The invention provides a novel approach to controlled delivery of biomolecules (e.g., lipids and proteins) by employing novel amphiphilic polymers that are effective delivery vehicles. These unique amphiphilic polymers may be employed as controlled delivery vehicles or tissue engineering scaffolds wherein the delivery of lipophilic or amphiphilic bioactive molecules can be achieved. An amphiphilic biodegradable polymer platform is disclosed herein for the stable encapsulation and sustained release of biomolecules, such as SIP.
AMPHIPHILIC DEGRADABLE POLYMERS FOR IMMOBILIZATION AND SUSTAINED DELIVERY OF BIOMOLECULES

Government Rights

[0001] The United States Government has certain rights to the invention pursuant to Grant No. R01AR055615 awarded by the National Institutes of Health to the University of Massachusetts.

Priority Claims and Cross Reference to Related Applications

[0002] This application claims the benefit of priority from U.S. Provisional Application Serial No. 61/945,117, filed February 26, 2014, the entire content of which is incorporated herein by reference in its entirety.

Technical Field of the Invention

[0003] The invention generally relates to controlled delivery of biomolecules. More particularly, the invention relates to amphiphilic degradable polymers, their preparation, and related methods for immobilization and controlled delivery of biomolecules (e.g., lipids, proteins) thereof.

Background of the Invention

[0004] The delivery of biomolecules, including lipids and therapeutic proteins, provides a promising vehicle for the treatment of many diseases and conditions, such as angiogenesis. Angiogenesis is essential for tissue development, function, maintenance, repair and regeneration. Impaired angiogenesis due to either injuries or diseases can severely impair these processes. (Carano et al. 2003 Drug Discov Today 8:980-9; Laschke et al. 2006 Tissue Eng 12:2093-104; Harris et al. 2013 Curr Pharm Des 19:3456-65; Novosel et al. 2011 Adv Drug Deliv Rev 63:300-11; Nguyen et al. 2012 Tissue Eng Part B Rev 18:363-82.) For instance, disruption of vascular network as a result of orthopedic trauma compromises the ability to vascularize bone grafts, resulting in high clinical failure rates of bone graft-mediated repair of traumatic bone defects. (Ito et al. 2005 Nat Med 11:291-7.) In pathological conditions such as diabetes, the microangiopathic complication/tissue ischemia also retards bone injury repair and graft healing as it disrupts the tightly coupled osteogenesis and angiogenesis processes. (Abaci et al. 1999 Circulation 99:2239-42; Waltenberger et al. 2001 Cardiovasc Res 49:554-60; Kanczler et al. 2008 Eur Cell Mater 15:100-14.)
Therapeutic strategies for promoting angiogenesis, particularly the formation of functional and stable vascular network, have long been sought after in scaffold-assisted tissue repair and regeneration. Angiogenesis involves a dynamic cascade of cellular and molecular events involving early-stage of lumen formation (e.g., increased blood vessel permeability, basement membrane degradation, endothelial cell (EC) migration, proliferation and further assembly into tubular structure) and later-stage of nascent EC tube stabilization and maturation (e.g., mural cells recruitment and new basement membrane deposition). (Carmeliet et al. 2011 Nature 473:298-307; Potente et al. 2011 Cell 146:873-87.) The entire angiogenesis process is tightly regulated by a dynamic balance of pro-angiogenic factors and vessel-stabilizing factors. (Jain 2003 Nat Med 9:685-93.)

Current strategies for recapitulating this process in-situ involve the delivery of angiogenic stimuli, of which angiogenic growth factor such as vascular endothelial growth factor (VEGF) is the most intensively studied. (Nguyen et al. 2012 Tissue Eng Part B Rev 18:363-82; Baiguera et al. 2013 Angiogenesis 16:1-14; Cenni et al. 2011 Acta Pharmacol Sin 32:21-30; Said et al. 2013 J VaseRes 50:35-51; Mehta et al. 2012 Adv Drug Deliv Rev 64:1257-76; Tayalia et al. 2009 Adv Mater 21:3269-85.) VEGF is a potent angiogenesis initiator that is also known to disrupt pericyte coverage and inhibit subsequent vessel stabilization, thus the delivery of exogenous VEGF alone often results in sub-optimal neovascularization characterized with immature "leaky" vessels. (Greenberg et al. 2008 Nature 456:809-13.)

Therefore, the delivery of alternative/complementary signaling molecules promoting the formation of more extensive, stable and functional vascular network are highly desired. Phospholipid sphingosine 1-phosphate (SIP) has emerged as such a promising candidate because of its dual role as angiogenic stimulant and blood vessel stabilizer.


There are few existing biomaterials that can adequately meet the requirements of the tunable and sustained delivery of such amphiphilic molecules. Poly(lactic-co-glycolic acid (PLGA) is commonly used for SIP delivery by physical blending or microsphere fabrication (Qi et al. 2010 Eur J Pharmacol 634:121-31; Sefcik et al. 2008 Biomaterials 29:2869-77; Petrie et al. 2010 Tissue Eng Part A 16, 1801-9). The release of SIP in these materials is mainly dominated by passive SIP diffusion and polymer scaffold hydrolytic degradation, which are poorly controlled by nature. The other material attempted for SIP delivery is polyethylene glycol (PEG)-based hydrogels cross-linked by albumin (Wacker et al. 2006 Biomacromolecules 7, 1335-43). The disadvantages of the system include multi-step chemical synthesis, complicated hydrogel formulation and the requirement of preloading of drug cargo in order to achieve reasonable eluting profiles. The hydrogel itself per se does not possess intrinsic structural tunability to enable manipulation of the SIP release kinetics.

Recently a cellulose hollow fiber-based system enabling timed delivery of SIP following earlier release of VEGF was shown to result in greater recruitment of ECs and higher maturation index of formed vessels in a Matrigel plug model. (Tengood et al. 2010 Biomaterials 31:7805-12.) However, this delivery system required external manual regulation, which complicates its implementation for in vivo tissue regeneration. Overall, synthetic scaffolds demonstrating significantly improved SIP loading efficiency and more tunable SIP release kinetics is still lacking.

Thus, a significant challenge for translating the SIP-based proangiogenic strategy to successful tissue repair is the lack of a tunable sustained release system enabling the optimization of its release kinetics for maximal stimulation of vessel formation and maturation. It is strongly desired that novel approaches and techniques be developed that enable controlled immobilization and delivery of biomolecules such as lipids and proteins.

Summary of the Invention

The invention provides a novel approach to controlled delivery of biomolecules (e.g., lipids and proteins) by employing novel amphiphilic degradable polymers as delivery vehicles. These
unique polymers may be utilized as tissue engineering scaffolds wherein the delivery of lipophilic or amphiphilic bioactive molecules can be effectively achieved.

[0014] An amphiphilic biodegradable polymer platform is disclosed herein for the stable encapsulation and sustained release of biomolecules, such as SIP. Mimicking the interaction between amphiphilic SIP and its binding proteins, a series of polymers with hydrophilic poly(ethylene glycol) core and lipophilic flanking segments of polylactide and/or poly(alkylated lactide) with different alkyl chain lengths were synthesized. These polymers were electrospun into fibrous meshes, and loaded with SIP in generally high loading efficiencies (>90%). Sustained SIP release from these scaffolds can be tuned by adjusting the alkyl chain length, blockiness and lipophilic block length, achieving 35-55% and 45-80% accumulative releases in the first 8 h and by 7 days, respectively. Furthermore, using endothelial cell tube formation assay and chicken chorioallantoic membrane (CAM) assay, it was shown that the different SIP loading doses and release kinetics translated into distinct pro-angiogenic outcomes.

[0015] In one aspect, the invention generally relates to an amphiphilic degradable block copolymer, which includes hydrophilic blocks; flanking lipophilic blocks; and lipophilic blocks having pendent alkyl chains of lengths from about C6 to about C24:

[0016] In another aspect, the invention generally relates to an amphiphilic degradable random copolymer, which includes hydrophilic monomer units, having the structure of

\[
\begin{align*}
\text{O-C}_2\text{H}_4 \quad &; \\
\text{O-} & \\
\text{O-C}_2\text{H}_4 \quad &; \\
\end{align*}
\]

lipophilic monomer units, having the structure of

\[
\begin{align*}
\text{O} & \\
\text{H} & \\
\text{O} & \\
\end{align*}
\]

lipophilic monomer units, having the structure of

\[
\begin{align*}
\text{O} & \\
\text{H} & \\
\text{O} & \\
\end{align*}
\]

wherein each of \( R_1 \) and \( R_2 \) is hydrogen or a \( \text{C}_i\text{-C}_j \) alkyl group;

\( R \) is a linear or substantially linear alkyl chain of a length from about \( C_e \) to about \( C_{24} \).
In certain preferred embodiments, the amphiphilic degradable random copolymer, each of \( R_1 \) and \( R_2 \) is a methyl group; and \( R \) is a linear alkyl chain of a length from about \( C_6 \) to about \( C_{18} \).

[0017] In another aspect, the invention generally relates to a fibrous scaffold made from an amphiphilic degradable copolymer of the invention.

[0018] In yet another aspect, the invention generally relates to a method for sustained release of a biomolecule to an \textit{in vivo} target location. The method includes: providing a fibrous scaffold prepared from an amphiphilic degradable copolymer of the invention; loading the fibrous scaffold with the biomolecule to be delivered \textit{in vivo}; placing the loaded fibrous scaffold at the target location; and causing sustain release of the biomolecule at the target location.

