolds enhanced the proliferation of human adipose tissue derived stem cells, yet while there were moderate increases in ALP activity on the scaffolds, electrical stimulation was not observed to have a significant effect on the adipose tissue derived stem cells,[15b] and Loboa and coworkers reported that electrical stimulation of human adipose tissue derived stem cells increased their proliferation and levels of calcium deposition,[15c] highlighting the potential of electroactive tissue scaffolds for bone tissue regeneration.

Tissue scaffolds allowing the behavior of the cells that reside within them to be controlled are of particular interest for tissue engineering. Herein we describe the preparation of conductive fiber-based bone tissue scaffolds (nonwoven mats of electrospun polycaprolactone with an interpenetrating network of polypyrrole and polystyrenesulfonate) that enable the electrical stimulation of human mesenchymal stem cells to enhance their differentiation towards osteogenic outcomes.

Here we describe the preparation of mats of electrospun PCL (an FDA-approved biodegradable polymer, PCL is frequently used as a base material for such scaffolds with encouraging results both *in vitro* and *in vivo*†[14,16] that were rendered conductive via growth of an interpenetrating network of polypyrrole,[9,17] and their use as instructive bone tissue scaffolds that enable electrical stimulation of human bone marrow derived mesenchymal stem cells, enhancing ALP activity and calcium deposition.

Experimental Section

Full experimental details are found in the supplementary information. In short, non-woven mats of PCL were electrospun from syringes containing a solution of PCL in chloroform and methanol followed by drying under high vacuum. Interpenetrating networks of polypyrrole and polystyrenesulfonate were grown within the PCL matrix in accordance with our previously described methodology.[17b] Tensile tests were performed using an Instron Materials Testing Machine 5543 Series Single Column System (Instron, Norwood, MA) with Bluehill 2 software. The electrical properties of the conductive PCL-based fibers

were characterized in accordance with our previously described methodology.[17b] Scanning Electron Microscopy (SEM) images were obtained using a Zeiss Supra 40 VP field emission scanning electron microscope. X-ray photoelectron spectroscopy (XPS) was performed on a Kratos Axis X-ray photoelectron spectrometer (Kratos Analytical Ltd., Manchester, UK).[17b] *In vitro* culture of human mesenchymal stem cells (Lonza, Walkersville, MD) on collagen-1 coated substrates was carried out for 21 days in osteogenic medium. Electrical stimulation of HMSCs was achieved employing a custom built setup (Figure SI). The DNA content and Alkaline Phosphatase (ALP) activity of samples were quantified concurrently, using the PicoGreen® assay (Life Technologies, Thermo Fisher Scientific Inc., USA) for DNA quantitation in accordance with the manufacturer's protocol, an ALP assay kit (Abeam®, Cambridge, MA, USA) for ALP activity in accordance with the manufacturer's protocol, and a Synergy HT Multi-Mode Microplate Reader (Bio-tek US, Winooski, VT). The calcium content of samples was quantified using a Calcium Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) in accordance with the manufacturer's protocol with a Synergy HT Multi-Mode Microplate Reader. Data were normalized to DNA quantity. Cells were stained with fluorescent dyes after by fixing the cells with paraformaldehyde, and staining the actin filaments and cell nuclei with Alexa Fluor 488® Phalloidin (Life Technologies, USA) and 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, USA), respectively. Fluorescence images of cells were obtained using an Olympus 1X70 inverted microscope equipped with an Olympus DP80 dual color and monochrome digital camera. Images were analyzed with Olympus cell Sens® imaging software.

Results and Discussion

Preparation and characterization of the tissue scaffolds:

Non-woven mats of PCL composed of nanofibers of 2.10 ± 0.49 μm diameter were electrospun from solutions of PCL (12 wt %) in a solution of chloroform and methanol (9:1 ratio v/v) onto non-stick aluminum foil after which they were dried under high vacuum. An interpenetrating network of polypyrrole and polystyrene sulfonate (Figures 1A-D) was generated within the PCL matrix by incubation of the PCL mats in an aqueous solution of pyrrole and polystyrenesulfonate in the presence of the initiators ammonium persulfate and ferric chloride.[17b, 18] When the mats were homogeneously colored, they were washed thoroughly with water and ethanol to remove the by-products (e.g. initiators, monomers, oligomers and polymers) and vacuum dried. SEM showed that the surface of the non-

conductive PCL fibers are somewhat rough (Figure 1A), and that the conductive fibers have a homogeneous coating of aggregates of polypyrrole and polystyrenesulfonate on their surface and are therefore notably rougher (Figure 1B).[9, 17b] X-ray photoelectron spectra of the non-conductive and conductive fibers confirms that the surface chemistry is different, with the appearance of peaks in the spectra of the conductive fibers at 400 eV (N Is, Figure 1C) and 168 eV (S 2p, Figure ID) resulting from the polypyrrole and polystyrenesulfonate,[17b] respectively.

