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(57) Abstract: The invention relates to a tissue culture scaffold comprising a crosslinked polyHIPE formed a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, wherein the scaffold has an outer surface comprising a plurality of open pores.

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Scaffolds

The present invention relates to a method of forming a tissue culture scaffold by polymerizing a High Internal Phase Emulsion (HIPE) via a thermally initiated, light-initiated or acoustically initiated polymerisation route. The characteristics of the polymerized HIPE scaffold may be varied depending on the composition and characteristics of the HIPE. The HIPE scaffolds produced from the method of the present invention are useful for cell and tissue culture.

BACKGROUND

Synthetic biomaterials that can be structured into porous scaffolds to support cell growth have played an influential role in developing the field of tissue engineering [B. Dhandayuthapaniet al., Polymeric scaffolds in tissue engineering application: a review, International Journal of Polymer Science 2011 (2011)]. An emerging research interest in this area is the combination of additive manufacturing technologies and emulsion templating to produce multiscale porosity materials [M. Sušec et al. Hierarchically Porous Materials from Layer-by- Layer Photopolymerization of High Internal Phase Emulsions, Macromolecular rapid communications (2013); D.W. Johnson, et al. Macrostructuring of Emulsion-templated Porous Polymers by 3D Laser Patterning, Advanced Materials (2013); R. Owen et al. Emulsion templated scaffolds with tunable mechanical properties for bone tissue engineering, Journal of the Mechanical Behavior of Biomedical Materials 54 (2016) 159-172]. A high level of pore interconnectivity and percentage porosity are essential requirements for a tissue engineering scaffold as they permit sufficient oxygen and nutrient transfer to support the growth and proliferation of cells [Q.L. Loh, C. Choong, Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size, Tissue Engineering Part B: Reviews 19(6) (2013) 485-502].

The pore size and interconnectivity is a crucial factor for cell ingrowth and 3D tissue generation [S.J. Hollister, Porous scaffold design for tissue engineering, Nature materials 4(7) (2005) 518-524]. However, many techniques that provide the required pore size and interconnectivity result in a closed surface porosity due to the formation of a surface 'skin', negating the benefits of the highly porous material.

Accordingly, there remains a need for a tissue engineering scaffold that has the required pore size and interconnectivity that does not suffer from the disadvantages associated with the existing methods.

BRIEF SUMMARY OF THE DISCLOSURE

In accordance with the present inventions there is provided a tissue culture scaffold comprising a crosslinked polyHIPE formed a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, wherein the scaffold has an outer surface comprising a plurality of open pores.

Suitably, said elastomeric biodegradable polyester is selected from the group consisting of Poly(glycerol sebacate) (PGS), Poly(caprolactone)(PCL), copolymers and polymer blends of PGS and PCL, or copolymers and polymer blends of PGS and PCL with poly(Lactic acid) (PLA) or Poly(Glycolic acid) (PGA).

Suitably, the scaffold has a microporosity of 1 to 50 μm.

Suitably, the scaffold has a macroporosity of 100μm or greater.

Suitably, the scaffold comprises a plurality of cells.

Suitably, the macrostructure of the scaffold is in the form of a particle, tube, sphere, strand, coiled strand, capillary network, film, fibre, mesh, sheet or combination thereof.

Suitably, wherein the tube is straight, bent or branched.

Suitably, the acrylate groups are methacrylate groups.

Suitably, the scaffold is a tube and comprises a plurality of smooth muscle cells.

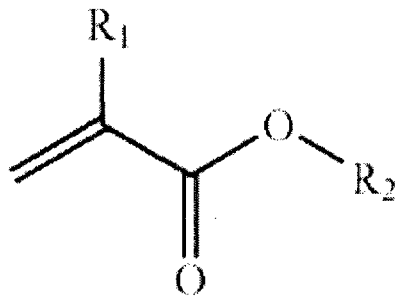
In a further aspect of the invention there is provide a method of forming a tissue culture scaffold comprising polymerizing a high internal phase emulsion having an external phase comprising a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, a solvent, and a surfactant and an internal phase comprising water, thereby crosslinking the acrylated elastomeric biodegradable polyester in the external phase.

Suitably, said elastomeric biodegradable polyester is selected from the group consisting of Poly(glycerol sebacate) (PGS), Poly(caprolactone)(PCL), copolymers and polymer blends of PGS and PCL, or copolymers and polymer blends of PGS and PCL with poly(Lactic acid) (PLA) or Poly(Glycolic acid) (PGA).

Suitably, said polymerizing comprises photopolymerization.

Suitably, the method comprises acrylating the elastomeric biodegradable polyester.

Suitably, the acrylate comprises the structure:



where R_1 is either a H or CH_3 and where R_2 is alkyl, aryl, heterocycles, cycloalkyl, aromatic heterocycles, multicycloalkyl, hydroxyl, ester, ether, halide, carboxylic acid, amino, alkylamino, dialkylamino, trialkylamino, amido, carbamoylthioether, thiol, alkoxy, or ureido groups, and branched and substituted versions thereof.

Suitably, the solvent is an organic hydrophobic solvent.

Suitably, the organic hydrophobic solvent is toluene, benzene, chloroform, dichloromethane, ethyl acetate, tetrahydrofuran, dimethylformamide, dichloroethane, carbon tetrachloride, diethyl ether, hexafluoroisopropanol or an aliphatic alcohol e.g. methanol, ethanol or isopropanol.

Suitably, the surfactant is a polymeric stabiliser.

Suitably, the stabiliser has a hydrophilic-lipophilic balance of 3-6, optionally wherein the stabiliser is Span 80 or Hypermer B246.

Suitably, said polymerizing is photopolymerizing and comprises UV excitation of said high internal phase emulsion in the presence of a photoinitiator.

Suitably, said polymerizing is heat induced polymerizing and comprises thermo excitation of said high internal phase emulsion in the presence of a thermal initiator.

Suitably, said polymerizing is sound induced polymerizing and comprises ultrasound excitation of said high internal phase emulsion in the presence of a redox based initiator.

Suitably, the photoinitiator, thermal initiator or redox-based initiator is a free radical generating photoinitiator.

Suitably, wherein the high internal phase emulsion is moulded prior to polymerization.

Suitably, said moulding comprises contacting said high internal phase emulsion with a hydrophobic surface.

Suitably, said a high internal phase emulsion is formed by mixing.

Suitably, said mixing occurs at a rotation speeds of 50-5000 rpm and temperature in between 0-100°C.

Suitably, said method further comprises washing the crosslinked acrylated elastomeric biodegradable polyester.

Suitably, said method further comprises seeding the washed crosslinked acrylated elastomeric biodegradable polyester with at least one cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows Melt polycondensation reaction performed under inert gas flow for 24 hours, then under vacuum for the remaining reaction duration.

Figure 2 shows Gel Permeation Chromatography of the PGS prepolymer synthesised

Figure 3 shows an overview of the result of methacrylation of PGS.

Figure 4 shows a diagrammatic representation of the outcome of photocuring.

Figure 5 shows 1H-NMR spectra showing successful incorporation of methacrylate groups into PGS prepolymers.

Figure 6 shows a graph showing that the degree of methacrylation is directly proportional to the reactant quantities used.

Figure 7 shows IR spectra showing the successful incorporation of the methacrylate groups into the PGS prepolymers.

Figure 8 shows enzymatic degradation of different PGS-M ore-polymers.

Figure 9 shows SEM of different PGS-M pre-polymers after enzyme treatment.

Figure 10 shows the mechanical properties of different PGS-M pre-polymers.

Figure 11 shows the cytocompatibility of the PGS-M pre-polymers in a resazurin reduction assay for cell metabolism.

Figure 12 shows cell proliferation on the PGS-M pre-polymers using a PicoGreen® assay for DNA quantification.

Figure 13 shows cell proliferation on the PGS-M pre-polymers using microscopy.

Figure 14 shows a schematic overview of Poly High Internal Phase Emulsion (PolyHIPE) generation.

Figure 15 shows SEM of PGS-M polyHIPE and a photograph of polyHIPE disks in a culture plate.

Figure 16 shows different shapes of PGS-M polyHIPE scaffolds that have been generated and can be used for tissue engineering.

Figure 17 shows tissue engineered blood vessels.

Figure 18 shows tissue engineered blood vessels cultured in a pulsatile flow bioreactor.

Figure 19 shows light microscopy of the tissue engineered blood vessels and a comparison of SMC invasion between polyHIPE PGS-M scaffolds and porous PGS-M scaffolds produced by an alternative common method in tissue engineering (porogen leaching) shows polyHIPE scaffolds allow superior cell invasion.

Figure 20 shows a schematic overview of PCL synthesis.

Figure 21 shows non-methacrylated and methacrylated 4 arm PCL.

Figure 22 shows the mechanical properties of PCL PolyHIPE.

Figure 23 shows SEM images of PCL PolyHIPE tubes on the exterior and interior of the tube surface.

Figure 24 shows SEM images of PCL PolyHIPE.

Figure 25 shows SEM images of PCL PolyHIPE.

Figure 26 shows box and whisker diagram showing the average pore diameter and the upper and lower quartiles of the measure pores in a PCL PolyHIPE. Different surfactant amounts were used (10, 20 and 30wt %) and the respective water volume ratios within each surfactant concentration, 1:4, 1:6 and 1:8 ratios.

Figure 27 shows a pore size Graph from PCL PolyHIPE with a ratio of 1:8 monomer to water phase. Here the monomer phase consisted of 0.4 g PCL and 0.6 g solvent.

DETAILED DESCRIPTION

An emulsion is a dispersion of one liquid in another liquid and generally is in the form of a water-in-oil mixture having an aqueous or water phase dispersed as droplets within a substantially immiscible continuous oil phase. Water-in-oil (or oil-in-water) emulsions having a high ratio of dispersed aqueous phase to continuous oil phase are known in the art as High Internal Phase Emulsions, also referred to as "HIPE" or HIPEs. At relatively high dispersed aqueous "internal" phase to continuous oil "external" phase ratios the external phase becomes a thin film separating and coating the droplet-like structures of the internal phase. The continuous phase of a water-in-oil HIPE may comprises one or more polymerizable prepolymers. These prepolymers can be polymerized, forming a cellular structure, for example a foam or a sponge, having a cell size distribution defined by the size distribution of the dispersed, aqueous phase droplets. In a preferred embodiment the water phase of the prepolymer emulsion is > 74% of the emulsion volume. Alternatively, the water phase of the prepolymer emulsion is>75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85% or more of the emulsion volume.

The pre-processing conditions of the emulsion directly affect the PolyHIPE morphology. Very high porosity can be achieved by increasing the droplet volume ratio in the emulsion, with ratios of up to 99% being reported [Richez, H. et al. Preparation of ultra-low-density microcellular materials, Journal of applied polymer science 96(6) (2005) 2053-2063]. Varying the surfactant concentration affects both the emulsion stability and the pore interconnectivity [J.M. Williams, A.J. Gray, M.H. Wilkerson, Emulsion stability and rigid foams from styrene or divinylbenzene water-in-oil emulsions, Langmuir 6(2) (1990) 437-444]. Furthermore, the preferential solubility of the initiator into the internal or continuous phase can determine whether open or closed porosity scaffolds are created [J.L. Robinson et al, Achieving interconnected pore architecture in injectable polyHIPEs for bone tissue engineering, Tissue Engineering Part A 20(5-6) (2014) 1103-1112].

The inventors have surprisingly identified that crosslinked polyHIPEs formed a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester have a scaffold architecture that makes them particularly useful in tissue engineering. The inventors have identified production methods for crosslinked polyHIPEs formed from crosslinked acrylated elastomeric biodegradable polyester which comprise an open pore structure and no closed porosity surface skin. The lack of a surface skin on the scaffolds allows cell ingrowth and permeation without the need for subtractive manufacturing to remove the outer skin layer.

Scaffolds

In one aspect the invention provides a tissue culture scaffold comprising a crosslinked polyHIPE formed a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, wherein the scaffold has an outer surface comprising a plurality of open pores.

As used herein, the term "scaffold" refers to a structural support facilitating cell infiltration and attachment in order to guide tissue growth. Cells and active agents can be incorporated into the scaffold as desired.