**Brief Description of the Drawings**

[0019] **FIG. 1.** (A) Schematic illustration of possible interactions between SIP and the amphiphilic polymers. (B) Synthetic schemes for the alkylated lactides and amphiphilic polymers. Reagents and conditions: (a) 2-bromopropionyl bromide (1.05 eq.), Et3N (2.0 eq.), acetone, rt for 0.5 h, then filtered; Et3N (2.0 eq.), 65 °C for 2 h. (b) PEG20K, Sn(Oct)2, 150 °C, 30 min. (c) D,L-lactide, 150 °C, 60 min. (d) PEG20K, D,L-lactide, Sn(Oct)2, 150 °C, 60 min.

[0020] **FIG. 2.** (A) GPC chromatograms of triblock copolymer intermediate P(C14LA)15-b-PEG454-b-P(C14LA)15 (Mn = 34,653, PDI = 1.13) and crude pentablock copolymer PLA312-b-P(C14LA)15-b-PEG454-b-P(C14LA)15-b-PLA312 (C14-L, Mn = 113,463, PDI = 1.47). (B) 1H NMR spectra of the pentablock and random copolymers.

[0021] **FIG. 3.** DSC spectra of PELA, pentablock and random copolymers.

[0022] **FIG. 4.** (A) SEM micrographs, (B) calculated mesh porosity (n = 3) and (C) water contact angles (n = 7) of electrospun fibrous meshes. Scale bar = 50 \( \mu \)m.

[0023] **FIG. 5.** (A) SIP loading efficiencies (n = 3) on polymeric fibrous meshes and (B) their cumulative releases over time (n = 3) in PBS with 0.2% FAF-BSA.

[0024] **FIG. 6.** Representative micrographs and total tube length quantifications (n = 3-4) of HUVEC-Matrigel cultures after 17 h exposure to free SIP solutions of varying concentrations (A & C) or polymer meshes preloaded with varying doses of SIP (B & D). Scale bar = 100 \( \mu \)m.

[0025] **FIG. 7.** Ex-ovo angiogenic effects of amphiphilic polymer meshes preloaded with 0.5^g SIP examined by CAM assay. (A) Representative photographs of the CAM surrounding the meshes with/without SIP (16x mag.) at day 0 and day 3, and the photographs of the flipped side of the...
harvested CAM on day 3 (25 x mag.) of the boxed area. (B) Quantification of microvessel numbers surrounding each scaffold (n = 4).

[0026] FIG. 8. DSC spectra (second heating cycle) of PEG20K vs. the C14- and C6-alkylated triblock copolymers.

[0027] FIG. 9. DSC spectra (first and second heating cycles) of PELA, pentablock and random polymers.

[0028] FIG. 10. Water contact angles (n = 10) of dense solvent-cast polymer films. **P<0.01 (Student's t-test).

[0029] FIG. 11. Water contact angles (n = 7) of as-spun meshes vs. lyophilized meshes following 24-h hydration.


[0032] FIG. 14. (A) Representative tube formation of HUVECs cultured with or without the supplements of SIP alone, and SIP in combination of a lower or higher dose of VEGF. (B) The total tube length in each well (n = 3) as quantified by ImageJ (NIH). Data are plotted as mean ± standard derivation. *P < 0.05, **P<0.01 (student t-test).

**Detailed Description of the Invention**

[0033] The invention provides a novel approach for controlled delivery of biomolecules. Amphiphilic degradable polymers disclosed herein can be employed to effectively immobilize and deliver biomolecules such as lipids and proteins. For example, emulating the amphiphilic interactions of angiogenic lipid SIP with its natural binding proteins, the electrospun amphiphilic degradable copolymer platform of the invention allows for highly efficient SIP loading and sustained release. It is demonstrated that SIP release profiles can be fine tuned by incorporating alkylated polylactides with side chains of select length to amphiphilic triblock copolymer PLA-PEG-PLA (PELA). Various spatial distributions (block vs. random) and clustering densities (high, medium and low) can be used in fine turning the desired release profile.

[0034] Structurally, SIP is an amphiphilic lysophospholipid comprised of a zwitterionic head group and a hydrophobic 18-carbon (CI 8) aliphatic tail. In circulating blood, SIP is released from platelets in micromolar concentrations and most of the released SIP is stored by binding with albumin and lipoproteins such as high-density lipoprotein (HDL). (Yatomi et al. 2000 Blood 96:3431-8; Rivera et al. 2008 Nat Rev Immunol 8:753-63; Aoki et al. 2005 J Biochem 138:47-55; Murata et al. 2000 Biochem J 352:809-15; Sachinidis et al. 1999 Arterioscler Thromb Vase Biol
Recent structural studies revealed that the interaction of SIP with HDL is mediated by HDL-associated apolipoprotein M (apoM). Specifically, apoM was shown to have an amphiphilic binding pocket with a polar entrance to grab the hydrophilic SIP headgroup and an inner lipophilic pocket to accommodate the C18 aliphatic tail. (Arkensteijn et al. 2013 IntJMol Sci 14:4419-31; Christoffersen et al. 2011 Proc Natl Acad Sci USA 108:9613-8.) This amphiphilic interaction pattern is also observed with the bindings of SIP antagonist with S1P1 receptor and SIP with SIP antibody. (Hanson et al. 2012 Science 335:851-5; Wojciak et al. 2009 Proc Natl Acad Sci USA 106:17717-22.)

The amphiphilic polymers of the invention represent a unique biomimetic strategy to realize biomolecule immobilization and tunable sustained release through reversible amphiphilic interactions. As disclosed herein, the amphiphilic polymer scaffold incorporating both hydrophobic and hydrophilic segments effectively binds SIP, mimicking the natural amphiphilic interaction pattern, which translates into improved SIP loading efficiency. Furthermore, the release kinetics of the encapsulated SIP can be tuned by adjusting the lipophilicity of the polymer, for example, with a PLA-PEG-PLA (PELA)-based amphiphilic block copolymer platform incorporating alkylated lactides. (Kutikov et al. 2013 Acta Biomater 9:8354-64.) By varying alkyl side chain lengths, blockiness and block lengths, one can utilize the impact of these factors on the encapsulation, release and angiogenic outcome of SIP delivery.

The polymers of the invention can be synthesized with conventional ring opening polymerization (ROP) and electrospun into fibrous meshes. Examples of synthesized polymers are three C14-alkylated pentablock copolymers (C14-H, C14-M, C14-L) containing high, medium and low C14-block lengths relative to the hydrophilic PEG core, one C6-alkylated pentablock copolymer (C6-M) containing medium C6 block length, one C14-alkylated random copolymer (rC14-L), and the triblock copolymer PELA without alkyl side chains. These polymers were subjected to detailed comparative studies.

Thermal analysis of the amphiphilic polymers by DSC revealed an endothermic peak at 10-12 °C ascribable to alkyl-alkyl aggregations in C14-block polymers, but not in the C6-block or C14-random copolymers (FIG. 3). These observations suggest that adequate interactions between clustered (blocky) alkyl side chains of critical length is required for creating the hydrophobic "pocket" desired for trapping the lipid tail of SIP. SEM micrographs of the electrospun meshes (FIG. 4A) and the porosities calculated from the weight ratios of the porous meshes to the dense films (FIG. 4B) revealed varying fiber morphologies and porosities.
While the C6-M, C14-M, C14-L and the unalkylated PELA meshes were characterized with medium (40-60%) porosity and well-defined fibers free of beading, the electrospun C14-H fibers exhibited some degrees of fusing, resulting in lower porosity (<20%). The random polymer rC14-L was unsuited for electrospinning, resulting in low-porosity (<10%) meshes lacing well-defined fiber morphology. The differential porosities, in combination with the intrinsic hydrophilicity of the amphiphilic copolymers as reflected by the water contact angles of the dense solvent-cast films (FIG. 10), translated into significant differences in the wettability of the electrospun meshes (FIG. 4C).

The SIP loading efficiency and release kinetics was governed by both the thermal and physical properties of the alkylated amphiphilic polymer meshes. At room or body temperature, the intramolecular alkyl-alkyl aggregation is expected to undergo a dynamic equilibrium of association and disassociation, allowing SIP to be reversibly sequestered/released from the aggregated hydrophobic "pocket", a characteristic desired for controlled and sustained release of SIP. Indeed, dynamic hydrophobic interactions appeared to have played a prominent role than the mesh porosity in ensuring high SIP loading efficiency with block copolymers with longer alkyl side chains (FIG. 5A, >90% loading efficiency for C14-L, C14-M and C14-H vs. -55-65% for C6-M and rC14-L). The relatively low mesh porosity and wettability of C14-H electrospun mesh did not compromise its ability to support high SIP loading efficiency.