The chemical modification process did not alter the mechanical properties of the mats significantly (Table 1). The electrical sheet resistance of the samples was measured in accordance with the method described by Schmidt[9, 17b] and Zhang,[19] and generating an interpenetrating network of polypyrrole and polystyrenesulfonate yielded mats with sheet resistances of 52.8 ± 4.7 kΩ square^{-1}, which is of a similar order of magnitude to analogously coated poly(lactic-co-glycolic acid) nanofibers (17 kΩ square^{-1})[9] or Dacron® 56 polyester fibers (16 kΩ square^{-1}).[19] While the electrochemical stability of the polypyrrole is known to decrease over long periods of time which may pose problems for biointerfaces implanted for long periods of time,[20] we and others have found it to be acceptable for the short term stimulation of cells residing in tissue scaffolds such as those reported here.[3f, 3g, 3h, 4, 9, 17b]

In vitro cell culture:

With a view to the application of the mats of fibers as bone tissue scaffolds, we rendered the surface of conductive PCL mats cell adhesive by incubation in a solution of collagen-1, which is the most abundant protein in bone. TCP and PCL controls were coated in a similar fashion to ensure the substrates had similar surface chemistry because cell-matrix interactions have been shown to modify cell behaviour.[21] Thereafter, we seeded bone marrow-derived HMSCs on the scaffolds and cultured them in osteogenic medium for 21 days. We studied four different systems: 1) cells seeded on commercially available tissue-culture treated Corning® Costar® tissue culture plates (TCP), 2) cells seeded on mats of electrospun PCL, 3) cells seeded on mats of conductive PCL without electrical stimulation, and 4) cells seeded on mats of conductive PCL with electrical stimulation (2 days without stimulation, 1 day with stimulation at 10 mV mm^{-1} for 8 hours, no stimulation thereafter) achieved employing a custom built setup (Figure 4).

After 21 days in culture, cells were fixed with paraformaldehyde and actin filaments and cell nuclei within cells were stained with Alexa Fluor® 488 Phalloidin and 4', 6-diamidino-2-phenylindole (DAPI), respectively. We observed that cells were homogeneously distributed on the TCP controls, and that cells had infiltrated the mats of electrospun fibres

(Figures 2A-D). Evidence for infiltration can be found in Figures 2B, 2C and 2D in which there are blue DAPI-stained nuclei with various sizes, the largest nuclei are in the focal plane of the microscope and the smaller nuclei are somewhat out of focus as they are located deeper in the mats of fibers; and moreover, in Figures 2C and 2D in which there are black conductive fibers both above and below the blue DAPI-stained nuclei. Such evidence is promising for the integration of such biomaterials in the body where infiltration of cells such as macrophages and osteoclasts facilitate remodelling of implanted biomaterials. Macrophages are known to produce enzymes such as cholesterol esterase [22] that degrade polyesters such as polycaprolactone, and we have previously shown that cholesterol esterase can efficiently degrade the polycaprolactone component of analogously prepared materials with interpenetrating networks of polypyrrole and polystyrenesulfonate. [17b] Such scaffolds are likely to degrade over the period of several years if administered *in vivo* [16a] leaving behind the residual water insoluble polyelectrolyte complex of polypyrrole and polystyrenesulfonate. Ramanavicius and coworkers have reported detailed investigations of the toxicity of nanoparticles composed of the polyelectrolyte complex formed by polypyrrole and polystyrenesulfonate. [23] While high concentrations were toxic to primary mouse embryonic fibroblasts, mouse hepatoma (MH-22A) cells, and human T lymphocyte Jurkat cells, affecting both cell viability and proliferation, this effect was dependent on the concentration of nanoparticles, and concentrations lower than 9.7 μg ml^{-1} did not affect cell viability or proliferation.' [23] Further, preclinical trials of such nanoparticles in mice carried out by the same group have shown that the nanoparticles did not display any detectable cytotoxicity towards mouse peritoneum cells, nor did they induce an allergic response, or affect spleen, kidney or liver indexes. [24] Moreover, there were no observable changes to immune-related haematological parameters, and no inflammation in the peritoneum after a 6-week period of treatment. [24] Likewise, histological studies of tissues in the proximity of polypyrrole-based materials in rats [25] showed immune cell infiltration comparable to FDA-approved poly(lactic acid-co-glycolic acid) [25a] or poly(D,L-lactide-co-glycolide), [25b] and no significant inflammation in the coronary artery after 5 weeks, [25c] sciatic nerve guidance channels implanted after 8 weeks, [25d] or electrodes in brains after 3 or 6 weeks. [25e]

Further, after 21 days we observed the differentiation of the FDVISCs towards osteogenic fates using biochemical assays for alkaline phosphatase (ALP) activity and Ca^{2+} deposition normalized to their DNA content. FDVISCs cultured on the non-conductive collagen- 1-coated tissue-culture treated Corning® Costar® TCP controls adhered and