As used herein, the term "prepolymer" refers to a composition that can be cured to form a polymer. Typically, it will refer to a composition comprising intermediate polymers or oligomers that can be reacted to form a final polymer product. Prepolymers may comprise monomers, oligomers or polymeric systems comprising monomers that have been reacted to an intermediate molecular weight state. This material is capable of further polymerization or cross-linking of suitable reactive groups to a fully cured state, e.g. a high molecular weight state. As such, mixtures of reactive polymers with un-reacted monomers may also be referred to as prepolymers. In a particular meaning, the term 'prepolymer' may refer to a polymeric backbone that comprises functional groups that can be further polymerised or cross-linked to form a product polymer. The functional groups may have been added to appropriate functionality on the polymer after the polymer has been formed. Thus, the prepolymer of the first aspect of the invention comprises acrylate groups attached to a polyester backbone. The acrylate groups can be subjected to free-radical polymerisation conditions to form cross-linking groups between the polyester molecules and/or between different portions of a single polyester molecules, this forming the product polymer.

As used herein, the term "polyester" refers to a polymer containing ester functional groups in its main chain. As used herein, "biodegradable" refers to polymers and prepolymers that degrade to monomeric species under physiological or endosomal conditions. In various preferred embodiments, the polymers, prepolymers and biodegradation by products thereof are biocompatible. Biodegradable polymers and prepolymers in the form of biodegradable polyesters are well known in the art. As used herein, the term "elastomeric" refers the ability to respond to stress with deformations that are fully recoverable and repeatable.

In one embodiment, the elastomeric, biodegradable polyester is a poly(hydroxyalkanoate), a poly(lactic acid), a poly(glycolic acid) or a poly(caprolactone). In one embodiment the prepolymer is poly(caprolactone)(PCL). In one embodiment the prepolymer is poly(glycerol sebacate) (PGS). In one embodiment the elastomeric biodegradable polyester is a copolymer or polymer blend of PGS and PCL. In one embodiment, the elastomeric biodegradable polyester is a copolymer or polymer blend of PGS and PCL with poly(Lactic acid) (PLA) or Poly(Glycolic acid) (PGA).

In some embodiments the elastomeric biodegradable polyester is a copolymer or blend of any of the aforementioned polyesters. In one embodiment the prepolymer may be a high molecular weight prepolymer, having a molecular weight from about 2500 Da to about 70000 Da. In one embodiment the prepolymer may be a low molecular weight prepolymer, having a molecular weight from about 250 Da to about 4999 Da.

The crosslinked polyHIPE of the invention is formed from a prepolymer functionalised with an acrylate. The term 'acrylate' is intended to encompass both acrylate itself (-$C(=O)CH=CH_2$) and groups that have substituent groups, e.g. C_1-C_4-alkyl groups, attached in place of one or more of the acrylate hydrogen atoms. Suitable acrylate groups include methacrylate (i.e. $C(=O)CMe=CH_2$). The crosslink density of the polyHIPE can be controlled by adjusting the degree of acrylation on the polyester, i.e. the proportion of the hydroxy groups on the polyester that have been used to attach acrylate groups to the polyester backbone.

The polyHIPE of the invention is crosslinked. As used herein, the terms "crosslinked" and "crosslinking" refer to the formation of a polymer network by the linking of one polymer chain to another via a bond, such as a covalent or ionic bond. Mixing of an unpolymerized monomer or partially polymerized prepolymer with crosslinking reagents results in a chemical reaction that forms crosslinks. In particular, the crosslinking groups may be formed by reaction of the acrylate groups, e.g. under free radical polymerisation

conditions. The mechanical properties of the resulting crosslinked polymer network will depend on the crosslink density. For example, mixing at rotation speeds of from about 50-about 5000 rpm and temperature at a temperature of from about 0 -100°C will produce a crosslinked polyHIPE with varying pore sizes.

The HIPE scaffolds of the present invention comprise open pores. By "open pores" is meant that the individual pores of the HIPE scaffold are in substantially unobstructed communication with adjoining cells, i.e. they are interconnected (e.g. as in open-cell foam or sponge). The pores in such substantially open-celled HIPE scaffold structures have intercellular openings that are large enough to permit ready fluid transfer from one pore to another within the HIPE foam structure. For purpose of the present invention, a HIPE scaffold is considered open-pored if at least about 50%, 60%, 70%, 80%, 90% or 95% of the cells in the HIPE scaffold are in fluid communication with at least one adjoining cell. In addition to being open-celled, the external surface of the HIPE scaffolds comprises open pores, without mechanical post treatment, in other words. The HIPE does not have a surface skin), i.e., a surface having no pores or very few pores only. A HIPE is considered to have a surface skin if the surface porosity is less than 25 % of the bulk porosity. Accordingly, the HIPEs of the present invention have a surface porosity of at least 25 %, 30%, 35%, 40%, 45% or 50 % of the bulk porosity.

The HIPE scaffold internal pore sizes may range from 1 to 100 µm and in certain embodiments may be more than 10 µm. The HIPE scaffold surface pore sizes may range from 1 to 100 µm and in certain embodiments may be more than 10 µm. Preferably, the pore size for cell ingrowth is greater than 10 microns. In one embodiment the scaffold has a microporosity of 0.5 to 150 µm. In one embodiment, the scaffold has a macroporosity of 100 µm or more.

Scaffold formation

In one aspect the invention provides a method of forming a tissue culture scaffold comprising polymerizing a high internal pressure emulsion having an external phase a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, a solvent, and a surfactant and an internal phase comprising water, thereby crosslinking the acrylated elastomeric biodegradable polyester in the external phase.

In certain embodiments an internal phase and external phase are combined in a ratio between about 2:1 and 20:1, and in certain embodiments in a ratio between 2.8:1 and 19:1. This ratio can be used to determine the density of the resulting HIPE scaffold.

In one embodiment the external phase may contain one or more prepolymers that are polymerized to form a HIPE scaffold, a surfactant to help stabilize the HIPE and a solvent. In certain embodiments the external phase comprises one or more photoinitiators. The water phase will contain water and in certain embodiments one or more components such as electrolytes.

In one embodiment the prepolymer component may be present in an amount of from about 25% to about 100% by weight of the oil phase.

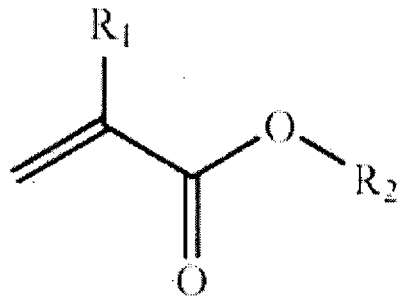
In one embodiment, the elastomeric, biodegradable polyester is a poly(hydroxyalkanoate), a poly(lactic acid), a poly(glycolic acid) or a poly(caprolactone). In one embodiment the prepolymer is poly(caprolactone)(PCL). In one embodiment the prepolymer is poly(glycerol sebacate) (PGS). In some embodiments the elastomeric biodegradable polyester is a copolymer or blend of any of the aforementioned polyesters.

In one embodiment the prepolymer may be a high molecular weight prepolymer, having a molecular weight from about 2500 Da to about 70 000 Da. In one embodiment the prepolymer may be a low molecular weight prepolymer, having a molecular weight from about 250 Da to about 4999 Da.

The crosslinked polyHIPE of the invention is formed from a prepolymer functionalised with a plurality of acrylate groups. Suitable acrylate groups include methacrylate. The crosslink density of the polyHIPE can be controlled by adjusting the degree of acrylation. A variety of techniques can be used to functionalize the pre-polymer with acrylate groups. In one embodiment a methacrylate functionalised prepolymer is provided by attaching methacrylate to a hydroxyl end group of the pre-polymer via reaction with methacrylic anhydride. In one embodiment an acrylate functionalised prepolymer is provided by attaching acrylate to a hydroxyl end group of the pre-polymer via reaction with acroyl chloride.

The degree of acrylation of the prepolymer influences the strength and degradation properties of the resulting polyHIPE. In one embodiment from about 20% to 90% of the hydroxyl groups in the prepolymer are acrylated, in another embodiment the prepolymer is from about 30% to about 80% acrylated, or from about 20% to about 40% acrylated.

In one embodiment the acrylate comprises the structure:



where R_1 is either a H or CH_3 and where R_2 is alkyl, aryl, heterocycles, cycloalkyl, aromatic heterocycles, multicycloalkyl, hydroxyl, ester, ether, halide, carboxylic acid, amino, alkylamino, dialkylamino, trialkylamino, amido, carbamoylthioether, thiol, alkoxy, or ureido groups, and branched and substituted versions thereof.

In one embodiment the solvent is an organic solvent. Suitable organic solvents for the prepolymer that can be used in the present invention include but are not limited to toluene, benzene, chloroform, dichloromethane, ethyl acetate, tethrahydrofuran, dimethylformamide, dichloroethane, carbon tetrachloride, diethyl ether, hexafluoroisopropanol or an aliphatic alcohol e.g. methanol, ethanol, isopropanol, or mixtures thereof. In certain embodiments of the present invention toluene is used. In other embodiments a mixture of toluene and chloroform is used. The polymer concentration in the organic solvent is from about 25 - 100% wt., and in certain embodiments 40-60% wt..

The solvent component may be present in the oil phase in an amount of from about 0 % to about 75 % by weight of the oil phase.

The emulsion may be formed at an emulsification temperature of from about 0° C to about 100° C and in certain embodiments from about 15° C to about 40° C.

Surfactants used include oil soluble polymeric stabilizers. Suitably, the stabiliser has a hydrophilic-lipophilic balance of 3-6, and is typically Span 80 or Hypermer B246. In one embodiment the emulsifier is PEG 30-dipolyhydroxystearic acid (Hypermer B246 ®).

Stabilizers used include particles to form Pickering HIPEs. The particles can be 1 nm up to 10 μm in size and can be ceramic or polymeric particles. In one embodiment the particles are made of a biodegradable polymer, such as polycaprolactone, polyglycerol sebacate, polylactide or polyglycolic acid or copolymers of these components.

The oil phase of the HIPE may comprise between about 1% and about 20%, in certain embodiments from about 2% to about 15%, and in certain other embodiments from about 3% to about 12% by weight stabilizer. In one embodiment the oil phase of the HIPE comprises about 10% by weight stabilizer.

The HIPE may be formed by combining the internal aqueous and external oil phases. Combining may involve mixing or agitation. In some embodiments combining involves subjecting these combined phases to shear agitation. The combined phases are subjected to shear agitation for a sufficient time to produce a HIPE having aqueous droplets of a desired size.

A HIPE scaffold is produced from the polymerization of the prepolymers of the external phase of a HIPE. Polymerization of the prepolymers of the HIPE involves the application of thermal energy or heat.

As used herein the term "polymerize" refers to both polymerization of prepolymers and formation of crosslinks between active sites on adjacent prepolymers. The polymerization step may take place before or after complete or partial removal of the solvent. Alternatively, an initial partial cross-linking step may be followed by solvent removal and a further cross-linking step.

In one embodiment, the combined internal aqueous and external oil phases are exposed to ultraviolet (UV) light, preferably in the presence of a photoinitiator. In one embodiment, the combined internal aqueous and external oil phases are exposed to UV light at a suitable intensity and for a suitable period of time sufficient to form a desired number of photocrosslinks. In one embodiment the UV intensity is from 0.1 W-100 W for time durations of 1 s to 30 mins.

The crosslinking may be achieved using chemical radical initiators. Preferably, however, the crosslinking may be achieved by photocrosslinking. Any suitable method for photocrosslinking can be used. As intended herein, photocrosslinking refers to the photoinduced formation of a covalent bond between two macromolecules or between two different parts of one macromolecule. In various embodiments, the photocrosslinking introduces a covalent bond between the double bond carbons of the unsaturated di-acid group, with a concomitant loss of the double bond in favour of the formation of an intermolecular carbon-carbon bond. Any suitable UV light source or lamp may be used and such systems are well known and readily available in the art. The time and intensity of the UV exposure may be adjusted as needed. In general, the number of photocrosslinks

will increase with any of: (a) increasing UV exposure time; (b) increasing the intensity of the UV light; or (c) a combination of increased time and light intensity.