The differential hydrophobic interactions between these amphiphilic alkylated polymers and SIP (FIG. 1A) also translated into distinct SIP release profiles (FIG. 5B). The less effective sequestering of SIP by the C6-M and rC14-L resulted in more rapid early release (-55% in the first 8 h) followed by substantial continued release (70-80% cumulative release in 7 days). C14-H displayed the most sustained and steady release of SIP, amounting to 35% in the first 8 h and 65% accumulative release by day 7. C14-M and C14-L exhibited very similar, and the slowest SIP release, totaling 30-35% in the first 8 h but no more than 10% additional SIP release in the next 7 days. The more substantial SIP release from mesh C14-H than those from C14-M or C14-L could in part be a result of its relatively low porosity (FIG. 4B), which may have resulted in SIP encapsulation more towards the surface (thus easier release). It is worth noting that due to the varying SIP loading methods, releasing conditions, detection methods adopted by literature reports, direct quantitative comparison of the SIP loading efficiency and release kinetics with literature carriers is difficult, although the high loading efficiency accomplished with the C14-alkylated system was excellent. (Qi

[0041] Interestingly, PELA displayed SIP loading and release kinetics similar to those of alkylated polymers C14-L and C14-M, despite its lack of alkylated side chains. One possible explanation may be that the amphiphilic PELA binds SIP through different mode of molecular interactions. Previous studies showed that PELA could undergo a conformation rearrangement upon contact with water, exposing the hydrophilic PEG blocks to the polymer/water interface. (Kutikov et al. 2013 Acta Biomater 9:8354-64.) Such a hydration-induced structural rearrangement may strengthen the hydrophobic interaction between its PLA blocks and the lipid tail of the SIP. For the alkylated amphiphilic copolymers, the mobility of the PLA segments may have been hindered due the steric constraints imposed by the aliphatic side chains, thereby minimizing SIP sequestration through this mechanism. This was supported by the differential changes in water contact angles of the amphiphilic meshes upon 24-h hydration (FIG. 11). Unlike PELA, which exposed its hydrophilic PEG segments upon prior hydration to result in significant reduction in water contact angles, the alkylated amphiphilic polymers exhibited similar or slightly higher water contact angles, supporting that their PEG segments did not effectively expose to surface upon hydration. (Kutikov et al. 2013 Acta Biomater 9:8354-64.)

[0042] The benefit of sustained delivery of SIP via a suitable scaffold was demonstrated in HUVEC-Matrigel tube formation assay and CAM assay. Unlike high doses (e.g., 50 µM) of free SIP solution that could pose inhibitory effect on tube formation, the same dose of SIP, when encapsulated/released by amphiphilic scaffolds, promoted EC tube formations. (Kohno et al. 2008 Genes Cells 13:747-57.) Such a benefit was more pronouncedly manifested by the C14-H and C14-M scaffolds than the C6-M mesh, which exhibited most rapid early release of SIP. Using a 3-day ex ovo CAM assay, it was further demonstrated that C14-H mesh, with more sustained SIP release kinetics, led to significantly more neovessel formation and capillary bending/infiltration than the C6-M mesh. Collectively, these observations support that controlled SIP release can be functionally translated into pro-angiogenic activities both in vitro and ex ovo.

[0043] The tunable SIP loading efficiency, release profile, and in vitro and ex ovo pro-angiogenic activities enabled by the amphiphilic copolymer platform presented in this study provides a unique opportunity for optimizing angiogenesis for tissue repair/regeneration. A recent study demonstrated superior aqueous stability, tensile elasticity, osteoconductive and osteoinductive properties of the bone mineral composites of amphiphilic copolymer PELA compared to those based on the
hydrophobic PLA. (Kutikov et al. 2013 Acta Biomater 9:8354-64.) These benefits, likely retained with the amphiphilic copolymer platform presented here, may be combined with the controlled SIP delivery to synergistically promote osteogenesis and angiogenesis, thereby improving the outcome of scaffold-assisted bone repair. However, the in vivo efficacy of such a strategy will need to be rigorously examined using suitable animal models.

[0044] The alkylated amphiphilic polymers disclosed in this invention can be used alone, or as a carrier for bioactive lipids such as SIP, proteins and other amphiphilic biomolecules. They can also be used in combination with other polymers, osteoconductive minerals, osteoinductive growth factors, or cells. Depending on specific applications, the polymers or their combination can be formulated into:

1. scaffolds such as bone graft, vascular graft and peripheral nerve graft;
2. biodegradable coatings for drug-eluting stents;
3. microspheres and hydrogels for localized drug delivery;
4. films as wound dressing for skin regeneration, diabetic foot ulcer or guided bone regeneration;
5. sutures, pins, plates, screws and other surgery tools.

[0045] Thus, in one aspect, the invention generally relates to an amphiphilic degradable block copolymer, which includes hydrophilic blocks; flanking lipophilic blocks; and lipophilic blocks having pendant alkyl chains of lengths from about C6 to about C24;

[0046] In certain preferred embodiments of the amphiphilic degradable block copolymer, the hydrophilic blocks comprise blocks of poly(ethylene glycol) having the structure of

\[ \left( \overset{\circ}\text{O} \overset{\circ}\text{C}_2\text{H}_4 \right)_i \]

The flanking lipophilic blocks comprise blocks of polylactide having the structure of

\[ \left( \overset{\circ}\text{O} \overset{\circ}\text{C} \overset{\circ}\text{H} \overset{\circ}\text{O} \overset{\circ}\text{C} \overset{\circ}\text{H} \right)_i \]

The lipophilic blocks having pendant alkyl chains comprise poly(alkylated lactide) having the structure of

\[ \left( \overset{\circ}\text{O} \overset{\circ}\text{C} \overset{\circ}\text{H} \overset{\circ}\text{O} \overset{\circ}\text{C} \overset{\circ}\text{H} \right)_i \]

Each of R_i and R_2 is independently hydrogen or a C1-C2 alkyl group (e.g., methyl, ethyl). Each R is independently a linear or substantially linear alkyl chain of a length from about C6 to about C24 (e.g.,
a Ce, C_7, C_8, C_9, C_10, C_11, C_12, C_13, C_14, C_15, C_16, C_17, C_18, C_19, C_20, C_21, C_22, C_23, C_24, C_25, or C_26 alkyl chain). i is an integer from about 10 to about 5,000 (e.g., from about 10 to about 4,000, from about 10 to about 3,000, from about 10 to about 2,000, from about 10 to about 1,000, from about 10 to about 500, from about 20 to about 5,000, from about 50 to about 5,000, from about 100 to about 5,000, from about 200 to about 5,000, from about 500 to about 5,000). m is an integer from about 1 to about 1,000 (e.g., from about 1 to about 800, from about 1 to about 500, from about 1 to about 300, from about 1 to about 200, from about 1 to about 100, from about 1 to about 50, from about 10 to about 1,000, from about 50 to about 1,000, from about 100 to about 1,000, from about 200 to about 1,000, from about 500 to about 1,000). n is an integer from about 10 to about 5,000 (e.g., from about 10 to about 4,000, from about 10 to about 3,000, from about 10 to about 2,000, from about 10 to about 1,000, from about 10 to about 500, from about 20 to about 5,000, from about 50 to about 5,000, from about 100 to about 5,000, from about 200 to about 5,000, from about 500 to about 5,000).

[0047] In certain preferred embodiments, the amphiphilic degradable block copolymer includes the structure of

\[
\begin{array}{c}
\text{O} \\
\text{R}_2 \\
\text{C}_n \\
\text{H}_2 \\
\text{O} \\
\text{R}_1 \\
\text{C}_m \\
\text{O} \\
\text{C}_2H_4 \\
\text{O} \\
\text{R}_2 \\
\text{C}_n \\
\text{H}_2 \\
\text{O} \\
\text{R}_1 \\
\text{C}_m \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\end{array}
\]

In certain preferred embodiments, each of R_i and R_2 is independently a C_1-C_2 alkyl group; R is a linear alkyl chain of a length from about C_12 to about C_24; i is an integer from about 10 to about 5,000; each m is an integer from about 1 to about 1,000; and each n is an integer from about 10 to about 5,000.

[0048] In certain preferred embodiments of the amphiphilic degradable block copolymer, each of R_i and R_2 is a methyl group; R is a linear alkyl chain of a length from about C_8 to about C_18; i is an integer from about 200 to about 800 (e.g., from about 200 to about 600, from about 200 to about 500, from about 200 to about 400, from about 300 to about 800, from about 400 to about 800, from about 500 to about 800); each m is an integer from about 10 to about 100 (e.g., from about 10 to about 80, from about 10 to about 60, from about 10 to about 50, from about 10 to about 30, from about 10 to about 20, from about 20 to about 100, from about 40 to about 100, from about 60 to about 100, from about 80 to about 100); and each n is an integer from about 100 to about 500 (e.g., from about 100 to about 400, from about 100 to about 300, from about 100 to about 200, from about 200 to about 500, from about 300 to about 500, from about 400 to about 500).

[0049] The ratio of i : m : n may be any suitable ratio, for example, ranging from about 1—50 : 1-50 : 1-50 to about 50-1 : 50-1 : 50-1 (e.g., from about 1-30 : 1-30 : 1-30 to about 30-1 : 30-1 :
In certain preferred embodiments, the amphiphilic degradable block copolymer has a molecular weight from about 10,000 to about 1,000,000 (e.g., from about 10,000 to about 500,000, from about 10,000 to about 300,000, from about 10,000 to about 200,000, from about 10,000 to about 100,000, from about 50,000 to about 1,000,000, from about 100,000 to about 1,000,000, from about 200,000 to about 1,000,000, from about 300,000 to about 1,000,000).

In certain preferred embodiments, the amphiphilic degradable block copolymer has a polydispersity from about 1.0 to about 2.0 (e.g., from about 1.0 to about 1.8, from about 1.0 to about 1.6, from about 1.0 to about 1.4, from about 1.2 to about 2.0, from about 1.4 to about 2.0, from about 1.6 to about 2.0).