proliferated most effectively and their DNA content was concomitantly highest (Figure 3A). To within experimental error, HMSCs adhered and proliferated equally well on the non-conductive collagen- 1-coated electrospun mats of PCL (Figure 3A). The lower DNA content of the conductive electrospun mats (either without or with electrical stimulation) is because cell adhesion on the surface of interpenetrating networks of PCL, polypyrrole and polystyrene sulfonate is weaker than on PCL alone; weak cell adhesion is commonly observed for such electroactive matrices and is the reason that their functionalization with cell adhesive moieties is common, either non-covalently during/after the synthesis/doping process, [26] or covalently. [27] Nevertheless, levels of ALP activity on the matrices confirm the viability of the cells after 3 weeks in culture (Figure 3B). Relative to FDVISCs cultured on the non-conductive tissue-culture treated Corning® Costar® TCP controls, those cultured on electrospun mats of PCL had slightly higher levels of ALP activity (Figure 3B) and notably higher levels of Ca^{2+} deposition (Figure 3C). Interestingly, both ALP activity and Ca^{2+} deposition were further increased on mats of electrospun PCL with an interpenetrating network of polypyrrole and polystyrenesulfonate, and we postulate this to be a result of differences in the surface chemistry and roughness altering non-specific interaction between proteins in the medium leading to their deposition onto the scaffolds. [28] Furthermore, we observed that electrical stimulation resulted in increased ALP activity and a 3-fold increase in Ca^{2+} deposition relative to the unstimulated controls, which is in line with reports by Langer and coworkers, [12] and Rouabhia and Zhang. [15a] Thus, quantitative biochemical analyses of the scaffolds reveal that, while the non-conductive scaffolds support differentiation of FDVISCs towards osteogenic outcomes, the application of an electrical stimulus to HMSCs residing in a conductive scaffold enhances their differentiation towards osteogenic outcomes, and the increased quantity of calcium in the scaffolds is an important step toward the formation of bone-like calcified extracellular matrix.

Osteoinduction in porous materials can be attributed to a variety of factors including: the incorporation and concentration of bone morphogenetic proteins (BMPs), a rough surface microstructure causing the asymmetrical division of mesenchymal cells that may produce osteoblasts, the surface charge of the substrate triggering cell differentiation, a bone-like apatite layer formed in vivo that mesenchymal cells recognize and respond to, and localized high levels of Ca^{2+} ions that trigger cell differentiation and bone formation. On smooth 2D non-conductive TCP controls, we observe that levels of ALP expression and calcium deposition are lower than on electrospun mats of non-conductive PCL (Figure 3B and 3C), and we attribute the increased levels of ALP expression and calcium deposition to the

differences in surface micro structure which alters cell-material interactions. We see further increases in both ALP expression and calcium deposition on the rougher conductive PCL-based scaffolds both without and with electrical stimulation (Figure 3B and 3C), and believe that this is a result of differences in the surface chemistry altering protein deposition from the medium onto the scaffolds, [21] which Bose and coworkers show to modify cell-material interactions in vitro, [29] yet it is noteworthy that Epinette and Manley conclude that microstructure and surface charge are not the sole factors at play in osteoinduction in the clinic. [30] Furthermore, our experimental data for non-conductive mats of PCL fibers, and conductive mats of PCL fibers without/with electrical stimulation enabled us to observe that electrical stimulation markedly enhanced both ALP expression and calcium deposition on these matrices (Figure 3B and 3C), supporting further investigations into the development of conducting biomaterials for bone tissue engineering.

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 instruct the behaviour of cells residing within them are particularly interesting for such applications. We report herein the preparation of conductive PCL fiber-based bone tissue scaffolds by a simple process, and while it would be desirable to improve cell adhesion to the scaffolds by chemical modification of the polymers, [26, 27] we have shown that the electrical stimulation of FDVISCs residing therein enhances their differentiation towards osteogenic outcomes, as confirmed by quantitative biochemical assays. Importantly, electrical stimulation increased quantities of alkaline phosphatase activity and calcium deposition, which represents an important step towards the formation of calcified bone-like extracellular matrix; such materials should be relatively non-immunogenic in vivo and represent interesting platforms for further development of conductive tissue scaffolds.

Supplmental Information:

Experimental Section

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.

Preparation of non-woven mats of PCL fibers:

Non-woven mats of PCL were electrospun from syringes containing a solution of PCL (12 wt %) in chloroform and methanol (9: 1 ratio v/v), tipped with a blunt tipped gauge 16 needle, with a flow rate of 1 ml hr^{-1}, an accelerating voltage of 18 kV and a working distance of 12 cm onto a non-stick aluminium foil. The resulting mats were dried under high vacuum for 96 hours.

Preparation of conductive PCL-based fibers with interpenetrating networks of polypyrrole and polystyrenesulfonate

Pyrrole was purified by passage over basic alumina. White PCL-based tissue scaffolds were placed in disposable 50 mL centrifuge tubes containing a solution of pyrrole (291 μï¿½, [84 mM], 1 eq.) and PSS (Mn 70 kDa, 0.799 g, [84 mM], 1 eq.) in distilled water (50 mL). Samples were sonicated for 5 minutes and cooled to 4 °C (for 1 hour). Thereafter, ferric chloride (1.848 g, [228 mM], 2.7 eq.) was added. The samples were shaken to assure dissolution of the ferric chloride and then incubated for a further 24 h at 4 °C. Black electroactive tissue scaffolds were removed from the reaction mixture, placed in fresh distilled water, sonicated for 5 min, and then exhaustively washed (to remove monomers, oligomers and initiators) with deionized water until the water used to wash the materials was clear, colorless and the pH was neutral (ca. 48 h). Electroactive tissue scaffolds (PCL with an interpenetrating network of PPy and PSS) were dried under high vacuum at 2 1 °C. Samples were cut to lengths appropriate for the various subsequent experiments using a razor blade.

Mechanical properties:

Tensile tests were performed using an Instron Materials Testing Machine 5543 Series Single Column System (Instron, Norwood, MA) with Bluehill 2 software. The dimensions of the scaffolds (length, width and thickness, average of 3 positions per scaffold) were recorded accurately with high precision digital calipers (ThermoFisher Scientific, Waltham, MA, USA) immediately prior to tensile extension testing in the dry state. The laboratory was 2 1 °C with a relative humidity of ca. 40%. The initial grip separation was set at 5 mm, and a 50 N load cell was used at a drawing rate of 30 mm per minute. The tensile properties reported are the average of at least 10 measurements.