In one embodiment, the prepolymer is crosslinked by free radical UV-light induced polymerization. In one such embodiments a photoinitiator is added to the solvent of the external phase. A photoinitiator is a compound especially added to a formulation to convert absorbed light energy, UV or visible light, into chemical energy in the form of initiating species, free radicals or cations. Based on the mechanism by which initiating radicals are formed, photoinitiators are generally divided into two classes: Type I photoinitiators undergo a unimolecular bond cleavage upon irradiation to yield free radicals; Type II photoinitiators undergo a bimolecular reaction where the excited state of the photoinitiator interacts with a co-initiator to generate free radicals. UV photoinitiators of both Type I and Type II are well known in the art and available commercially. Suitable photoinitiators (PI) include 2-hydroxy-1-[4(hydroxyethoxy)phenyl]-2-methyl-1 propanone (Irgacure 2959),), 2-hydroxy-2-methylpropiophenone, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide or mixtures thereof. Other photoinitiators are well known in the art.

In one embodiment the prepolymer is are moulded prior to crosslinking, e.g. moulded into a specific two-dimensional or three-dimensional shape that is suitable for the intended tissue engineering application. The HIPE prepolymers of the present invention be processed into a wide range of formats and geometries. The shape of the compositions and materials of the present inventions can be manipulated for specific tissue engineering applications. Exemplary macrostructures include particles, tubes, spheres, strands, coiled strands, films, sheets, fibers, meshes, and combinations thereof. In various embodiments, microfabrication can be used to form capillary networks from compositions and materials of the present inventions.

In a preferred embodiment, the prepolymers are moulded in a hydrophobic material, suitably hydrophobic materials having surface energies of 50 dynes/cm or less, for example waxes and hydrophobic polymers.

Following polymerization the resulting HIPE scaffold requires removal of the aqueous phase in order to obtain a substantially dry HIPE scaffold. Removal of the aqueous phase may be achieved by any suitable means, for example by exposure to compression, heat or vacuum, freeze drying or solvent exchange (to low boiling point organic solvents, e.g. methanol, acetone, ethanol, isopropanol), preferably followed by above mentioned drying methods.

In one embodiment the prepolymer is methacrylated polyglycerol sebacate (PGS). In one embodiment the methacrylated polyglycerol sebacate (PGS) is methacrylated by reacting the PGS prepolymer with methacrylic anhydride. In one embodiment the degree of methacrylation of the of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%. In one embodiment the PGS prepolymer is be a high molecular weight prepolymer, having a molecular weight from about 5000 Da to about 70000 Da. In one embodiment the PGA prepolymer may be a low molecular weight prepolymer, having a molecular weight from about 250 Da to about 4999 Da. In one embodiment the PGS prepolymer is a high molecular weight prepolymer having a molecular weight from about 5000 Da to about 70000 Da and a degree of acrylation, preferably methacrylation of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%. In one embodiment the PGS prepolymer is a low molecular weight prepolymer having a molecular weight from about 250 Da to about 4999 Da and a degree of acrylation, preferably methacrylation of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%.

In one embodiment the prepolymer is methacrylated polyglycerol sebacate (PGS) and the organic solvent is Toluene. In one embodiment the methacrylated polyglycerol sebacate (PGS) is methacrylated by reacting the PGS prepolymer with methacrylic anhydride and the organic solvent is Toluene. In one embodiment the degree of methacrylation of the of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the organic solvent is Toluene. In one embodiment the PGS prepolymer is be a high molecular weight prepolymer, having a molecular weight from about 5000 Da to about 70000 Da and the organic solvent is Toluene. In one embodiment the PGA prepolymer may be a low molecular weight prepolymer, having a molecular weight from about 250 Da to about 4999 Da, and the organic solvent is Toluene. In one embodiment the PGS prepolymer is a high molecular weight prepolymer having a molecular weight from about 5000 Da to about 70000 Da and a degree of acrylation, preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the organic solvent is Toluene. In one embodiment the PGS prepolymer is a low molecular weight prepolymer having a molecular weight from about 250 Da to about 4999 and a degree of acrylation, preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the organic solvent is Toluene.

In one embodiment the prepolymer is methacrylated polyglycerol sebacate (PGS), and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the methacrylated polyglycerol sebacate (PGS) is methacrylated by reacting the PGS prepolymer with methacrylic anhydride, and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the degree of methacrylation of the of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is be a high molecular weight prepolymer, having a molecular weight from about 5000 Da to about 70000 Da, and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGA prepolymer may be a low molecular weight prepolymer, having a molecular weight between from about 250 Da to about 4999 Da, and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is a high molecular weight prepolymer having a molecular weight from about 5000 Da to about 70000 Da and a degree of acrylation, preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is a low molecular weight prepolymer having a molecular weight from about 250 Da to about 4999 Da and a degree of acrylation, preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, and the stabilizer is PEG 30-dipolyhydroxystearic acid.

In one embodiment the prepolymer is methacrylated polyglycerol sebacate (PGS), the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the methacrylated polyglycerol sebacate (PGS) is methacrylated by reacting the PGS prepolymer with methacrylic anhydride, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the degree of methacrylation of the of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is be a high molecular weight prepolymer, having a molecular weight from about 5000 Da to about 70000 Da, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGA prepolymer may be a low molecular weight prepolymer, having a molecular weight from about 250 Da to about 4999 Da, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is a high molecular weight prepolymer having a molecular weight from about 5000 Da to about 70000 Da and a degree of acrylation,

preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid. In one embodiment the PGS prepolymer is a low molecular weight prepolymer having a molecular weight from about 250 Da to about 4999 Da and a degree of acrylation, preferably methacrylation, of the hydroxyl groups PGS prepolymers is from about 20% to 90%, from about 30% to about 80%, or from about 20% to about 40%, the organic solvent is Toluene and the stabilizer is PEG 30-dipolyhydroxystearic acid.

Tissue culture scaffolds

The scaffolds of the present inventions can find application in engineering a wide variety of tissues. The open pore structure of the surface of the scaffolds of the invention allow improved cell ingrowth, meaning that the scaffolds are particularly suitable for the production of biocompatible and biodegradable elastomeric foams for medical applications such as tissue engineering.

The scaffolds of the invention find particular utility in various methods, including, but not limited to i) the provision of implantable engineered tissues for use in regenerative medicine for treating damaged and/or diseased tissues and ii) the testing of the efficacy, safety and toxicity of experimental pharmacologic agents and screening and characterization of pharmacologic agents.

The scaffolds of the present inventions can also be seeded with a variety of other cells, for example, tenocytes, fibroblasts, ligament cells, endothelial cells, epithelial cells, muscle cells, nerve cells, kidney cells, bladder cells, intestinal cells, chondrocytes, bone-forming cells, stem cells such as human embryonic stem cells or mesenchymal stem cells, and others.

Cells may be integrated into the scaffolds of the invention using a variety of methods. In one embodiment, the scaffold may be submersed in an appropriate growth medium for the cells of interest, and then directly exposed to the cells. The cells are allowed to proliferate on the surface and interstices of the scaffold. The matrix is then removed from the growth medium, washed if necessary, and where appropriate implanted into a patient. In an alternative embodiment, cells of interest are dissolved into an appropriate solution (e.g., a growth medium or buffer) and then sprayed onto the scaffolds of the invention. These integration processes are only possible with a scaffold having an open-pore surface, having no skin.

In one embodiment, the scaffold is fabricated into a vascular graft, whereby the scaffold is a tubular member which acts as an artificial vessel.

A vascular graft can include a single material, a blend of materials, a weave, a laminate or a composite of two or more materials.

In one embodiment, the scaffolds are fabricated into a tube to facilitate nerve regeneration, whereby the tube serves to guides the migration of axons.

In one embodiment the scaffolds are fabricated into a network of tubes that mimic a blood vessel and capillary network that mimics tissue structures of liver. For example, scaffolds are fabricated into networks imitating the arrangements of extracellular matrix in liver tissue and seeded with hepatocytes.

In one embodiment, the scaffolds of the present inventions are fabricated into a fibrous network, seeded with islet cells, and used to tissue engineer pancreas.

In various embodiments, growth factors can be incorporated into scaffolds of the present inventions to recruit cells to and/or promote specific metabolic and/or proliferative behaviour in cells that are at the site and/or seeded within the matrix. Exemplary growth factors include, without limitation, TGF-β, acidic fibroblast growth factor, basic fibroblast growth factor, epidermal growth factor, IGF-I and II, vascular endothelial-derived growth factor, bone morphogenetic proteins, platelet-derived growth factor, heparin-binding growth factor, hematopoetic growth factor, and peptide growth factor. In various embodiments, integrins and cell adhesion sequences (e.g., the RGD sequence) can be attached to the scaffolds of the present inventions to facilitate cell adhesion. In various embodiments, extracellular matrix components, e.g., collagen, fibronectin, laminin, elastin, etc., can be combined with scaffolds of the present inventions to manipulate cell recruitment, migration, and metabolism and the degradation and mechanical properties of the material. In various embodiments, proteoglycans and glycosaminoglycans can be covalently or non-covalently attached to scaffolds of the present inventions.

EXAMPLES

METHODS

PGS/PCL/PGCL PolyHIPE

The pre-polymer PGCL (poly-Glycolic Caprolacatone), PCL (poly-Caprolactone) or PGS (poly-Glycerolsebacate) is dissolved in a solvent in the ratio of 1:0.5, 1:1 or 1:1.5 between the pre-polymer and solvent. Different ratios are used depending on the viscosity of the pre-polymer used. The solvent is either toluene or a blend of toluene and chloroform that is mixed in the ratio of toluene to chloroform as being either 100%s Toluene or 100% chloroform and the blends between including (90:10, 80:20, 70:30, 60:40, 50:50, 40:60). Dissolving the pre-polymer in this solvent (or solvent blend) reduces its viscosity so that it is runny enough for it to be created into an emulsion.

The surfactant Hypermer B246 is added relative to the weight of the pre-polymer (for every 1 g of pre-polymer 0.1 g of surfactant is added, so the total weight of the pre-polymer / surfactant is 1.1 g, so surfactant weight relative to this is around 9 wt% of the total weight) The surfactant is added to the pre-polymer /solvent solution from 2 wt% to 20 wt% relative to the weight of the pre-polymer. Increasing or decreasing the surfactant amount directly affects the size and interconnectivity of the resulting PolyHIPE.

A photoinitiator is added to the pre-polymer /solvent/surfactant solution in a weight of 2 to 25wt% relative to the weight of the pre-polymer in the solution.

The solution of pre-polymer /surfactant/solvent/photo initiator is put into a container and mixed using a stirrer normally around 350 rpm. The speed of the mixing can be controlled decreased or increased to control the water droplet size in the PolyHIPE. Either as low as 50 rpm or as high as 3000 rpm.

Water is added dropwise during this mixing. The ratio of water to pre-polymer solution is at least 75% relative to the total amount of the pre-polymer /solvent/surfactant/photoinitiator (with the water its normally the water volume ratio, so its 75% of water is added relative to the volume of the pre-polymer /solvent/surfactant/photoinitiator solution). The emulsion is mixed continuously as the water is added dropwise.
75% water volume ratio is the minimum requirement for the emulsion to be classified as a High internal phase emulsion, the water volume ratio can be increased to 80%, 85% 90% or 95%. Increasing the water increases the interconnectivity of the PGS PolyHIPE.

The emulsion is exposed to UV light, to crosslink the HIPE into a PolyHIPE. The emulsion can be poured into a mould, cured as a sheet, or selectively polymerised by UV light by stereolithography.

RESULTS

Extending the polycondensation reaction time for PGS prepolymer synthesis increases the molecular weight, measured as Number average (Mn) or Weight average (Mw).

Molecular weights (Mw) have been reported up to ~70,000 Da.

In our work we used two different molecular weights of PGS prepolymer, based on the polycondensation reaction times used to synthesise them:

48 hrs reaction time = Low M_w PGS

72 hrs reaction time = High M_w PGS

(Figure 2)

PGS prepolymer is methacrylated by reacting with methacrylic anhydride.

The secondary hydroxyl group of the glycerol subunit in the PGS prepolymer backbone is targeted for methacrylation. This is favoured as the primary hydroxyl groups of the glycerol subunit are more reactive than the secondary and thus favour polymer chain extension during prepolymer synthesis. (Figure 3).

PGS-M prepolymer is photocured into an insoluble polymer matrix by mixing with a free-radical generating photoinitiator and exposing to an appropriate wavelength of light. Our research has used a photoinitiator that reacts with UV light (Figure 4).

Proton NMR shows the incorporation of the methacrylate groups into the PGS prepolymers.

Peaks f, g and h are associated with the methacrylate group and are not present in the PGS prepolymer alone.