In another aspect, the invention generally relates to an amphiphilic degradable random copolymer, which includes hydrophilic monomer units, having the structure of

\[
\begin{align*}
\text{lipophilic monomer units, having the structure of } & \\
\text{and } & \\
\text{wherein } & \\
\text{each of } R_i \text{ and } R_2 \text{ is hydrogen or a } C_i-C_2 \text{ alkyl group; } & \\
\text{R is a linear or substantially linear alkyl chain of a length from about } C_6 \text{ to about } C_{24}. & \\
\end{align*}
\]

In certain preferred embodiments, the amphiphilic degradable random copolymer, each of \( R_i \) and \( R_2 \) is a methyl group; and \( R \) is a linear alkyl chain of a length from about \( C_6 \) to about \( C_{18} \).

In certain preferred embodiments of the amphiphilic degradable random copolymer, the ratio of hydrophilic units : lipophilic units : lipophilic units with alkyl chains ranges from about 1-50 : 1-50 : 1-50 to about 50-1 : 50-1 : 50-1. In certain preferred embodiments, the amphiphilic
degradable random copolymer has a molecular weight from about 10,000 to about 1,000,000 (e.g., from about 10,000 to about 500,000, from about 10,000 to about 300,000, from about 10,000 to about 200,000, from about 10,000 to about 100,000, from about 50,000 to about 1,000,000, from about 100,000 to about 1,000,000, from about 200,000 to about 1,000,000, from about 300,000 to about 1,000,000).

[0054] In certain preferred embodiments, the amphiphilic degradable random copolymer has a polydispersity from about 1.0 to about 2.0 (e.g., from about 1.0 to about 1.8, from about 1.0 to about 1.6, from about 1.0 to about 1.4, from about 1.2 to about 2.0, from about 1.4 to about 2.0, from about 1.6 to about 2.0).

[0055] In another aspect, the invention generally relates to a fibrous scaffold made from an amphiphilic degradable copolymer of the invention.

[0056] The fibrous scaffold may be loaded with a biomolecule, for example, a lipid or a protein. In certain preferred embodiments, the fibrous scaffold is loaded with SIP at a loading efficiency greater than about 70% (e.g., greater than about 80%, greater than about 90%, greater than about 95%). In certain preferred embodiments, the fibrous scaffold is loaded with rhVEGF at a loading efficiency greater than about 70% (e.g., greater than about 80%, greater than about 90%, greater than about 95%). In certain preferred embodiments, the fibrous scaffold is loaded with rhBMP at a loading efficiency greater than about 70% (e.g., greater than about 80%, greater than about 90%, greater than about 95%).

[0057] The fibrous scaffold may take any suitable physical form, for example in the form of selected from fibrous meshes (e.g., by electrospinning), dense films (e.g., by solvent casting), porous 3-D scaffolds (e.g., by salting leaching, gas foaming), dense 3-D scaffolds (e.g., by pressing or extrusion), and macroporous 3-D scaffolds (e.g., fabricated by 3-D prototyping/3-D printing).

[0058] In yet another aspect, the invention generally relates to a method for sustained release of a biomolecule to an in vivo target location. The method includes: providing a fibrous scaffold prepared from an amphiphilic degradable copolymer of the invention; loading the fibrous scaffold with the biomolecule to be delivered in vivo; placing the loaded fibrous scaffold at the target location; and causing sustain release of the biomolecule at the target location.

[0059] Any suitable biomolecules (e.g., lipids or proteins) may be delivered according to the method of the invention. The biomolecule may be a lipid selected from SIP, ceramide, sphingosine, omega-3 fatty acids such as EPA and DHA. The biomolecule may be a protein selected from VEGF, BMP, FGF, EGF, PDGF and IGF. Any suitable target locations may be selected, for example, bone.
defect, dental bone defect, craniofacial defect, soft tissue defects such as cartilage and skin defect, composite tissue defects such as osteochondral defect, and any wound surfaces. The sustained releases of the biomolecule ranges from about 8 h to more than 60 days (e.g., from about 8 h to about 60 days, from about 8 h to about 45 days, from about 8 h to about 30 days, from about 8 h to about 14 days, from about 8 h to about 7 days, from about 8 h to about 3 days, from about 12 h to about 60 days, from about 1 day to about 60 days, from about 3 to about 60 days, from about 7 to about 60 days, from about 14 to about 60 days, from about 30 to about 60 days).

Examples

[0060] In general, experiments showed that alkylated random copolymers were found to exhibit inferior electrospinability and SIP loading efficiency (~50% vs. >90%) compared with the block copolymers. Furthermore, C6-alkylated block copolymers were found to lead to more rapid early release of SIP (55% in 8 h and 80% in 7 days) comparing with C14-alkylated block copolymers. More sustained and steady release of SIP (35% in 8 h and 65% in 7 days) was accomplished with the C14- block copolymer with long alkylated block length. Much slower release (30-35% in 8 h and 45% in 7 days) was observed with C14-alklyated block copolymer with medium alkylated block length and the unmodified PELA. The interactions between the alkylated amphiphilic copolymers and the SIP appeared to be primarily governed by the tendency of the alkylated side chains and the SIP lipid tail to aggregate (with longer alkyl chains and higher clustering density being more effective in sequestering SIP). By contrast, in the absence of the alkylated side chains, enhanced aggregation of the hydrophobic PLA blocks of PELA upon hydration may be a more dominant factor for the hydrophobic encapsulation of SIP. These distinctive SIP release profiles also translated into varying pro-angiogenic effects in vitro (HUVEC tube formation assay) and ex ovo (CAM assay) in a SIP dose-dependent manner. The benefit of more sustained release of SIP was clearly demonstrated with relatively high SIP encapsulation dose where high concentration of SIP resulting from their burst release could result in inhibitory rather than stimulatory effect on EC tube formations. CAM assay over three days confirmed the proangiogenic and chemotactic effect of SIP-bearing amphiphilic scaffolds. The C14- block copolymer mesh with longer alkylated block length, when encapsulated with SIP and placed on CAM, most effectively induced local neovessel formation and infiltration. Overall, this amphiphilic degradable copolymer platform represents a promising tool for mechanistic investigations of dose and temporal effects of SIP delivery on angiogenesis outcome. Furthermore, it
can be exploited for controlled delivery of SIP and other hydrophobic or amphiphilic biomolecules for a wide range of guided tissue regeneration applications.

*Synthesis and characterization of alkylated monomers and amphiphilic copolymers*

[0061] The mono-alkylated lactides CeLA and C_{14}LA were prepared using a two-step process (FIG. IB) with an overall moderate yield (largely limited by the intramolecular condensation step) that is consistent with literature. With a targeted molecular weight of 120 kD for all pentablock and random copolymers, melt ROP using PEG20K as a macromolecular initiator and Sn(Oct)$_2$ as catalyst (FIG. IB) was carried out by simultaneous (for random polymer) or sequential (for block copolymers) addition of alkylated lactides and *D,L*-lactide (FIG. IB). PELA of the same targeted molecular weight was also prepared. All polymers were obtained in good yields with high monomer conversions (>90%) and reasonable molecular weight distributions, with PDI of 1.1-1.2 for most triblock copolymers (Table 2) and 1.4-1.5 for pentablock copolymers (Table 1). Decreased number-average molecular weights were observed for triblock copolymers containing increasing theoretical lengths of C14-alkylated blocks (Table 2). GPC comparison of a typical triblock intermediate and final crude pentablock product supported the narrow molecular distributions and high conversions (FIG. 2A). $^1$H NMR integration also supported an overall excellent (80-100%) incorporation of alkylated monomers (FIG. 2B and Table 1). It appeared to be more challenging to obtain high molecular weight random copolymers than the block copolymers (e.g., $M_n^{GPC}$ of rC14-L was 1.5-fold lower than that of the C14-L of same targeted molecular weights).

**Table 1. Properties of amphiphilic pentablock and random copolymers**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Polymer Composition $^a$</th>
<th>C$_6$/i$_4$LA: EG (mol: mol)</th>
<th>Yield (%)</th>
<th>$M_n^{GPC}$</th>
<th>PDI $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentablock copolymers</strong></td>
<td></td>
<td>Feeding: Incorporated $^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14-L</td>
<td>LA$<em>{3i2}$-(C$</em>{14}$LA)$<em>{15}$-EG$</em>{54}$-(C$<em>{14}$LA)$</em>{15}$-LA$_{3i2}$</td>
<td>1: 15.1: 17.2</td>
<td>91.7</td>
<td>107,282</td>
<td>1.47</td>
</tr>
<tr>
<td>C14-M</td>
<td>LA$<em>{278}$-(C$</em>{14}$LA)$<em>{3i}$-EG$</em>{54}$-(C$<em>{14}$LA)$</em>{3i}$-LA$_{278}$</td>
<td>1: 7.3: 8.3</td>
<td>86.9</td>
<td>79,687</td>
<td>1.51</td>
</tr>
<tr>
<td>C14-H</td>
<td>LA$<em>{23i}$-(C$</em>{14}$LA)$<em>{5i}$-EG$</em>{54}$-(C$<em>{14}$LA)$</em>{5i}$-LA$_{23i}$</td>
<td>1: 4.4: 5.4</td>
<td>90.2</td>
<td>66,963</td>
<td>1.55</td>
</tr>
</tbody>
</table>
The thermal properties of polymers were examined by DSC to reveal hydrophobic chain-chain interactions, characterized by a thermal transition associated with the aggregation and disassociation of the alkylated side chains. As shown in FIG. 3 and FIG. 9, an endothermic peak at 10.86 °C, 11.63 °C and 11.75 °C was detected for C14-alkylated pentablock copolymer C14-H, C14-M and C14-L, respectively, supporting the hydrophobic chain-chain interactions within these amphiphilic pentablock copolymers. However, no such thermal transition was detected in PELA, short chain polymer C6-M or random polymer rC14-L. This thermal transition was also observed in
the C14-triblock polymers, but not in the C6-triblock copolymer or PELA (FIG. 8), besides the major endothermic peak at 55-65 °C attributable to PEG crystallization/melting. (Kutikov et al. 2013 Acta Biomater 9:8354-64.)