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:

$$R_S = RW/L \quad (1)$$

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:

Samples were mounted on a Scanning Electron Microscopy (SEM) stub and sputter coated with Pt/Pd (15 nm) using a Cressington 208 Benchtop Sputter Coater. All samples were imaged using a Zeiss Supra 40 VP field emission scanning electron microscope. Fiber diameters were determined with the measure tool (plugin) in the open source program "ImageJ". The data presented are the average of 60 measurements and the errors quoted are standard deviations.

X-ray photoelectron spectroscopy (XPS):

XPS was carried out on the samples to confirm that the surface chemistry of the scaffolds had changed after the growth of an interpenetrating network of the EAP within the PCL matrix. XPS was performed on a Kratos Axis X-ray photoelectron spectrometer (Kratos Analytical Ltd., Manchester, UK). The binding energy was calibrated using the C 1s photoelectron peak at 284.6 eV as a reference. The CasaXPS computer program was used for peak fitting of the N 1s and S 2p peaks in the XPS spectra. The reported spectra are representative of two measurements at different positions on a sample.

In vitro culture of human mesenchymal stem cells without electrical stimulation: HMSCs were supplied by Lonza (Walkersville, MD). Electrospun scaffolds were punched into small discs with a diameter of 11 mm to fit in 48-well cell culture plates. Samples were rendered cell adhesive by incubation in a solution of collagen-1 in 0.02N acetic acid (50 μg mL^{-1}) and washed extensively with sterile ultrapure water to remove any non-adherent collagen and residual acetic acid. 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 5,000 cells cm^{-2}, and incubated at 37 °C, 95 % humidity, and a $C0_2$ content of 5 %. After 3 days the medium was aspirated and the scaffolds were 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 (n = 6).

In vitro culture of human mesenchymal stem cells with electrical stimulation: Electrical stimulation of HMSCs 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 approximately 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.

Samples were rendered cell adhesive with collagen-1 and sterilized as described above, and HMSCs were seeded and cultured as described above. After 48 hours, 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). A potential step of 10 mV mm^{-1} was placed across the samples for the duration of 8 h 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 (n = 6).

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, an ALP assay kit (Abeam®, Cambridge, MA, USA) for ALP activity in accordance with the manufacturer's protocol, and a Synergy HT Multi-Mode Microplate Reader (Bio-tek US, Winooski, VT). The calcium content of samples was quantified using a Calcium Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) in accordance with the manufacturer's protocol with a Synergy HT Multi-Mode Microplate Reader (Bio-tek US, Winooski, VT). Data were normalized to DNA quantity.

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. Images were analyzed with Olympus cellSens® imaging software, Version 1.1 1, and images presented are representative of at least 3 samples.

Supplementary Data

Table 1. Physicochemical properties of the materials.

Sample	Tensile modulus [MPa]	Yield Strength [MPa]	Ultimate Tensile Strength [MPa]	Resistance [kΩ square^{-1}]
PCL	2.12 ± 0.63	1.34 ± 0.23	2.07 ± 0.57	N/A
Conductive PCL	1.99 ± 0.07	1.05 ± 0.18	1.47 ± 0.38	52.8 ± 4.7

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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 method of differentiation of human mesenchymal stem cells, comprising:

providing a nonwoven mat of fibers, wherein interpenetrating networks of a conductive polymer and a dopant are on the surface of the fibers;

introducing human mesenchymal stem cells to the nonwoven mat, wherein the nonwoven mat 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 human mesenchymal stem cells towards osteogenic outcomes.

2. The method of claim 1, wherein periodically providing electrical stimulation causes increased ALP activity and increased Ca^{2+} deposition on the fibers relative to not periodically providing electrical stimulation.

3. The method of claim 1, wherein the diameter of the fibers is about 10 nanometers to 500 micrometers.

4. The method of claim 1, further providing: forming osteoblasts in the nonwoven mat.

5. The method of claim 1, wherein the fibers are polycaprolactone fibers, the conducting polymer is polypyrrole, and the dopant is polystyrenesulfonate.

6. The method of claim 5, wherein periodically providing electrical stimulation causes increased ALP activity and increased Ca^{2+} deposition on the fibers relative to not periodically providing electrical stimulation.

7. The method of claim 5, wherein the diameter of the fibers is about 10 nanometers to 500 micrometers.

8. The method of claim 5, further providing: forming osteoblasts in the nonwoven mat.

9. The method of claim 5, wherein the fibers are selected from the consisting of one or more of the following: polycaprolactone, polyester, polyamide, PCL, PLLA, PLGA, protein, polysaccharide, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, and chitosan.

10. The method of claim 5, wherein the conductive polymer is selected from the group consisting of one or more of the following: polypyrrole, polyaniline, polythiophene, poly(3,4-ethylenedioxythiophene), polyfluorene, polyphenylene, polypyrene, polyazulene, polynapthalene, polyindole, polyazepine, poly(p-phenylene sulfide), poly(p-phenylene vinylene), and polyfuran.