30%, 50% and 80% refers to the degree of methacrylation (proportion of hydroxyl groups in the prepolymer that have been methacrylated). (Figure 5)

NMR data shows that the degree of methacrylation is controllable and directly proportional to the reactant quantities used.

Adding an amount of methacrylic anhydride sufficient to methacrylate 30% of the PGS prepolymer results in a prepolymer that is ~30% methacrylated (R^2 = 0.993).

NMR data shows that the degree of methacrylation is controllable and directly proportional to the reactant quantities used.

Adding an amount of methacrylic anhydride sufficient to methacrylate 30% of the PGS prepolymer results in a prepolymer that is ~30% methacrylated (R^2 = 0.993). (figure 6)

IR spectra shows the incorporation of the methacrylate groups into the PGS prepolymers.

C=C peaks from the methacrylate group and are not present in the PGS prepolymer alone. (Figure 7)

PGS-M is degraded by enzymes (Cholesterol esterase and Lipase) over 8 days. Degree of methacrylation has a significant effect on degradation. (Figure 8)

Degradation of 30% degree of methacrylation PGS-M by Cholesterol esterase is visible as pits and craters on polymer surface.

Little evidence of degradation in other PGS-M variants or with other treatments (Figure 9).

Degree of methacrylation has a significant effect on the mechanical properties of PGS-M.

Range of mechanical properties varies from kPa to MPa for 30% to 80% degree of methacrylation. (Figure 10)

2D culture on 30% Low M_w PGS-M surfaces.

Support continued metabolism in various human primary cell types: Dermal fibroblasts, Adipose-derived stem cells (ADSCs) and Vascular smooth muscle cells (SMCs). (Figure 11)

2D culture on 30% Low M_w PGS-M surfaces.

Support cell proliferation in various human primary cell types: Dermal fibroblasts, Adipose-derived stem cells (ADSCs) and Vascular smooth muscle cells (SMCs). (Figure 12)

Human Dermal fibroblasts and ADSCs visibly proliferate on 2D PGS-M surfaces. Human Dermal fibroblasts and ADSCs visibly proliferate on 2D PGS-M surfaces (Figure 13)

PGS-M prepolymer is mixed with toluene (solvent), water and a surfactant to form an emulsion. This is a "water in oil" emulsion with a solvated PGS-M prepolymer continuous/external phase and a water dispersed/internal phase. The water phase must account for >74% of the emulsion volume to be considered a polyHIPE.

A free radical generating photoinitiator is also added to the emulsion to initiate the polymerisation reaction on exposure to an appropriate wavelength of light (UV in our case).

Photopolymerisation results in the PGS-M prepolymer continuous/external phase forming an insoluble matrix. The water dispersed/internal phase, along with any remaining surfactant, photoinitiator and toluene can then be washed out leaving a porous polyHIPE structure.

Variation in the emulsion processing can affect the properties of the produced polyHIPE. Shear used during the emulsion mixing step affects the size of the water droplets contained within the emulsion and the size of the pores in the resulting polyHIPE. (Figure 14)

Scanning electron microscopy of PGS-M polyHIPE. 2 mm thick PGS-M polyHIPE disks in a standard 24-well tissue culture plate (Figure 15)

The PGS-M emulsion can be shaped in moulds and then photocured to produce various polyHIPE scaffold geometries. Disks and tubular shapes; including straight, bending, and branched; have all been produced.

The surface properties of the mould affect the porosity of the polyHIPE surface that is photocured against them. A hydrophobic mould material, such as silicone, is required to produce an open surface porosity. This is advantageous for tissue engineering applications to allow cell and nutrient movement into the polyHIPE PGS-M scaffolds. (Figure 16)

HIPE PGS-M scaffolds seeded with vascular smooth muscle cells (SMCs) to produce tissue engineered blood vessels (Figure 17)

Tissue engineered blood vessels based on PGS-M polyHIPE scaffolds cultured in a pulsatile flow bioreactor (Figure 18)

HIPE PGS-M scaffolds seeded with SMCs – 7 day bioreactor culture (H&E stained) Comparing polyHIPE PGS-M scaffolds with porous PGS-M scaffolds produced by an alternative common method in tissue engineering (porogen leaching) shows polyHIPE scaffolds allow superior cell invasion.

This is also true when compared to published data on a PGS (not PGS-M) polymer scaffold produced by porogen leaching. (Figure 19)

PCL Mn 900 g/mol is purchased from sigma Aldrich. This PCL is then methacrylated to make it photocurable.

The 4 arm PCL is made in house by a ring opening polymerisation reaction using pentaerythritol and carprolactone. tin 2-ethylhexanoate is used as a catalyst for this reaction. (Figure 20)

Non methacrylated 4 arm PCL and methacrylated 4 arm PCL (Figure 21).

Tensil75,50 and 25% PCL blends with glycolide testing of Pure solid polymer

Tensile test of 75,50 and 25% PCL polyHIPE blends.

The 25%PCL ones were brittle. Repeat also needed with the Pure PCL polyHIPE. 75%PCL tensile test of polymer, the Youngs modulus is around 230 MPaa and polymer version around 6MPa 50% PCL polymer is around 470 MPa and polyHIPE version around 12MPa.(Figure 22)

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or

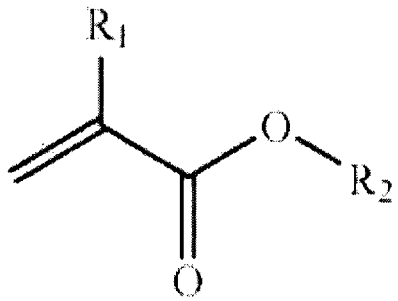
process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

CLAIMS

1. A tissue culture scaffold comprising a crosslinked polyHIPE formed a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, wherein the scaffold has an outer surface comprising a plurality of open pores.
2. The tissue culture scaffold according to claim 1, wherein said elastomeric biodegradable polyester is selected from the group consisting of Poly(glycerol sebacate) (PGS), Poly(caprolactone)(PCL), copolymers and polymer blends of PGS and PCL, or copolymers and polymer blends of PGS and PCL with poly(Lactic acid) (PLA) or Poly(Glycolic acid) (PGA).
3. The tissue culture scaffold according to claim 1 or claim 2, wherein the scaffold has a microporosity of 1 to 50 μm.
4. The tissue culture scaffold according to any one of the preceding claims, wherein the scaffold has a macroporosity of 100μm or greater.
5. The tissue culture scaffold according to any one of the preceding claims, wherein the scaffold comprises a plurality of cells.
6. The tissue culture scaffold according to any one of the preceding claims, wherein the macrostructure of the scaffold is in the form of a particle, tube, sphere, strand, coiled strand, capillary network, film, fibre, mesh, sheet or combination thereof.
7. The tissue culture scaffold according to claim 6, wherein the tube is straight, bent or branched.
8. The tissue culture scaffold according to any one of the preceding claims, wherein the acrylate groups are methacrylate groups.
9. A vascular graft comprising a tissue culture scaffold according to any one of the preceding claims, wherein the scaffold is a tube and comprises a plurality of smooth muscle cells.
10. A method of forming a tissue culture scaffold comprising polymerizing a high internal phase emulsion having an external phase comprising a prepolymer comprising a plurality of acrylate groups attached to an elastomeric biodegradable polyester, a solvent, and a surfactant and an internal phase comprising water, thereby crosslinking the acrylated elastomeric biodegradable polyester in the external phase.
11. The method of claim 10, wherein said elastomeric biodegradable polyester is selected from the group consisting of Poly(glycerol sebacate) (PGS), Poly(caprolactone)(PCL), copolymers and polymer blends of PGS and PCL, or copolymers and polymer blends of PGS and PCL with poly(Lactic acid) (PLA) or Poly(Glycolic acid) (PGA).
12. The method of claims 10 or 11, wherein said polymerizing comprises photopolymerization.
13. The method of any one of claims 10 to 12, wherein the method comprises acrylating the elastomeric biodegradable polyester.

14. The method of claim 13, wherein the acrylate comprises the structure:



where R_1 is either a H or CH_3 and where R_2 is alkyl, aryl, heterocycles, cycloalkyl, aromatic heterocycles, multicycloalkyl, hydroxyl, ester, ether, halide, carboxylic acid, amino, alkylamino, dialkylamino, trialkylamino, amido, carbamoylthioether, thiol, alkoxy, or ureido groups, and branched and substituted versions thereof.

15. The method according to any one of claims 10 to 14, wherein the solvent is an organic hydrophobic solvent.
16. The method according to claim 15, wherein the organic hydrophobic solvent is toluene, benzene, chloroform, dichloromethane, ethyl acetate, tetrahydrofuran, dimethylformamide, dichloroethane, carbon tetrachloride, diethyl ether, hexafluoroisopropanol or an aliphatic alcohol e.g. methanol, ethanol or isopropanol.
17. The method according to any one of claims 9 to 13, wherein the surfactant is a polymeric stabiliser.
18. The method according to claim 17, wherein the stabiliser has a hydrophilic-lipophilic balance of 3-6, optionally wherein the stabiliser is Span 80 or Hypermer B246.
19. The method according to any to any one of claims 10 to 18 wherein said polymerizing is photopolymerizing and comprises UV excitation of said high internal phase emulsion in the presence of a photoinitiator.
20. The method according to any to any one of claims 10 to 18 wherein said polymerizing is heat induced polymerizing and comprises thermo excitation of said high internal phase emulsion in the presence of a thermal initiator.
21. The method according to any to any one of claims 10 to 18 wherein said polymerizing is sound induced polymerizing and comprises ultrasound excitation of said high internal phase emulsion in the presence of a redox based initiator.
22. The method according to any one of claims 19 to 21, wherein the photoinitiator, thermal initiator or redox-based initiator is a free radical generating photoinitiator.

23. The method according to any one of claim 10 to 22, wherein the high internal phase emulsion is moulded prior to polymerization.
24. The method according to claim 23, wherein said moulding comprises contacting said high internal phase emulsion with a hydrophobic surface.
25. The method according to any one of claims 10 to 24, wherein said a high internal phase emulsion is formed by mixing.
26. The method according to claim 25, wherein said mixing occurs at a rotation speeds of 50-5000 rpm and temperature in between 0-100°C.
27. The method according to any one of claims 10 to 26, further comprising washing the crosslinked acrylated elastomeric biodegradable polyester.
28. The method according to claim 27, further comprising seeding the washed crosslinked acrylated elastomeric biodegradable polyester with at least one cell.