Polymer fibrous mesh fabrication and characterization

[0063] The amphiphilic polymers were electrospun into fibrous meshes. As shown in FIG. 4A, PELA, C6-M, C14-L and C14-M meshes were composed of randomly arranged microfibers free of beading, with an average fiber diameter of 1.76 ± 0.25 μm, 2.03 ± 0.60 μm, 1.18 ± 0.46 μm and 1.66 ± 0.37 μm, respectively. High-content alkyl side chain incorporation as in the case of C14-H resulted in partial fusion of the fibers, likely driven by hydrophobic interactions between the alkylated segments of contacting fibers at the ambient temperature. The random copolymer rC14-L mesh did not exhibit distinctive fiber morphology, but rather appeared to be composed of fused beading structures, suggesting that this copolymer did not possess optimal physical characteristics (e.g., viscosity) for electrospinning. The quantification of porosity of the electrospun meshes relative to the respective dense solvent-cast films by weight (FIG. 4B) revealed the highest porosity (~60%) for the C14-L mesh, 40-50% porosity for the C14-M, C6-M and PELA meshes, whereas <20% and <10% porosity for C14-H and rC14-L, consistent with the morphologies revealed by SEM micrographs (FIG. 4A).

[0064] To examine how the varying surface morphology/porosity and intrinsic hydrophilicity of the polymers translate into differential water wettability, the water contact angles of both electrospun meshes and the respective dense solvent-cast films were measured. Among all dense solvent-cast films (FIG. 10), PELA and rC14-L exhibited significantly lower water contact angle than others, suggesting relatively higher hydrophilicity for PELA and rC14-L. The difference in water contact angles among the dense C14-L, C14-M and C14-H solvent-cast film, however, was not dramatic. The electrospun C14-L, C14-M and C14-H meshes, on the other hand, exhibited significant increases in water contact angles (FIG. 4C), accompanying the decreasing porosity of these electrospun fiber meshes (FIG. 4B), as the content of C14-alkyl side chains increased, supporting significant contributions of surface porosity to the water wettability of the meshes. Overall, C14-L and PELA meshes were the most wettable by water (contact angles ~30°), while the C14-H and C6-M meshes were the least wettable among all (water contact angle ~100°). The least porous (<10%) yet one of the most hydrophilic (FIG. 10) rC14-L electrospun mesh exhibited a water contact angle (~80°) between that of C14-H and C14-M meshes, supporting that surface porosity and polymer hydrophilicity synergistically contribute the overall wettability of the fibrous mesh.
In-vitro SIP loading and release

[0065] The SIP loading efficiency and release profile were determined using SIP competitive ELISA ($R^2 = 0.970$ for standard curve). As shown in FIG. 5A, the SIP loading efficiencies for PELA and C14-alkylated block copolymer meshes, determined as the percentage of SIP retained on the meshes after 5-min incubation in PBS with 0.2% FAF-BSA, were all above 90%, while the rC14-L and C6-M meshes displayed a much lower loading efficiency of 64% and 54%, respectively.

[0066] The percentages of cumulative release of SIP at various time points were determined relative to the amount retained on the respective meshes at 5 min. As shown in FIG. 5B, mesh rC14-L exhibited similar SIP early release kinetics as C6-M while meshes C14-L, C14-M shared similar profiles as that of PELA. However, in consideration of its inferior electrospinnability (e.g. tendency to bead) and poor SIP loading efficiency, the random copolymer rC14-L was deemed unsuitable for SIP delivery and excluded from further investigations in the current study. Among the rest of the electrospun meshes, C6-M released significantly more SIP (-55%) than C14-block copolymer (C14-L, -M, -H) or PELA meshes (30-35%) during the first 8 h, followed by a slower yet continuing release (FIG. 5B). A total of -80% SIP was released from C6-M, -65% from C14-H, and -45% from PELA and C14-M by day 7 was accomplished (FIG. 5B).

[0067] Overall, PELA, C14-L and C14-M (FIG. 5B, bar symbols) exhibited the slowest releases over 7 days, C14-H exhibited the most sustained release (FIG. 5B, square symbols), while C6-M led to a higher burst release of SIP (FIG. 5B, circle symbols). Among the alkylated copolymers well-suited for electrospinning, C14-M, C14-H and C6-M were thus chosen for further investigation as to whether/how the three distinctive release profiles may translate into differential in vitro angiogenic outcomes by tube formation assays. Given the unalkylated nature, the PELA mesh, although with similar SIP release profile as C14-M, was also included in the tube formation assay.

Tube formation assay

[0068] HUVEC tube formation assay was employed to evaluate the pro-angiogenic activity of released SIP in vitro. (Lee et al. 1999 Biochem Biophys Res Commun 264:743-50.) It was first showed that in the absence of a polymer carrier, the total tube length increased with the direct supplement of 1-µM SIP, however, such proangiogenic effect was compromised at the higher concentration of 10-µM SIP. Furthermore, the direct exposure of HUVEC-Matrigel culture to a very high concentration of 50-µM SIP significantly inhibited tube formation (resulted from a dramatic inhibition of cell mobility) compared to no-SIP control (FIGs. 6A and 6C).
When SIP was delivered via the amphiphilic polymer scaffolds, more robust and uniform tube formations were observed. When SIP was loaded on C14-alkylated copolymer meshes at a dose equivalent to 10-µM upon 100% release, total tube lengths observed were equivalent to that observed upon supplementation of 1-µM free SIP (FIGs. 6B and 6D). This observation supports that the amphiphilic polymer scaffolds effectively prevented the burst-release of high doses of SIP that could have been inhibitory to tube formations, with the C14-alkylated (C14-M) slightly more effective than the C6-alkylated counterpart (C6-M) or PELA. The benefit of slower and more sustained releases of SIP from C14-alkylated copolymer (C14-M & C14-H) meshes were more profoundly reflected when a higher loading dose of SIP was applied (equivalent to 50-µM SIP upon 100% release). The total tube length observed was longer than those stimulated with 10-µM free SIP. The C6-M and PELA meshes preloaded with the same high-dose SIP resulted in significantly shorter total tube lengths, although still comparable to that observed with the culture supplemented with 10-µM free SIP.

**Ex-ovo chicken chorioallantoic membrane (CAM) assay**

To further explore the effect of SIP release kinetics on angiogenesis over a longer period (several days as opposed to 17 h in the HUVEC tube formation assay), three SIP-bearing amphiphilic groups (PELA, C6-M and C14-H) with distinct SIP release kinetics were subjected to the ex-ovo CAM assay. As representatively shown in FIG. 7A (top and middle row), the SIP-loaded C14-H group induced the most noticeable shift of surrounding vessels towards the implant. This is also accompanied by the most pronounced neovessel growth observed with the group implanted with SIP-loaded C14-H mesh, as supported by the quantification of total microvessels surrounding the implants (FIG. 7B). In comparison, SIP-bearing C6-M meshes, which led to more burst early release of SIP than C14-H, induced less potent neovessel growth (FIG. 7B). The morphologies of the neovessels beneath the meshes were more clearly visualized from the flipped CAM images (FIG. 7A, bottom row; note that the flipped image for C14-H without SIP was not shown as the CAM was damaged during the “flipping” process).

**Retention/release of protein therapeutics**

Experiments also demonstrated that the amphiphilic polymer platform of the invention can be employed for the retention/release of protein therapeutics, for example, rhVEGF165 or rhBMP-2 (R&D systems) on alkylated amphiphilic polymers (C14-M and C14-H) vs. non-alkylated amphiphilic polymer PELA vs. hydrophobic polymer PLA.
Initial loading doses were 10-ng rhVEGF or 10-ng rhBMP-2 per mesh (6.3 mm in diameter), shown in FIG. 12 and FIG. 13, respectively. Commercial ELISA kits (R&D systems) were used for the quantification. The protein-loaded meshes were incubated in 1-mL PBS at 37 °C and retrieved at predetermined time points. The PBS was collected for ELISA while the retrieved mesh was transferred into a fresh 1-mL PBS for continued incubation up to 7 days. The cumulative protein released into the PBS was quantified by ELISA (n=3). These data show that both C14-H and C14-M exhibited outstanding retention and the slowest release of rhVEGF165 among the four, whereas C14-M and C14-H exhibited the slowest and fastest release of rhBMP-2 among the four, respectively.

**Co-delivering SIP in combination with VEGF**

Also demonstrated was the synergistic effect of co-delivering SIP in combination with VEGF in promoting tube formation of HUVECs in some dose combinations (FIG. 14).