11. A structure, comprising:

a nonwoven mat of fibers, wherein interpenetrating networks of a conductive polymer and a dopant are on the surface of the fibers, wherein human mesenchymal stem cells are disposed within the nonwoven mat.

12. The structure of claim 11, wherein osteoblasts are present within the nonwoven mat after exposure of the nonwoven mat to an osteogenic medium.

13. The structure of claim 11, wherein the nonwoven mat includes ALP and Ca^{2+} deposition on the fibers.

14. The structure of claim 11, wherein the diameter of the fibers is about 10 nanometers to 500 micrometers.

15. The structure of claim 11, wherein the fibers are polycaprolactone fibers, the conducting polymer is polypyrrole, and the dopant is polystyrenesulfonate.

16. The structure of claim 15, wherein osteoblasts are present within the nonwoven mat.

17. The structure of claim 15, wherein the nonwoven mat includes ALP and Ca^{2+} deposition on the fibers.

18. The stmcture of claim 11, wherein the fibers are selected from the consisting of one or more of the following: polycaprolactone, polyester, polyamide, PCL, PLLA, PLGA, protein, polysaccharide, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, and chitosan.

19. The structure of claim 11, wherein the conductive polymer is selected from the group consisting of one or more of the following: polypyrrole, polyaniline, polythiophene, poly(3,4-ethylenedioxythiophene), polyfluorene, polyphenylene, polypyrene, polyazulene, polynapthalene, polyindole, polyazepine, poly(p-phenylene sulfide), poly(p-phenylene vinylene), and polyfuran.