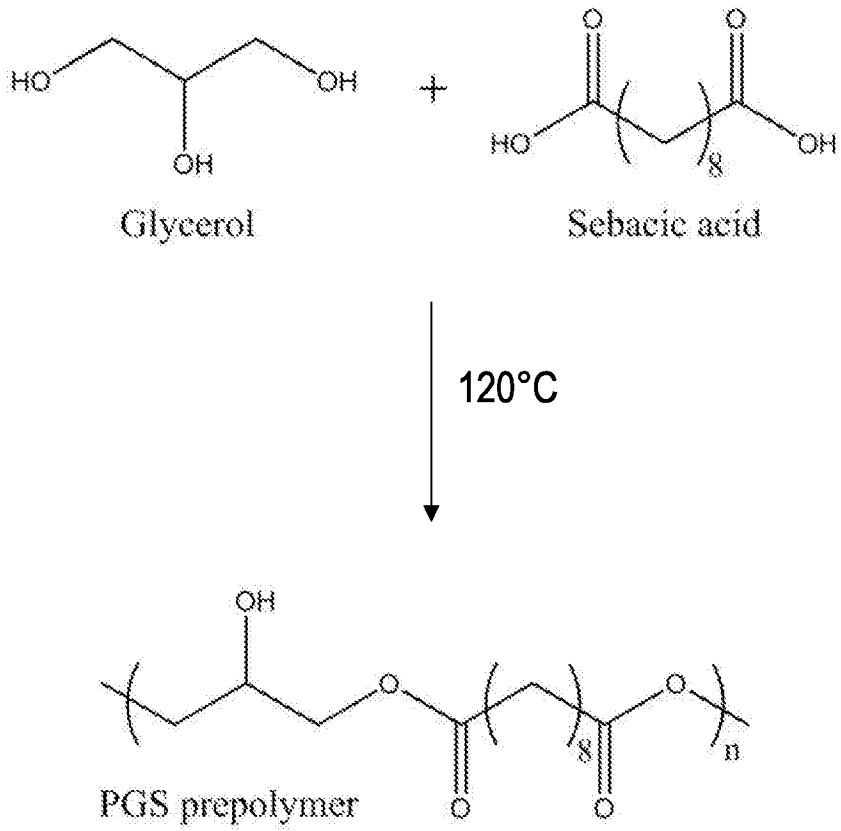

HO
OH
OH
+
HO
OH
8
Glycerol
Sebacic acid
120°C
OH
O
O
O
8
n
PGS prepolymer

Fig. 1

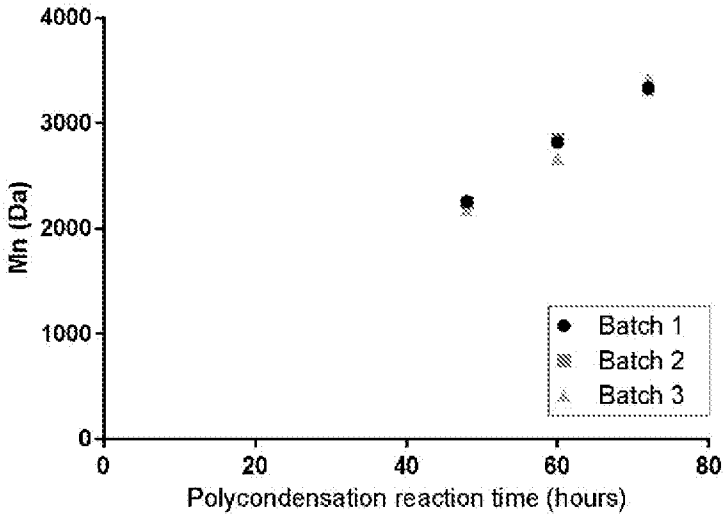

4000
3000
2000
1000
0
Mn (Da)
0
20
40
60
80
Polycondensation reaction time (hours)
Batch 1
Batch 2
Batch 3

Fig. 2A

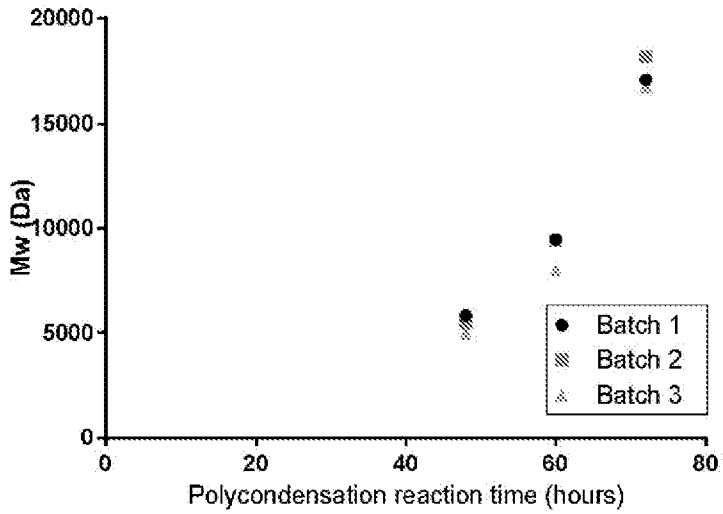

20000
15000
10000
5000
0
Mw (Da)
0
20
40
60
80
Polycondensation reaction time (hours)
Batch 1
Batch 2
Batch 3

Fig. 2B

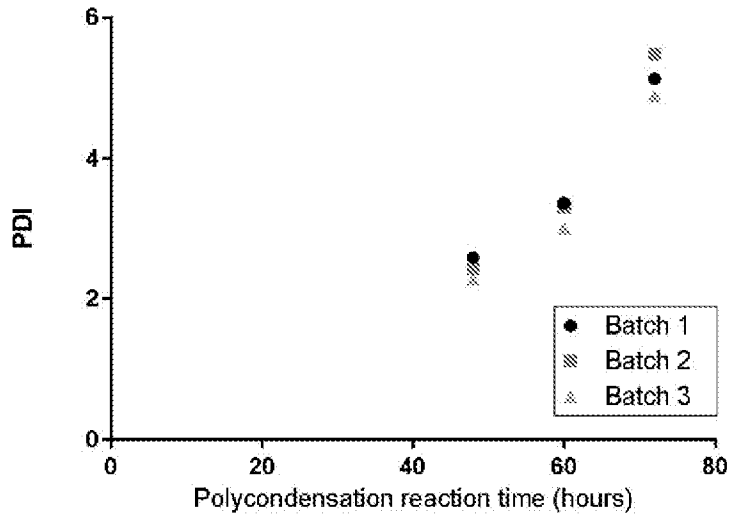

PDI
6
4
2
0
0
20
40
60
80
Polycondensation reaction time (hours)
Batch 1
Batch 2
Batch 3

Fig. 2C (cont.)

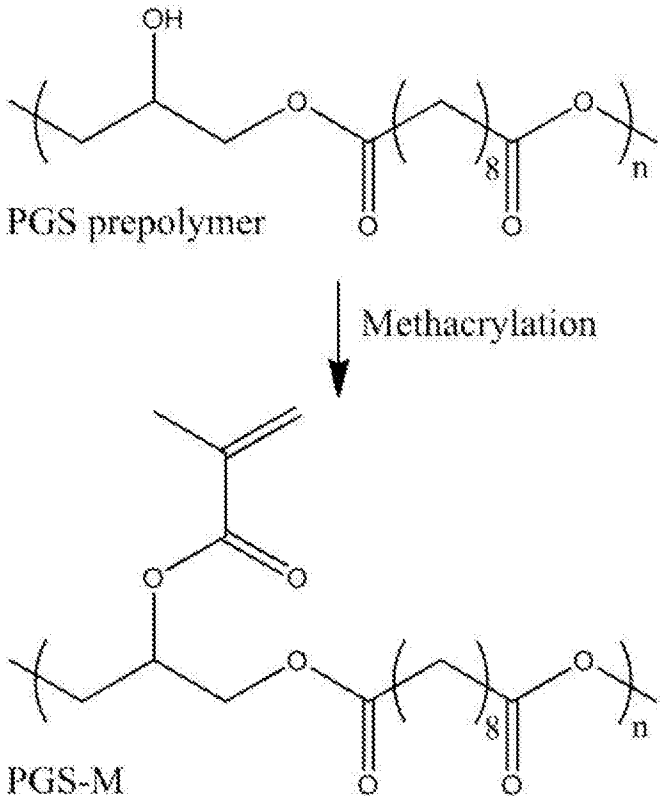


Fig. 3

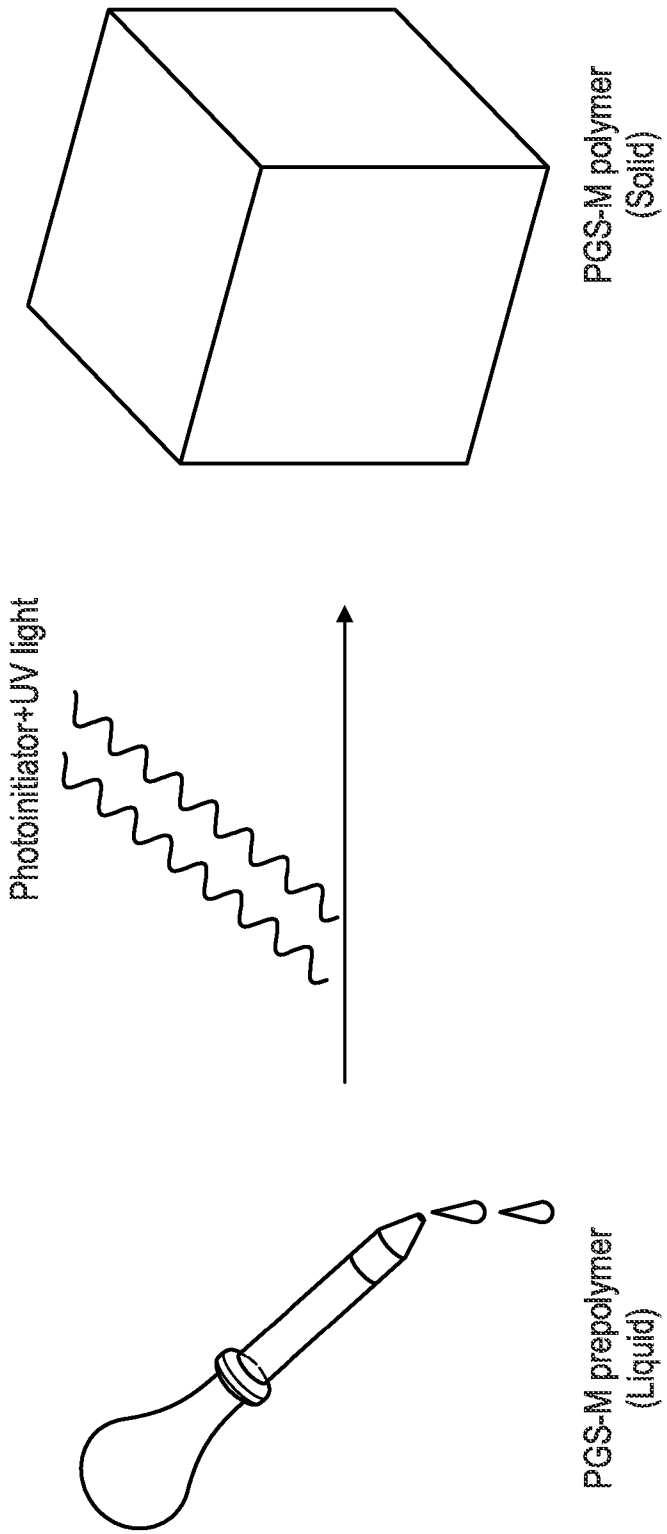

PGS-M polymer
(Solid)
Photoinitiator+UV light
PGS-M prepolymer
(Liquid)

Fig. 4

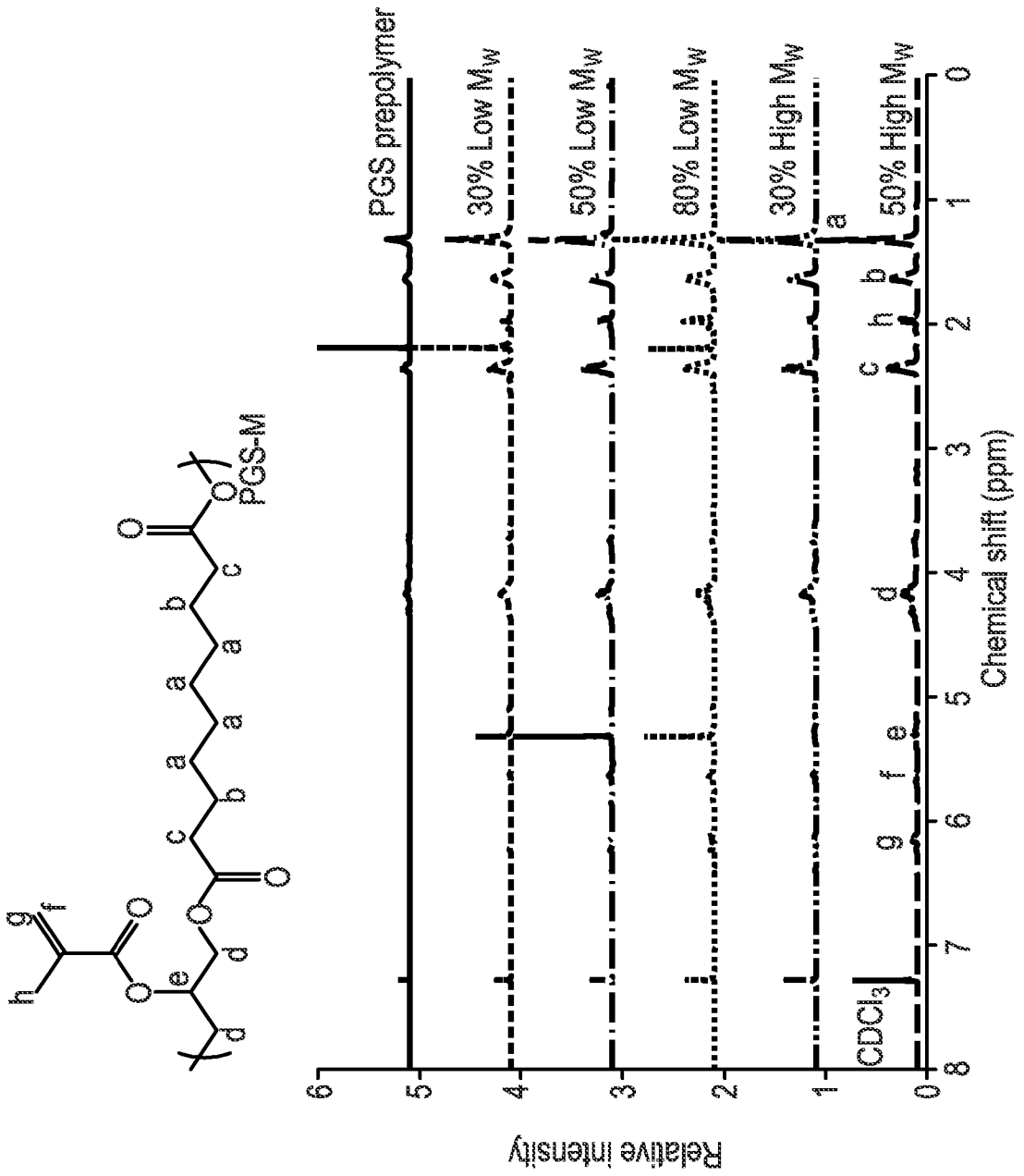

PGS-M
PGS prepolymer
30% Low M_W
50% Low M_W
80% Low M_W
30% High M_W
50% High M_W
$CDCl_3$
Relative intensity
Chemical shift (ppm)