Human umbilical vein endothelial cells (HUVECs, ATCC) were cultured on gelatin-coated plates in M199 medium with 20% fetal bovine serum (FBS), 3 ng/mL bFGF, 5 units/mL heparin and 100 U/100 μg/mL Pen/Strep at 37 °C under 5% CO<sub>2</sub>. The 96-well culture plate was coated with 50 μL/well growth factor reduced Matrigel and incubated at 37 °C for 0.5 h to allow Matrigel to solidify. Then HUVECs suspended in 100 mL of M199 medium with 0.1% FBS and 100 U/100 μg/mL Pen/Strep were seeded on the Matrigel at 2 x 10<sup>4</sup> cells/well. The SIP and/or VEGF solutions were carefully added to each well, followed by continued incubation at 37 °C for 17 h. After removing culture media, the HUVECs were fixed with 10% formalin saline solution and imaged with an Axiovert 40 CFL microscope equipped with a QImaging camera at 25x magnifications (representatively shown in A). The total tube length in each well (n = 3) was quantified by ImageJ (NIH) as shown in Figure B. All quantitative data are plotted as mean ± standard derivation. Student's t-tests were employed for statistical analysis. *P < 0.05, **P<0.01. These data support synergistic delivery of 10-mM SIP in combination with a lower dose of VEGF (10 ng/ml) more effectively promoted the tube formation than SIP alone, while the synergistic delivery higher dose of VEGF (50 ng/ml VEGF) in combination of this dose of SIP did not. Synergistic effect of the co-delivery of VEGF along with higher dose of SIP was not as significant.

**Materials and general instrumentation**

Sphingosine-1-phosphate (SIP) was purchased from Cayman Chemical (Ann Arbor, MI). Growth factor reduced Matrigel was obtained from BD Biosciences (Bedford, MA). Fertile chicken
eggs were supplied by Charles River Labs (Wilmington, MA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used as received unless otherwise stated. 2-Hydroxyhexadecanoic acid was synthesized from 2-bromohexadecanoic acid per literature protocols.

[0076] NMR spectra were recorded on a Varian INOVA-400 spectrometer. Molecular weights and polydispersity of polymers were determined by gel permeation chromatography (GPC) on a Varian Prostar HPLC system equipped with two 5-mm PLGel MiniMIX-D columns and a PL-ELS2100 evaporative light scattering detector. Calibrations were performed with polystyrene standards (polymer laboratories). THF was used as the eluent at a flow rate of 0.3 mL/min.

**Design rationale of the amphiphilic polymers and alkylated lactide monomers**

[0077] PLA-PEG-PLA (PELA)-based amphiphilic copolymer-based platform is designed to enable interactions between the polar SIP headgroup and the hydrophilic PEG segment, as well as the lipophilic SIP tail with the hydrophobic PLA blocks. Alkylated polylactides to PELA was inserted either in discrete blocks between the PEG core and the PLA ends or randomly with the PLA blocks to further enhance SIP binding via hydrophobic interactions between the aliphatic side chains and the SIP lipid tail (**FIG. 1A**). It is worth noting that complete elimination of PLA from the amphiphilic copolymers (*i.e.*, substituting two PLA blocks in PELA with alkylated polylactides) tended to result in copolymers with lower molecular weight liquids (**Table 2**) that are unsuitable for electrospinning fabrication of bulk scaffolds. Three distinct design elements were altered to allow the scaffolds to interact with SIP with varied affinities: the alkyl side chain lengths (C6 vs. C14), distribution (random copolymers with alkyl side chains spreading out vs. block copolymers with the alkyl side chains more densely clustered), and presentation density (low, medium and high alkylated repeating units relative to PEG core).

[0078] The design of 3-methyl-6-alkyl-1,4-dioxane-2,5-diones as alkylated lactide monomers was motivated by their biocompatible degradation products, a-hydroxyl fatty acids, that are present in plants and mammals. (Kishimoto *et al.* 1963 *J Lipid Res* 4:139-43; Foulon *et al.* 2005 *J Biol Chem* 280:9802-12.) The choice of mono- instead of bi-alkylated lactides was due to the concern that the excessive steric hindrance of the latter that may compromise the ring-opening polymerization efficiency.

**Monomer syntheses**
3-Methyl-6-hexyl-1,4-dioxane-2,5-dione (CeLA): The monomer synthesis was carried out following a protocol modified over literature. To an ice-bath chilled acetone solution (150 mL) of 2-hydroxyoctanoic acid (5.0 g, 31.21 mmol) and Et_3N (8.71 mL, 62.42 mmol) was slowly added 2-bromopropionyl bromide (3.43 mL, 32.77 mmol). The white suspension was then stirred at room temperature for 0.5 h before it was filtered. The obtained white residue was further washed with acetone twice to give a combined light yellow filtrate of a total volume of 300 mL, to which was added Et_3N (8.71 mL, 62.42 mmol). The mixture was stirred at 65 °C for 2 h before it was cooled to room temperature and concentrated to 50 mL under reduced pressure. The concentrate was filtered, further concentrated and diluted with a mixture of n-hexane and EtOAc (n-hexane/EtOAc ≈ 3/1, 150 mL), and passed through a short silica gel column to give the crude product, which was recrystallized twice with n-hexane to yield a white solid racemic monomer (1.85 g, 27.7% yield). ¾ NMR (400 MHz, CDCl_3) δ 5.02 (m, 1H), 4.90 (m, 1H), 2.00 (m, 2H), 1.67 (m, 3H), 1.61 (m, 2H), 1.52 (m, 6H), 0.88 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl_3) δ 167.77, 167.15, 77.11, 76.03, 72.73, 72.48, 32.15, 31.68, 31.62, 30.23, 28.97, 28.74, 24.84, 24.52, 22.71, 22.68, 17.77, 16.07, 14.24, 14.21 ppm.

3-Methyl-6-tetradecyl-1,4-dioxane-2,5-dione (C₁₄LA): The monomer C₁₄LA was prepared in a similar fashion from 2-hydroxyhexadecanoic acid instead of 2-hydroxyoctanoic acid. Recrystallized racemic product (white solid) was obtained in a 43.1% yield. ¹H NMR (400 MHz, CDCl_3) δ 5.01 (m, 1H), 4.89 (m, 1H), 2.02 (m, 2H), 1.68 (m, 3H), 1.52 (m, 2H), 1.30 (m, 22H), 0.87 (t, J = 6.4 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl_3) δ 167.71, 167.08, 77.13, 76.05, 72.49, 32.15, 30.26, 29.91, 29.90, 29.88, 29.87, 29.82, 29.79, 29.71, 29.67, 29.59, 29.53, 29.47, 29.33, 29.10, 24.90, 24.59, 22.92, 17.80, 16.10, 14.36 ppm.

Polymer syntheses

The synthesis of amphiphilic copolymers was conducted using one-pot ROP by sequential (for block copolymer) or simultaneous (for random copolymer) addition of the respective monomers. The polyethylene glycol (PEG, 20,000 Dalton) was dried by azeotropic distillation with toluene. The D,L-lactide was freshly purified by recrystallization with ethyl acetate twice. Catalyst stannous octoate, Sn(Oct)$_2$, was prepared as a stock solution in anhydrous toluene, and added in equivalent molar ratio to PEG. The feeding ratio of D,L-lactide, CeLA or C14LA monomers to PEG varied based on the target polymer compositions as described in Table 2 and Table 1. ¹H NMR spectra of intermediates and crude products were taken to ensure that the monomer conversions were >90% for each step. The yields and GPC characterizations (Mₙ, PDI) of the triblock and pentablock copolymers are summarized in Table 2 and Table 1.
Pentablock copolymer PLA₁₋₁²⁻b-P(C₁₄-LA)₁₅⁻b-PEG₁₅⁻b-P(C₁₄-LA)₁₅⁻b-PLA₁₁² (C₁₄-L).

PEG (600 mg, 0.03 mmol) and C₁₄LA (300 mg, 0.92 mmol) were combined in a Schlenk vessel (10 mL), which was dried at 150 °C for 0.5 h under vacuum. After being cooled to room temperature, the reaction vessel was purged with argon, and Sn(Oct)₂ solution (0.03 mmol) was added and the solvent was evaporated. The mixture was heated at 150 °C for 0.5 h to allow polymerization of the alkylated lactide, followed by the addition of D₃L-lactide (2700 mg, 18.73 mmol) under argon. The melt mass was allowed to polymerize at 150 °C for another 1 h before it was quenched by exposure to air at room temperature. The crude product was purified by dissolving in chloroform and precipitating in ice-cold methanol. ³¹ NMR (400 MHz, CDC₁₃) δ 5.17 (m, 90H), 3.64 (s, 138H), 1.51 (m, 345H), 0.87 (t, J = 6.80 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDC₁₃) δ 169.81, 169.62, 169.56, 169.52, 169.34, 70.77, 69.39, 69.20, 32.14, 29.93, 29.59, 22.91, 16.96, 16.89, 14.35 ppm.

Pentablock copolymer PLA₂₇₈⁻b-P(C₁₄LA)₃₁⁻b-PEG₄₅₄⁻b-P(C₁₄-LA)₃₁⁻b-PLA₂₇₈ (C₁₄-M):
¹H NMR (400 MHz, CDC₁₃) δ 5.18 (m, 38H), 3.64 (s, 66H), 1.54 (m, 179H), 0.87 (t, J = 6.80 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDC₁₃) δ 169.81, 169.56, 169.34, 70.78, 69.39, 69.20, 32.15, 29.93, 29.59, 22.91, 16.97, 16.89, 14.35 ppm.