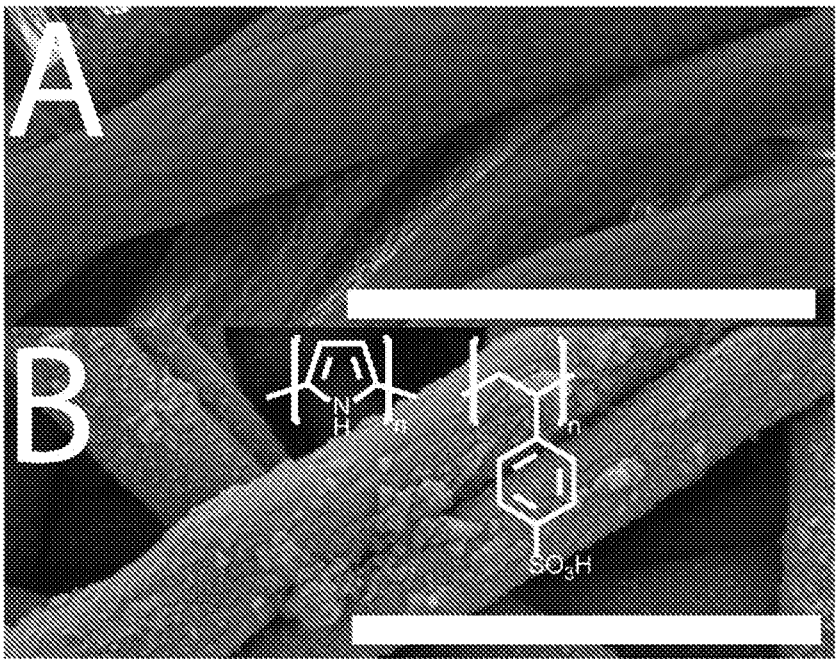

Fig. 1A

Fig. 1B

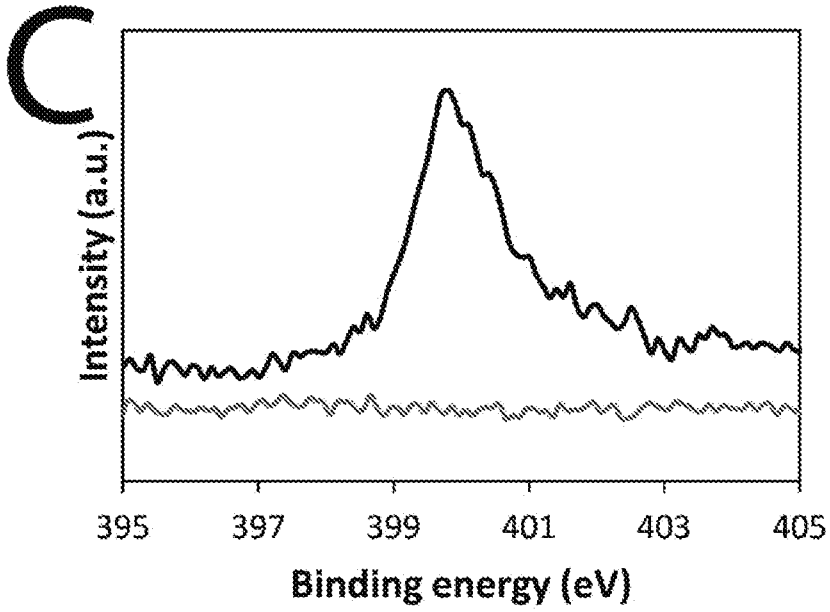

Fig. 1C

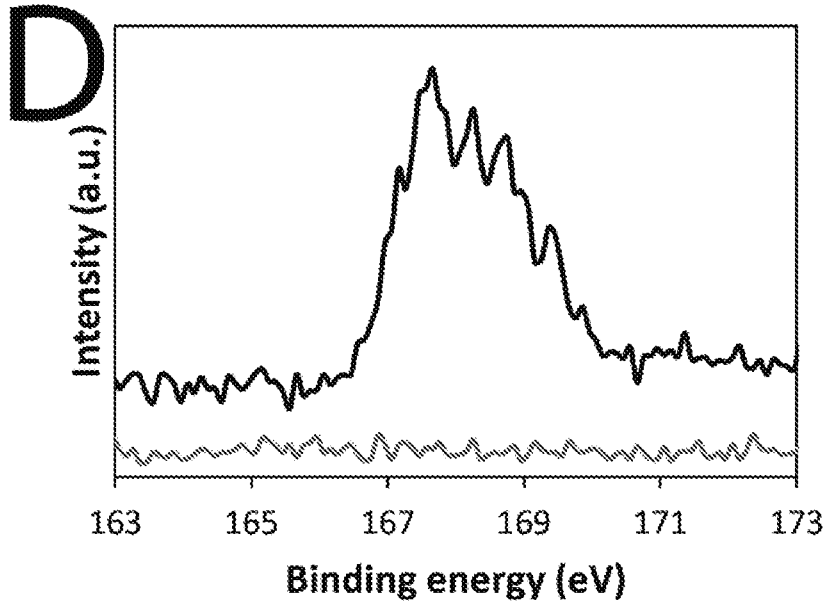

Fig. 1D

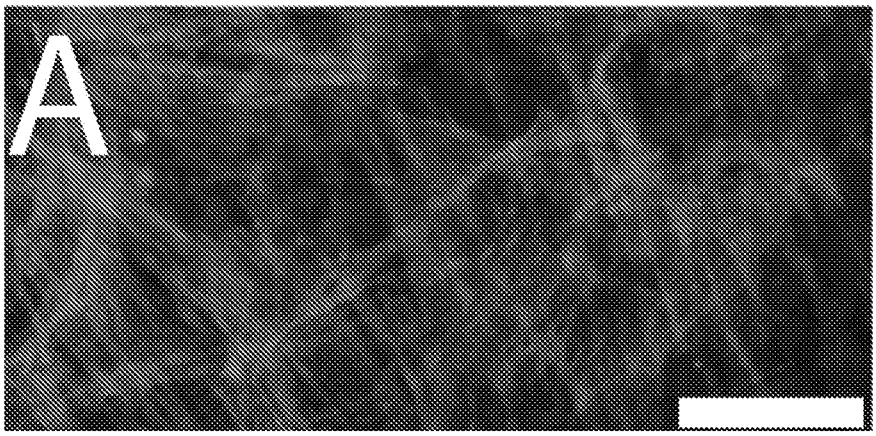

Fig. 2A

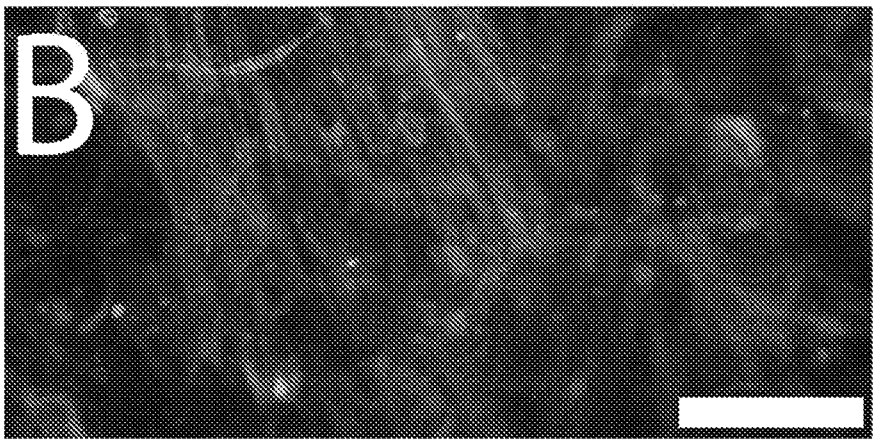

Fig. 2B

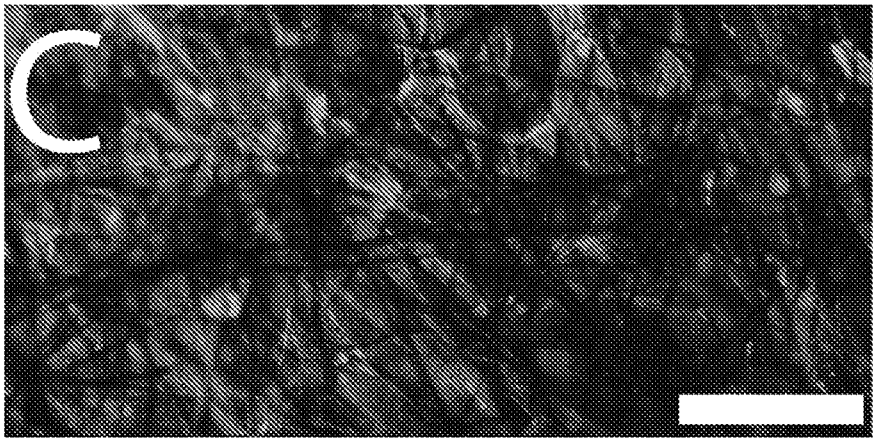

Fig. 2C

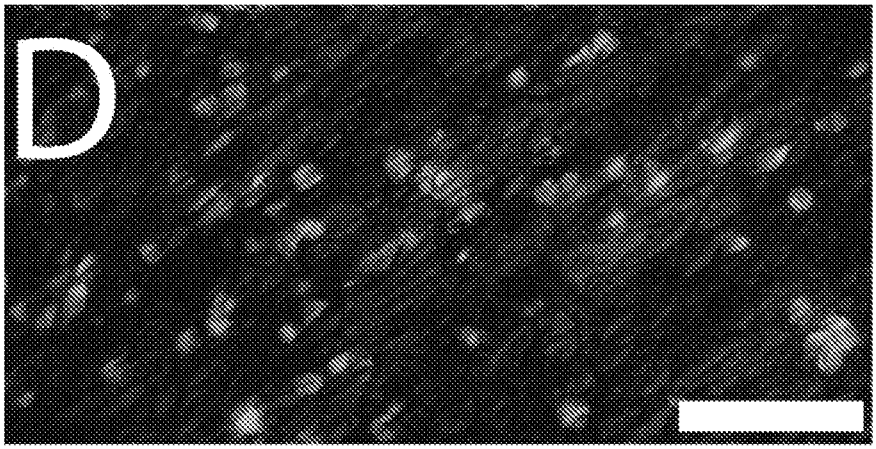

Fig. 2D

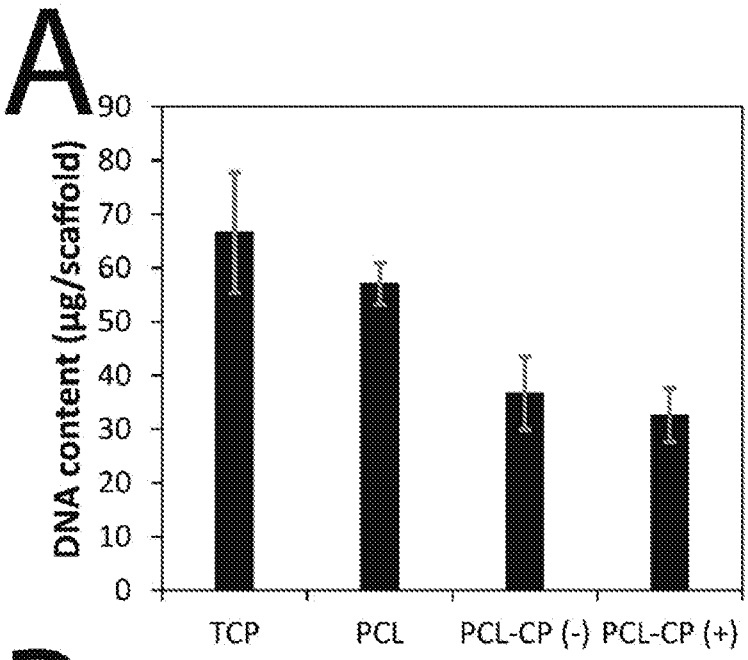

Fig. 3A

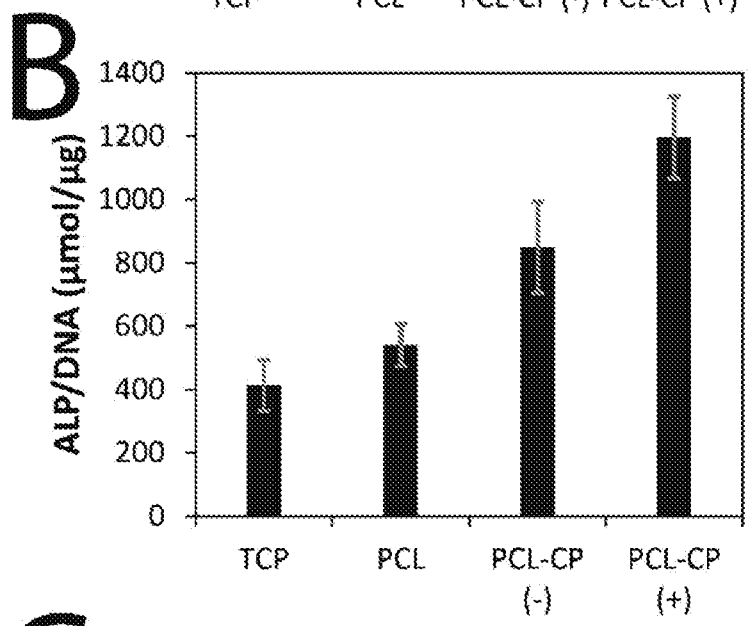

Fig. 3B

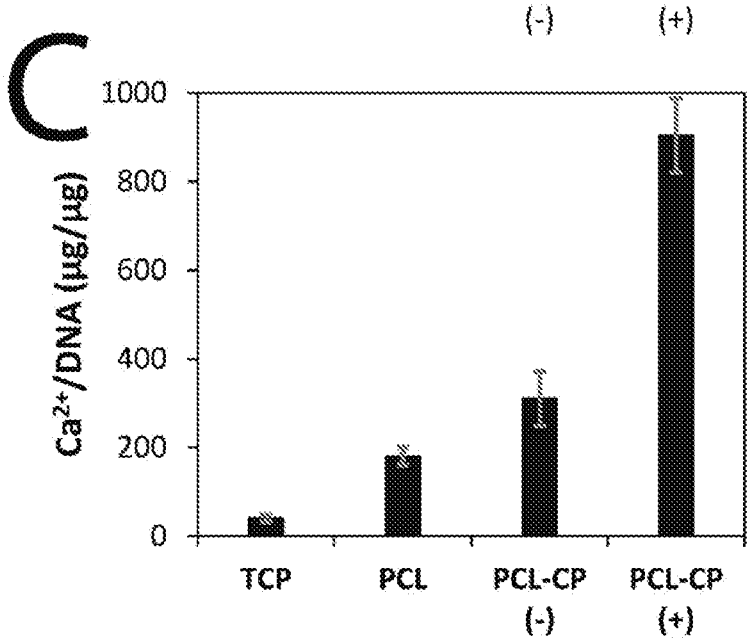

Fig. 3C

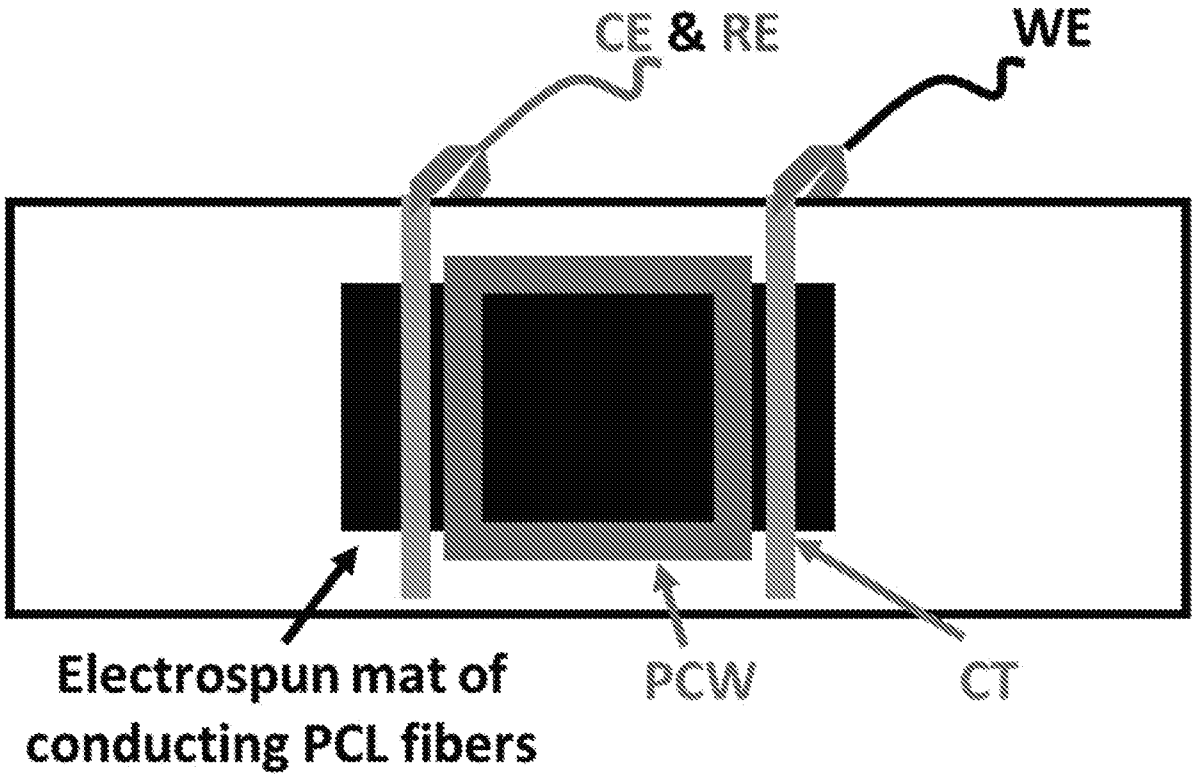

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/039230

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12M 1/00; C12M 3/00; C12N 5/0775 (2016.01)