Fig. 5

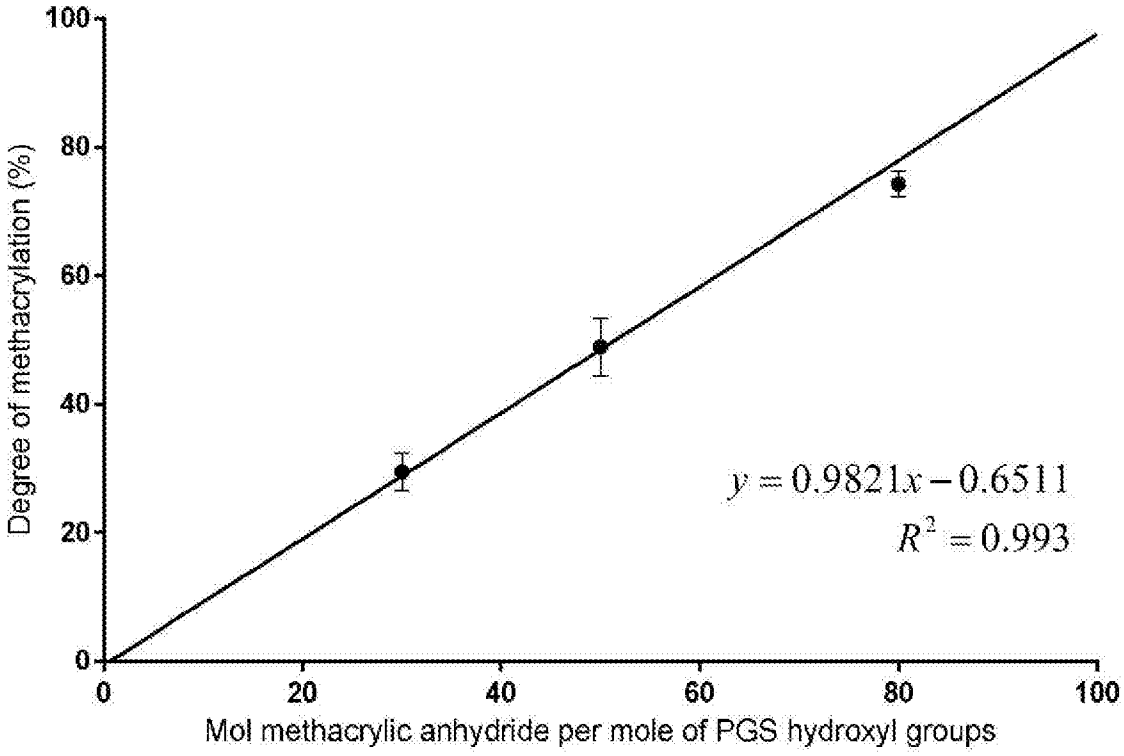

Degree of methacrylation (%)
100
80
60
40
20
0
0
20
40
60
80
100
Mol methacrylic anhydride per mole of PGS hydroxyl groups
$y = 0.9821x - 0.6511$
$R^2 = 0.993$

Fig. 6

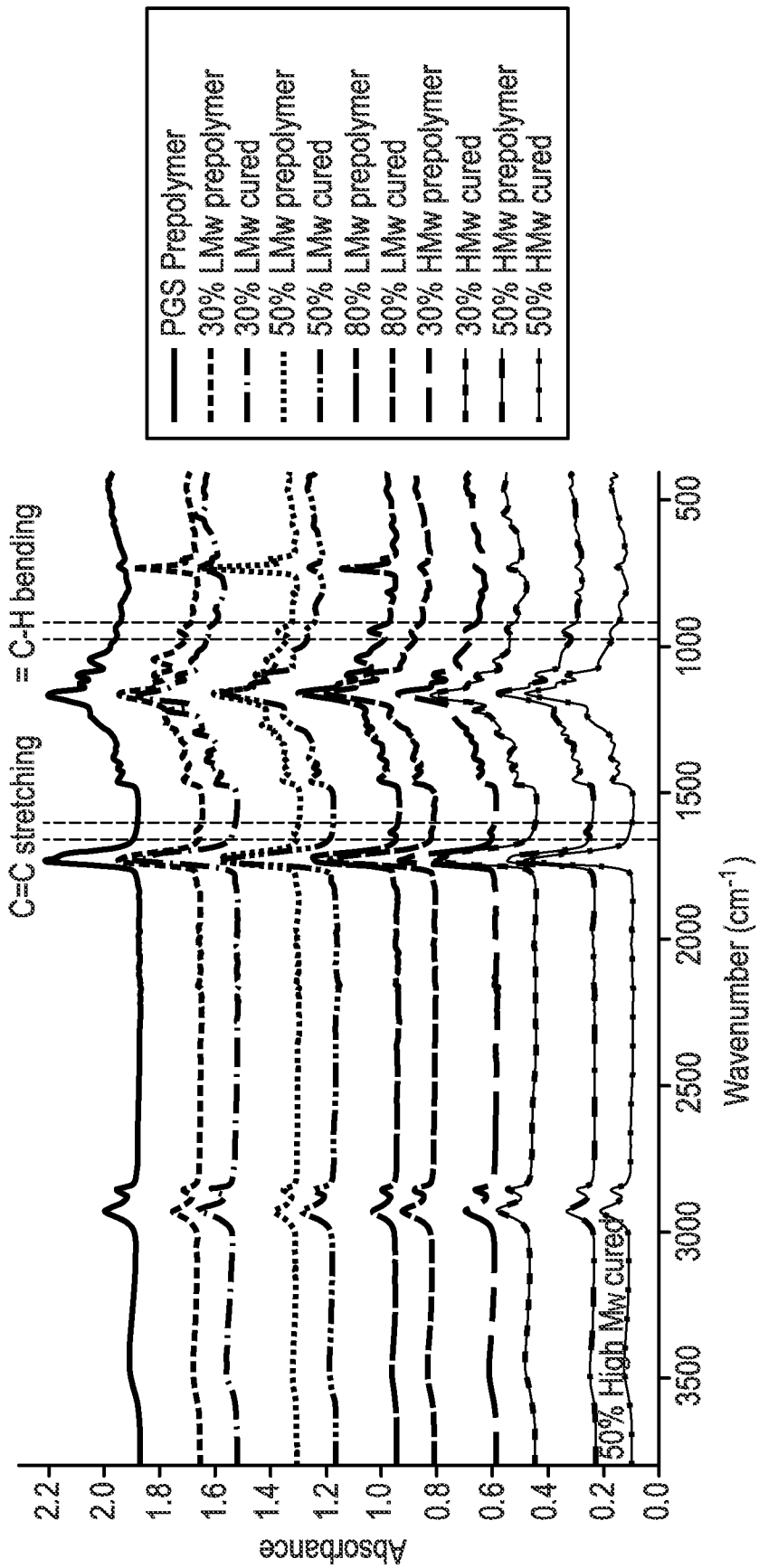

PGS Prepolymer
30% LMw prepolymer
30% LMw cured
50% LMw prepolymer
50% LMw cured
80% LMw prepolymer
80% LMw cured
30% HMw prepolymer
30% HMw cured
50% HMw prepolymer
50% HMw cured
C=C stretching
= C-H bending
50% High Mw cured
Absorbance
Wavenumber (cm-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
3500
3000
2500
2000
1500
1000
500

Fig. 7

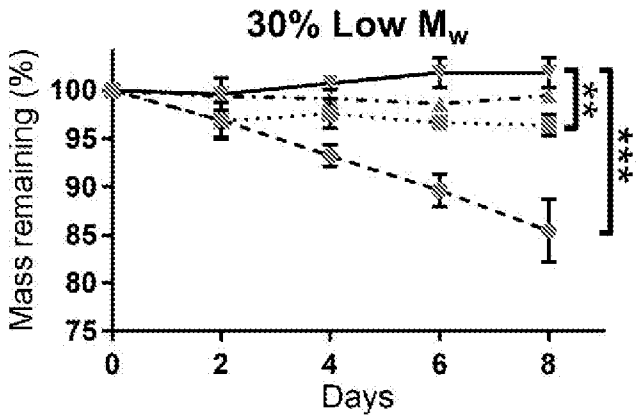


Fig. 8A

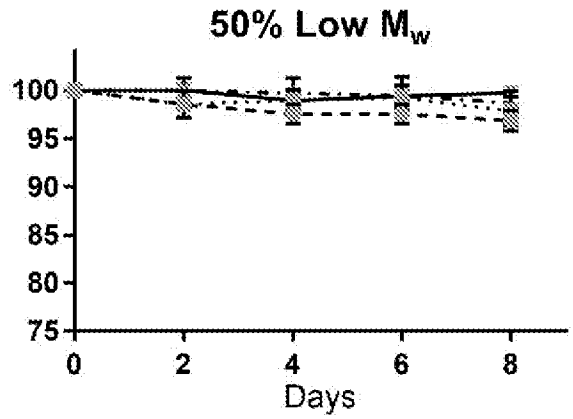


Fig. 8B

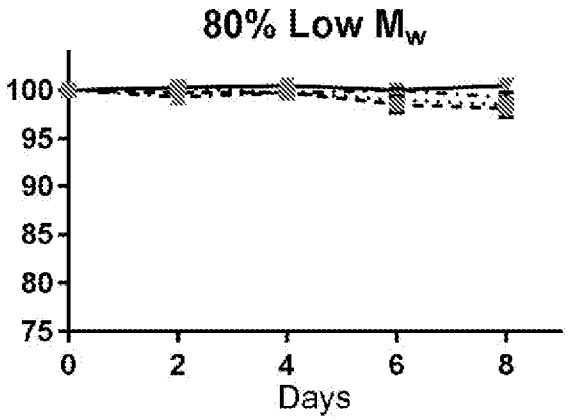
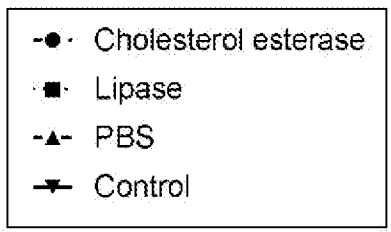