Pentablock copolymer PLA₂₁⁻b-P(C₁₄LA)₃₁⁻b-PEG₄₅₄⁻b-P(C₁₄-LA)₃₁⁻b-PLA₂₁ (C₁₄-H):
¹H NMR (400 MHz, CDC₁₃) δ 5.17 (m, 26H), 3.63 (s, 43H), 1.54 (m, 147H), 0.87 (t, J = 6.80 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDC₁₃) δ 169.81, 169.56, 169.53, 169.34, 70.78, 69.39, 69.20, 32.14, 29.93, 29.59, 22.91, 16.95, 16.89, 14.34 ppm.

Pentablock copolymer PLA₁₉₀⁻b-P(C₁₄LA)ₚₛ⁻b-P(C₁₄-LA)ₚₛ⁻b-PLA₁₉₀ (C₆-M):
³¹ NMR (400 MHz, CDC₁₃) δ 5.15 (m, 38H), 3.63 (s, 58H), 1.58 (m, 155H), 0.86 (t, J = 6.80 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDC₁₃) δ 169.83, 169.62, 169.55, 169.53, 169.34, 70.76, 69.38, 69.19, 31.70, 22.72, 16.94, 16.87, 14.23 ppm.

Random copolymer P[LA₁₁²⁻b-(C₁₄-LA)₁₅⁻b-EG₄₅₄⁻b-(C₁₄-LA)₁₅⁻b-LA₁₁²] (tC₁₄-L): PEG (600 mg), C₁₄LA (300 mg) and D₃L-lactide (2700 mg) were combined in a Schlenk vessel, and dried at 150 °C under vacuum for 0.5 h. The Sn(Oct)₂ solution (0.03 mmol) was then added and the solvent was evaporated. The mixture was allowed to polymerize under argon at 150 °C for 1 h. The polymerization was quenched by exposure to air at room temperature and purified by dissolving in chloroform and precipitating in ice-cold methanol. ¹H NMR (400 MHz, CDC₁₃) δ 5.17 (m, 93H), 3.63 (s, 152H), 1.54 (m, 370H), 0.87 (t, J = 6.80 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDC₁₃) δ 169.83, 169.56, 169.35, 70.75, 69.38, 69.19, 32.12, 29.89, 29.56, 22.89, 16.95, 16.88, 14.34 ppm.
[0086] **Triblock copolymer PLA** 34-P**EG** 5-P**LA** 34 (PELA): PEG (1000 mg, 0.05 mmol) and D,L-lactide (5000 mg, 34.69 mmol) were combined in a Schlenk vessel and dried under vacuum for 0.5 h. The mixture was allowed to polymerize at 150 °C for 45 min, following the addition of Sn(Oct)₂ solution (0.05 mmol) under argon. The polymerization was quenched and the polymer was purified as described above. H NMR (400 MHz, CDCI₃) δ 5.17 (m, 3.87H), 3.64 (s, 5.77H), 1.55 (m, 12H) ppm.

**Differential scanning calorimetry (DSC) analysis**

[0087] The DSC analysis was carried out on a Q200 Modulated DSC (TA Instruments), which was calibrated with indium and sapphire standards prior to use. Under nitrogen atmosphere, each specimen (10 mg) was heated at a rate of 10.00 °C/min from -90 °C to 150 °C, then cooled to -90 °C at the same rate before being heated back to 150 °C. Thermal transitions detected during the second heating cycle were used for data interpretation.

**Mesh fabrication by electrospinning**

[0088] The dried polymers were dispersed in a mixed solvent of chloroform and D,D-dimethylformamide (4:1 v/v) at a concentration of 25% (w/v) overnight. The polymers were electrospun into fibrous meshes by ejecting respective polymer solution through a blunt 22 gauge needle at a rate of 1.7 mL/h under 12 kV to a grounded Al-receiving plate set at 15 cm apart from the needle tip. ¹H NMR analysis of the mesh dissolved in CDCI₃ confirmed that there was no detectable residue D,D-dimethylformamide. The meshes were further dried in house vacuum overnight.

**Scanning electronic microscopy (SEM)**

[0089] The dried meshes were sputter coated with 4-nm gold and imaged on a Quanta 200 FEG MKII scanning electron microscope (FEI Inc., Hillsboro, OR) under high vacuum at 5 kV. The average fiber diameter was quantified from 50 randomly selected fibers in the micrograph acquired under 1000x magnification using ImageJ (NIH).

**Fabrication of dense polymer films by solvent casting**

[0090] In a typical procedure, the respective polymer (200 mg) was first dissolved in chloroform (4 mL) to obtain a clear solution, which was then cast into a 40 mm × 70 mm rectangle Teflon mold. The solvent was allowed to evaporate at room temperature overnight before the casted film was further dried under house vacuum and lifted off from the mold.

**Water contact angle measurements**
The water contact angle was determined with the sessile drop technique on a CAM 200 goniometer (KSV Instruments, Finland) connected with a charge-coupled device (CCD) camera. Deionized water droplets were deposited on the surface of electrospun or solvent-cast specimen. The contact angles from left and right side of each droplet were recorded at 30 sec following the initial water contact. Seven measurements from 3 specimens were taken for each electrospun mesh type and ten measurements were taken for each solvent-cast film.

Mesh porosity determination

To determine the porosity of the electrospun mesh, circular specimens of the same diameter (6.3 mm; n = 3) were cut from electrospun meshes and the respective solvent-cast films using a puncher. The porosity (%) of each mesh was calculated based on its weight relative to that of the solvent-cast film, adjusted by their respective thickness measured by a digital caliper: porosity (%) = \[1 - \frac{(\text{Weight}_{\text{mesh}} \times \text{thickness}_{\text{film}})}{(\text{weight}_{\text{film}} \times \text{thickness}_{\text{mesh}})}\] × 100%.

SIP loading

The electrospun meshes were punched into circles of 6.3 mm in diameter, weighed and sterilized with UV irradiation (254 nm, 1 h for each side). Fresh SIP solutions in PBS were carefully loaded onto each mesh (for SIP release study: 5 μL of 0.76 g/L solution to achieve 3.8 g SIP loading per mesh; for tube formation assay: 2 or 10 μL of 0.19 g/L solution to achieve 0.38 g or 1.90 g SIP loading per mesh). The SIP-loaded meshes were incubated at 37 °C for 1 h and 4 °C for another 4 h before they were air-dried in laminar flow hood overnight.

For CAM assay, pre-hydrated meshes were lyophilized, UV-sterilized, cut into circles of 3 mm in diameter and loaded with 0.5 g SIP (1 μL of 0.5-μg/μL SIP solution in PBS) per mesh. The SIP-loaded meshes were then incubated and air-dried as described above.

SIP release

The SIP-loaded specimens (n = 3) were placed in 200 μL of PBS solution containing 0.2% fatty acid-free-bovine serum albumin (FAF-BSA) and incubated at 37 °C with 5% CO₂. FAF-BSA was added to stabilize the released SIP and prevent them from aggregating to ensure their detection by ELISA. It was shown that in the absence of BSA, SIP standards became less detectable by ELISA. With the supplementation of 0.2% or 0.4% BSA, SIP standard remained consistently detected by ELISA. Thus, 0.2% FAF-BSA was chosen for the release study. At specific time points (5 min, 8 h, 1, 3, 5 and 7 days), the release solutions were collected, and fresh PBS solution with 0.2% FAF-BSA was added for continued incubation. The collected solutions were stored at -80 °C until time of...
quantification. The released SIP concentration was quantified using a sphingosine 1 phosphate assay kit (Echelon Biosciences, Salt Lake City, UT) following the vendor's instruction. The SIP loading efficiency (%) was calculated as: (initial SIP loading - released SIP at 5 min) / initial SIP loading x 100%

**Tube formation Assay**

[0096] Human umbilical vein endothelial cells (HUVECs, ATCC, passage 6), were cultured on gelatin-coated plates in M 199 medium with 20% fetal bovine serum (FBS), 3 ng/mL bFGF, 5 units/mL heparin and 100 U/100 µg/mL Pen/Strep at 37 °C under 5% CO₂. The 96-well culture plate was coated with 50 µL/well growth factor reduced Matrigel and incubated at 37 °C for 0.5 h to allow Matrigel to solidify. Then HUVECs suspended in 100 µL of M 199 medium with 0.1% FBS and 100 U/100 µg/mL Pen/Strep were seeded on the Matrigel at 2 x 10⁴ cells/well. After 30 min of cell attachment, PBS solutions of SIP or SIP-loaded meshes were carefully added to each well, followed by continued incubation at 37 °C for 17 h. After removing the meshes and culture media, the HUVECs were fixed with 10% formalin saline solution and imaged with an Axiocvert 40 CFL microscope equipped with a QImaging camera at 25x and 100x magnifications. The total tube length in each well (n = 3-4) was quantified by ImageJ (NIH).

**Chicken chorioallantoic membrane (CAM) assay**

[0097] An ex-ovo CAM assay was used to examine the pro-angiogenic effects of SIP-loaded meshes. Briefly, fertile chicken eggs were incubated blunt side up at 37 °C in 70% humidity for 3 days, and rotated three times daily. Then the eggs were wiped with 70% ethanol, carefully cracked into 100-mm Petri dishes, and incubated for another 7 days. Sterilized circular meshes loaded with 0.5 g SIP or PBS control were carefully placed on the CAM, and the embryos were cultured for 3 more days. The morphology of blood vessels surrounding the implants was photo-documented via a stereomicroscope by a digital camera (DFC 295, Leica) at 16x magnification. The CAM surrounding the mesh was then fixed with 10% formalin solution in PBS, flipped, and imaged at 25x magnification.