CPC - C12M 1/00; C12M 3/00; C12N 5/0654; C12N 5/0663 (2016.08)
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) - C12M 1/00; C12M 3/00; C12N 5/0775 (2016.01)
CPC - C12M 1/00; C12M 3/00; C12N 5/0654; C12N 5/0663 (2016.08)

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

USPC - 435/366; 435/377; 435/283.1 ; IPC(8) - C12M 1/00; C12M 3/00; C12N 5/0775; CPC - C12M 1/00; C12M 3/00; C12N 5/0654; C12N 5/0663 (keyword delimited)

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

Orbit, Google Scholar
Search terms used: conductive, polymer, mat, nonwoven, cells, stem, mesenchymal, differentiate, differentiation, ostegenic, osteoblasts, fiber, fibrous, dopant, ALP, interpenetrating network

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/0306137 A1 (AIZMAN) 15 December 2011 (15.12.2011) entire document	1-19
A	US 2003/0211602 A1 (ATALA) 13 November 2003 (13.11.2003) entire document	1-19
A	US 2015/0093363 A1 (BIOMATCELL AB) 02 April 2015 (02.04.2015) entire document	1-19
A	US 7,829,335 B2 (INOUE et al) 09 November 2010 (09.11.2010) entire document	1-19
A	US 2010/0240121 A1 (SENECAL et al) 23 September 2010 (23.09.2010) entire document	1-19

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
15 August 2016	**08 SEP 2016**
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774