Fig. 8C



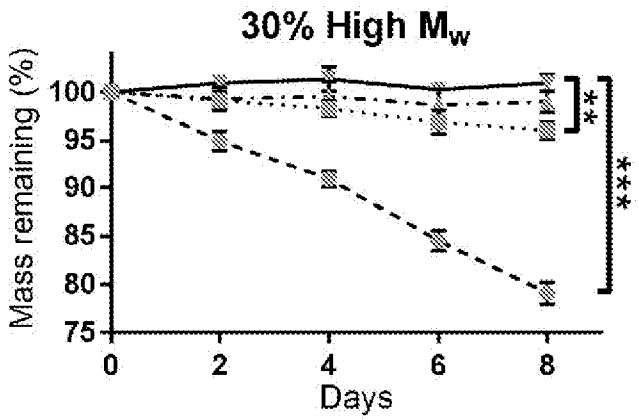


Fig. 8D

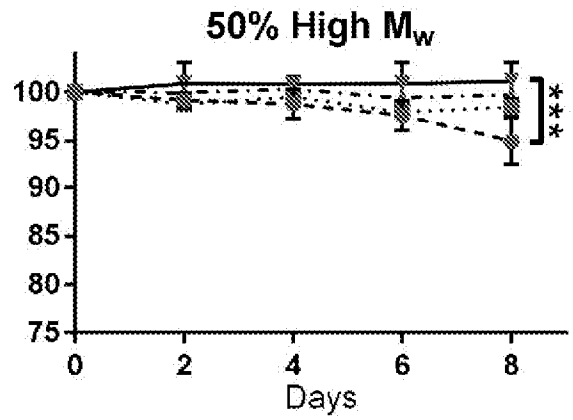


Fig. 8E

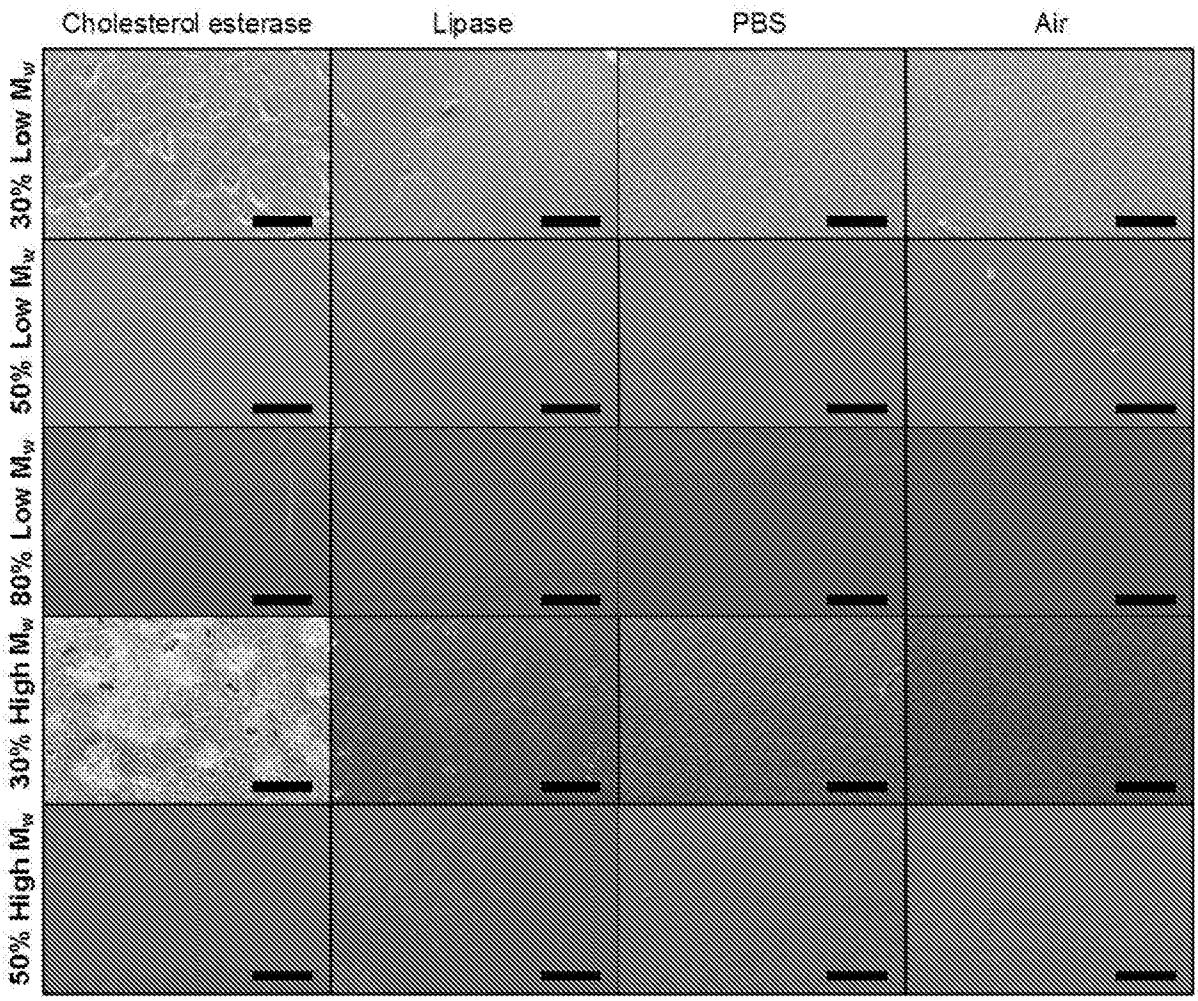

Cholesterol esterase
Lipase
PBS
Air
30% Low M_w
50% Low M_w
80% Low M_w
30% High M_w
50% High M_w