**Data analysis**

[0098] All quantitative data are plotted as mean ± standard derivation. Student's /-tests were employed for statistical analysis. Significance level was set as p < 0.05.
In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

**Incorporation by Reference**

References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

**Equivalents**

The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.
What is claimed is:

CLAIMS

1. An amphiphilic degradable block copolymer, comprising
   hydrophilic blocks;
   lipophilic blocks; and
   lipophilic blocks having pendant alkyl chains of lengths from about C₆ to about C₂₄;
2. The amphiphilic degradable block copolymer of Claim 1, wherein
   the hydrophilic blocks comprise blocks of poly(ethylene glycol) having the structure of
   \[
   \left[\begin{array}{c}
   O \\
   C_2H_4
   \end{array}\right]^i;
   \]
   the lipophilic blocks comprise blocks of polylactide having the structure of
   \[
   \left[\begin{array}{c}
   O \\
   H \\
   R_2
   \end{array}\right] \quad \left[\begin{array}{c}
   O \\
   C \quad R_1 \\
   O \\
   H \\
   R_2
   \end{array}\right]^n; \text{ and}
   \]
   the lipophilic blocks having pendant alkyl chains comprise poly(alkylated lactide) having the structure of
   \[
   \left[\begin{array}{c}
   O \\
   H \\
   R
   \end{array}\right] \quad \left[\begin{array}{c}
   O \\
   C \quad R_1 \\
   O \\
   H \\
   R_2
   \end{array}\right]^m,
   \]
   wherein
   each of R₁ and R₂ is independently hydrogen or a Cᵢ-C₂ alkyl group;
   each R is independently a linear or substantially linear alkyl chain of a length from about C₆ to about C₂₄;
   \( i \) is an integer from about 10 to about 5,000;
   \( m \) is an integer from about 1 to about 1,000; and
   \( n \) is an integer from about 10 to about 5,000.
3. The amphiphilic degradable block copolymer of Claim 1 or Claim 2, comprising the structure of
wherein

each of $R_i$ and $R_2$ is independently a $C_i-C_2$ alkyl group;

$R$ is a linear alkyl chain of a length from about $C_2$ to about $C_{24}$;

$i$ is an integer from about 10 to about 5,000;

each $m$ is an integer from about 1 to about 1,000; and

$R$ is a linear alkyl chain of a length from about $C_2$ to about $C_{12}$;

$i$ is an integer from about 200 to about 800;

each $m$ is an integer from about 10 to about 100; and

$R$ is a linear alkyl chain of a length from about $C_2$ to about $C_8$;

$i$ is an integer from about 10 to about 5,000.

4. The amphiphilic degradable block copolymer of any of Claims 1-3,

each of $R_i$ and $R_2$ is a methyl group;

$R$ is a linear alkyl chain of a length from about $C_2$ to about $C_{12}$;

$i$ is an integer from about 200 to about 800;

each $m$ is an integer from about 10 to about 100; and

$R$ is a linear alkyl chain of a length from about $C_2$ to about $C_8$;

$i$ is an integer from about 100 to about 500.

5. The amphiphilic degradable block copolymer of any of Claims 1-4, wherein the ratio of $i : m : n$ ranges from about $1:50:1$—50 to about $50:1:50$—1.

6. The amphiphilic degradable block copolymer of any of Claims 1-5, having a molecular weight from about 10,000 to about 1,000,000.

7. The amphiphilic degradable block copolymer of any of Claims 1-6, having a polydispersity from about 1.0 to about 2.0.

8. An amphiphilic degradable random copolymer, comprising

hydrophilic monomer units, having the structure of

$$\begin{array}{c}
\text{O} \\
\text{C}_2\text{H}_4
\end{array}$$

lipophilic monomer units, having the structure of

$$\begin{array}{c}
\text{R}_2
\end{array}$$

lipophilic monomer units, having the structure of
wherein

each of R_i and R_2i is hydrogen or a C_i-C_2 alkyl group; and
R is a linear or substantially linear alkyl chain of a length from about C_6 to about C_{24}.

9. The amphiphilic degradable random copolymer of Claim 8, wherein

each of R_i and R_2i is a methyl group; and
R is a linear alkyl chain of a length from about C_e to about C_{18}.

10. The amphiphilic degradable random copolymer of Claim 8 or Claim 9, wherein the ratio of hydrophilic units : lipophilic units : lipophilic units with alkyl chains ranges from about 1-50 : 1-50 : 1-50 to about 50-1 : 50-1 : 50-1.

11. The amphiphilic degradable random copolymer of any of Claims 8-10, having a molecular weight from about 10,000 to about 1,000,000.

12. The amphiphilic degradable random copolymer of any of Claims 8-11, having a polydispersity from about 1.0 to about 2.0.

13. A fibrous scaffold of made from an amphiphilic degradable copolymer of any of Claims 1-12.

14. The fibrous scaffold of Claim 13, loaded with a lipid or protein.

15. The fibrous scaffold of Claim 14, loaded with SIP at a loading efficiency greater than about 70%, and preferably greater than 90%.

16. The fibrous scaffold of Claim 14, loaded with rhVEGF at a loading efficiency greater than about 70%, and preferably greater than 90%.

17. The fibrous scaffold of Claim 14, loaded with rhBMP at a loading efficiency greater than about 70%, and preferably greater than 90%.

18. The fibrous scaffold of Claim 13, being in a form selected from electrospun fibrous meshes, dense films, porous or macroporous 3-D scaffolds and dense 3-D scaffolds.

19. A method for sustained release of a biomolecule to an in vivo target location, comprising

   providing a fibrous scaffold prepared from an amphiphilic degradable copolymer of any of Claims 1-12;

   loading the fibrous scaffold with the biomolecule to be delivered in vivo;

   placing the loaded fibrous scaffold at the target location; and

   causing sustain release of the biomolecule at the target location.
20. The method of Claim 19, wherein the biomolecule is a lipid or a protein.
21. The method of Claim 19 or Claim 20, wherein the biomolecule is a lipid selected from SIP, ceramide, sphingosine, omega-3 fatty acids such as EPA and DHA.
22. The method of any of Claims 19-21, wherein the biomolecule is a protein selected from VEGF, BMP, FGF, EGF, PDGF, IGF.
23. The method of any of Claims 19-22, wherein the target location is selected from bone defect, dental bone defect, craniofacial defect, soft tissue defects such as cartilage and skin defect, composite tissue defects such as osteochondral defect, and any wound surfaces.
24. The method of any of Claims 19-23, wherein sustained releases of the biomolecule ranges from about 8 h to more than 60 days.
FIG. 1

SUBSTITUTE SHEET (RULE 26)
FIG. 3
FIG. 5
FIG. 6
FIG. 7
FIG. 8. DSC spectra (second heating cycle) of PEG20K vs. the C14- and C6-alkylated triblock copolymers.
Polymer C14-H

FIG. 9 DSC spectra (first and second heating cycles) of PELA, pentablock and random polymers.
Polymer C14-M

FIG. 9 (Cont'd)
Polymer C14-L

FIG. 9 (Cont’d)
Polymer rC14-L

FIG. 9 (Cont'd)
Polymer C6-M

FIG. 9 (Cont'd)
Polymer PELA

FIG. 9 (Cont’d)
FIG. 10. Water contact angles (n = 10) of dense solvent-cast polymer films. **P<0.01 (Student’s t-test).

FIG. 11. Water contact angles (n = 7) of as-spun meshes vs. lyophilized meshes following 24-h hydration.
FIG. 12. Loading dose: 10-ng rhVEGF165/mesh

FIG. 13. Loading dose: 10-ng rhBMP-2/mesh
FIG. 14. (A) Representative tube formation of HUVECs cultured with or without the supplements of S1P alone, and S1P in combination of a lower or higher dose of VEGF. (B) The total tube length in each well (n = 3) as quantified by ImageJ (NIH). Data are plotted as mean ± standard derivation. *P < 0.05, **P<0.01 (student t-test).
# INTERNATIONAL SEARCH REPORT

**INTERNATIONAL APPLICATION**

International application No.
PCT/US 15/17640

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
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<th>IPC(8)</th>
<th>CPC</th>
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<tr>
<td>A61K 47/34; C08G 6308; A61F 250 (2015.01)</td>
<td>A61L 2300/604; A61L 31/148; A61L 31/16; C08G 63/08</td>
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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: A61K 47/34; C08G 6308; A61F 250 (2015.01)
- CPC: A61L 2300/604; A61L 31/148; A61L 31/16; C08G 63/08

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

- USPC: 424/426; 525/411; 424/423; 51/772.7

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

Minesoft Patbase, Google Scholar: drug/biomolecule, delivery, tissue engineering scaffold, amphiphilic block copolymer, hydrophilic, lipophilic, PEG, polylactide, poly(alkylated lactide)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>US 2013/0331464 A1 (MOLLER et al.) 12 December 2013 (12.12.2013) para [0005], [0006], [0057], [0234], [0259]-[0262]; Table 8.</td>
<td>1-3, 8-10</td>
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<tr>
<td>Y</td>
<td>US 2006/0165987 A1 (Hildgen et al.) 27 July 2006 (27.07.2006) Fig 6</td>
<td>1-3, 8-10</td>
</tr>
</tbody>
</table>

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

- "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
17 May 2015 (17.05.2015)

Date of mailing of the international search report
16 JUN 2015

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Form PCT/ISA/210 (second sheet) (January 2015)

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. LEI Claims Nos.: 4-7 and 11-24
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.

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<td>☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.</td>
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<td>☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.</td>
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Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)