Fig. 9

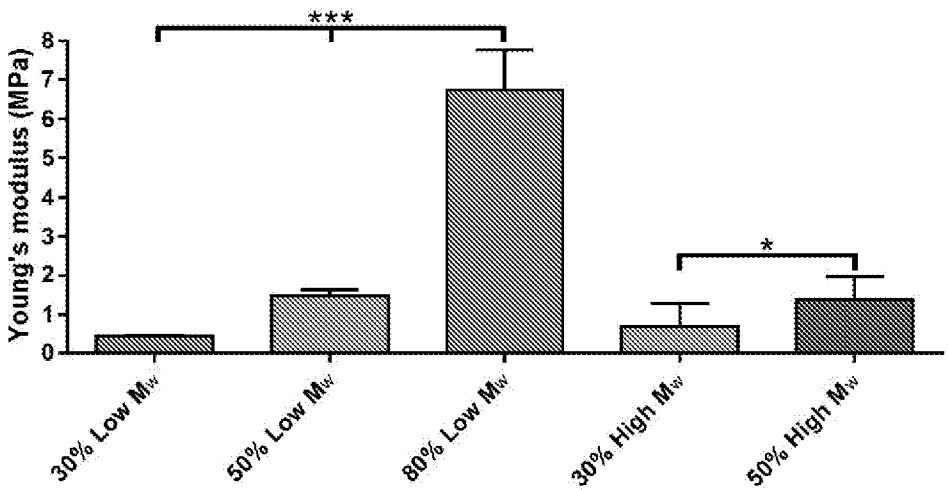


Fig. 10A

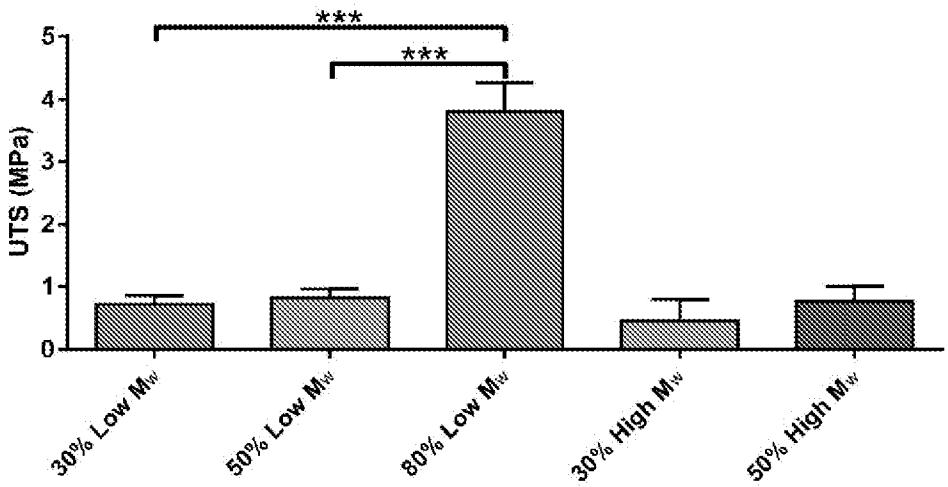


Fig. 10B

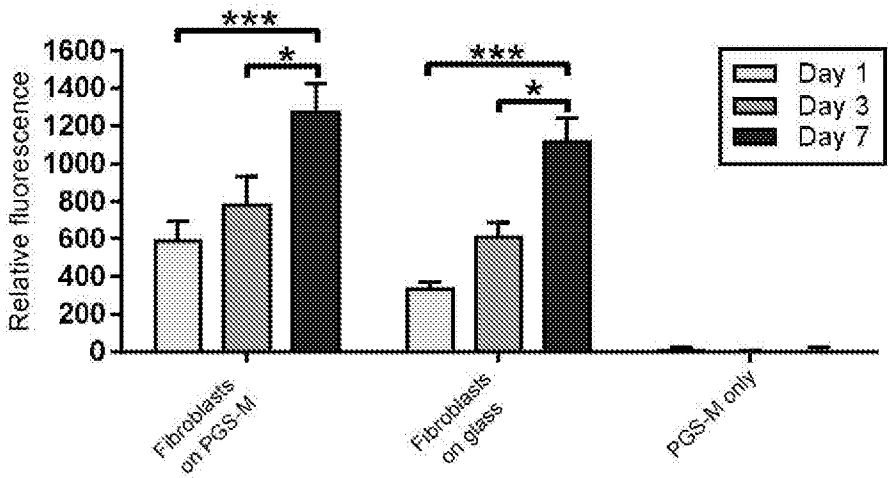


Fig. 11A

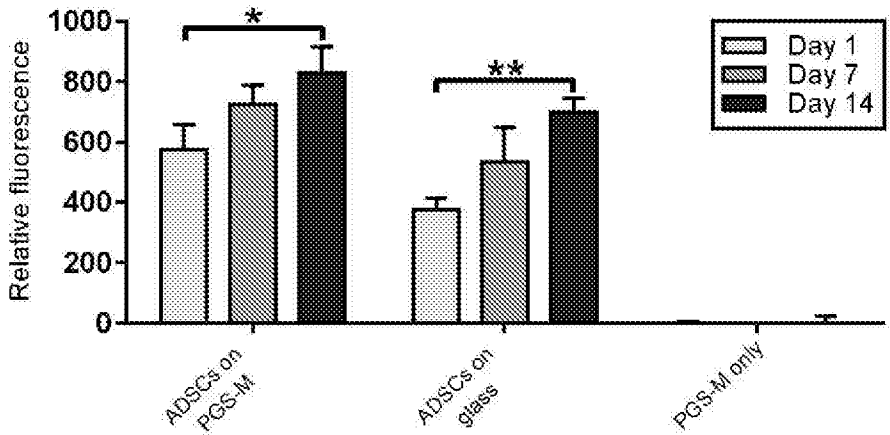


Fig. 11B

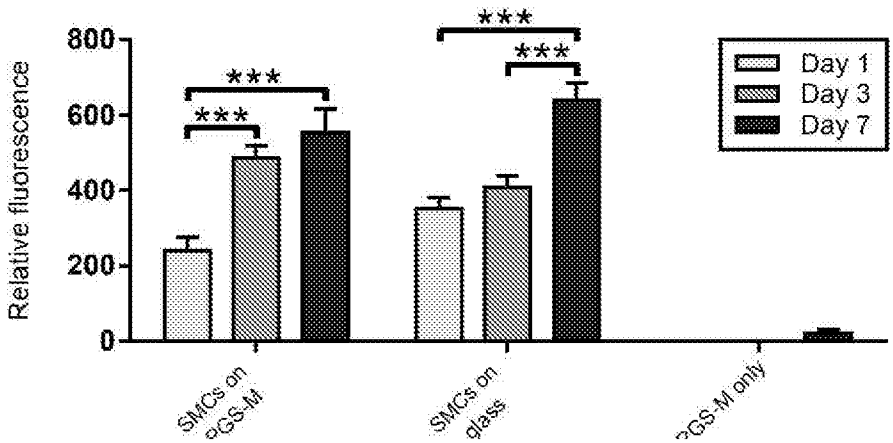


Fig. 11C

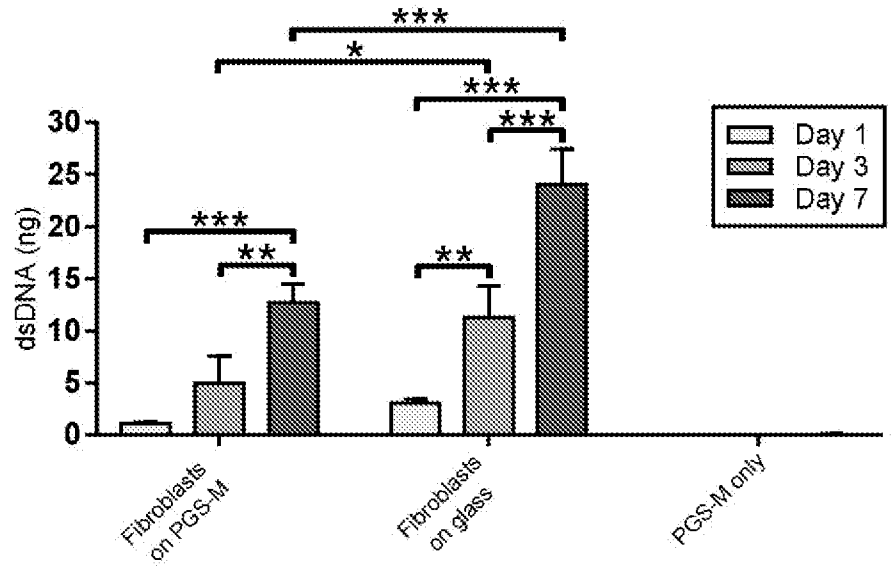


Fig. 12A

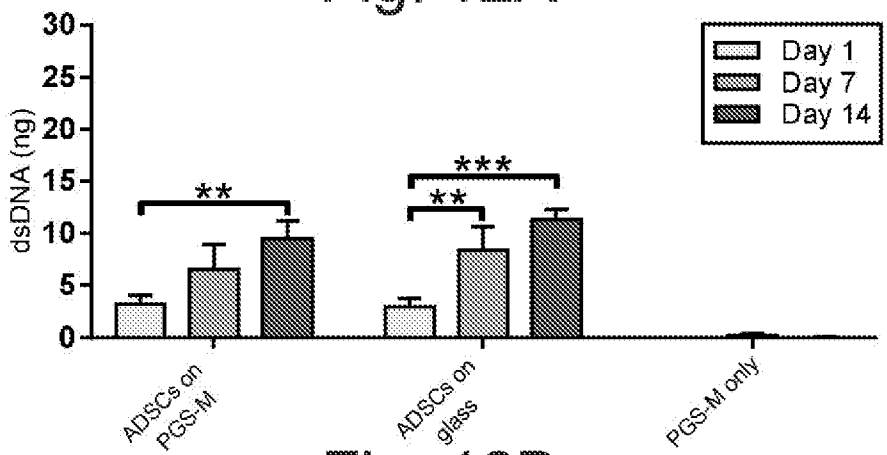


Fig. 12B

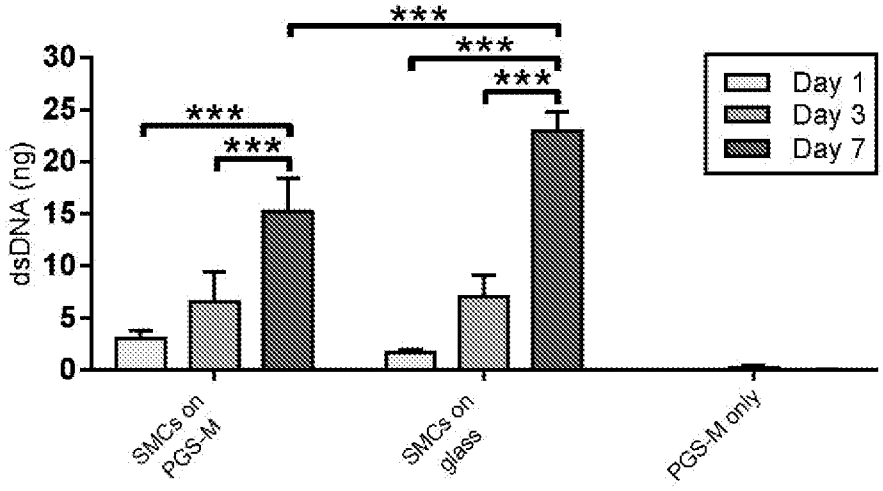


Fig. 12C

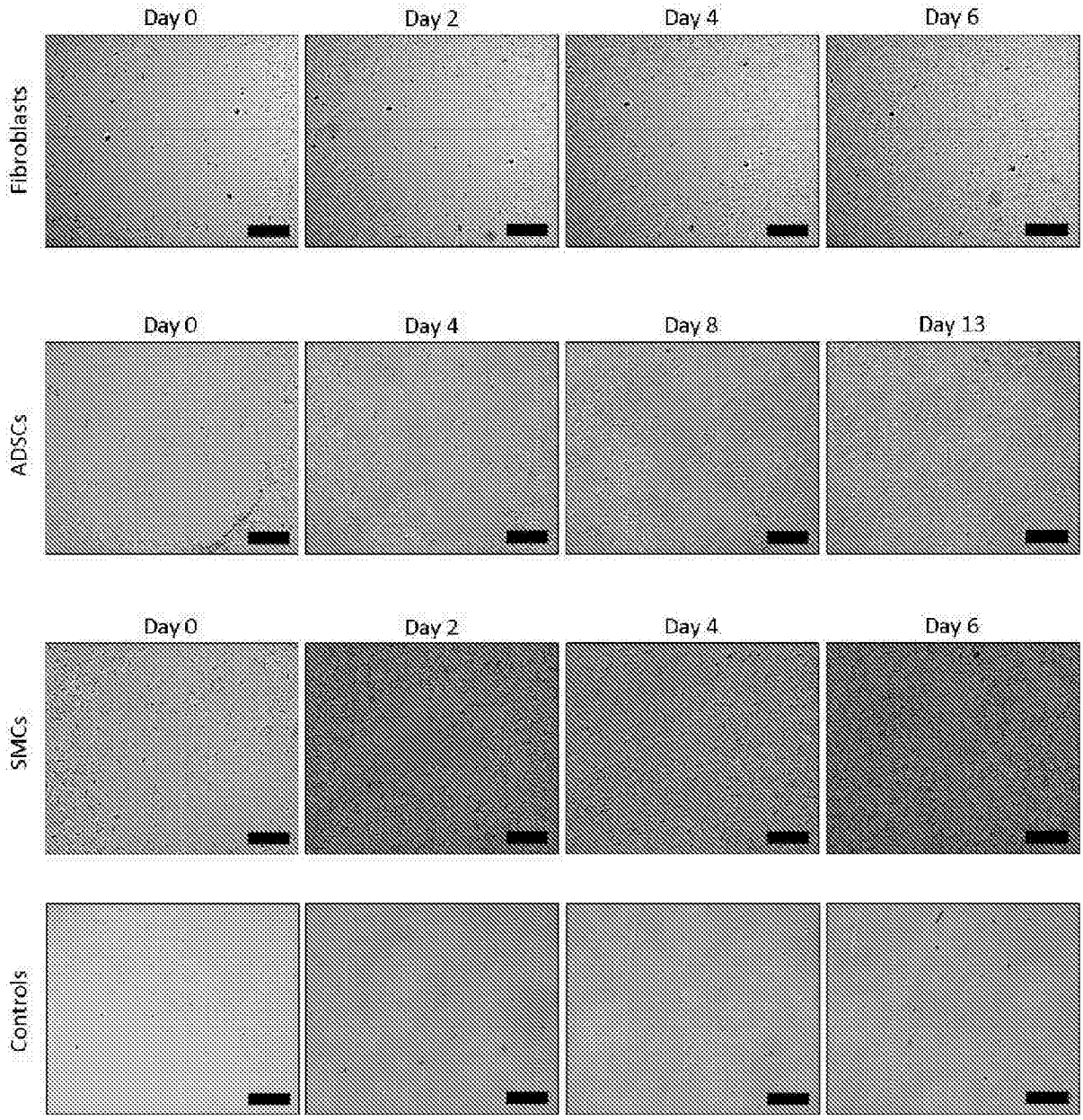

Day 0
Day 2
Day 4
Day 6
Fibroblasts
Day 0
Day 4
Day 8
Day 13
ADSCs
Day 0
Day 2
Day 4
Day 6
SMCs
Controls
Bar = 500 µm

Fig. 13

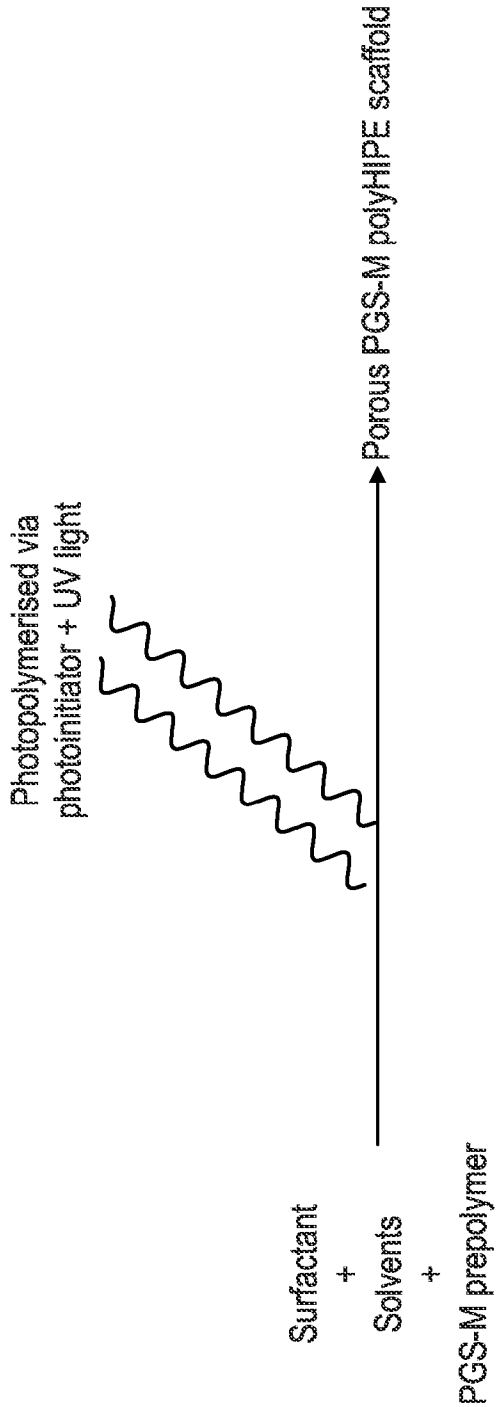

Porous PGS-M polyHIPE scaffold
Photopolymerised via photoinitiator + UV light
Surfactant
+
Solvents
+
PGS-M prepolymer

Fig. 14

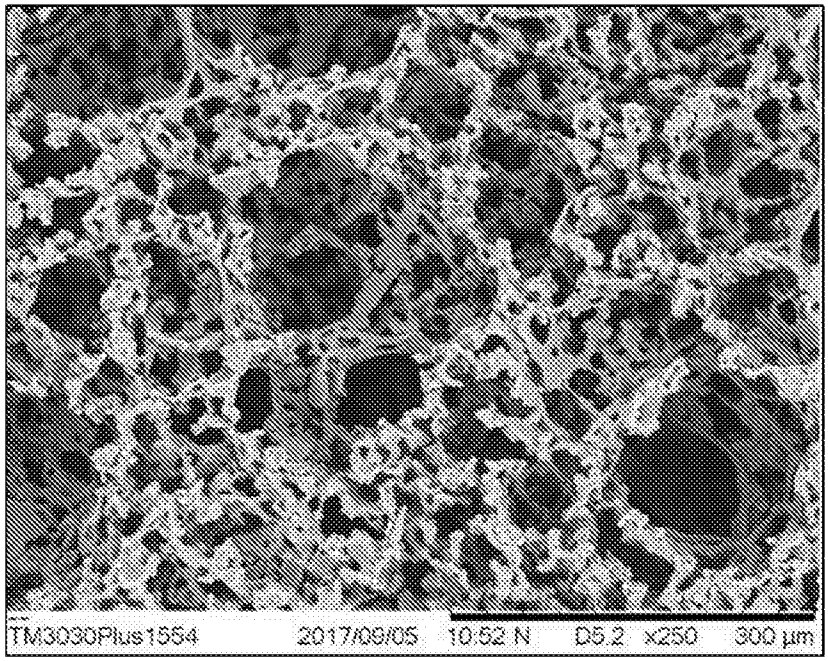


Fig. 15A

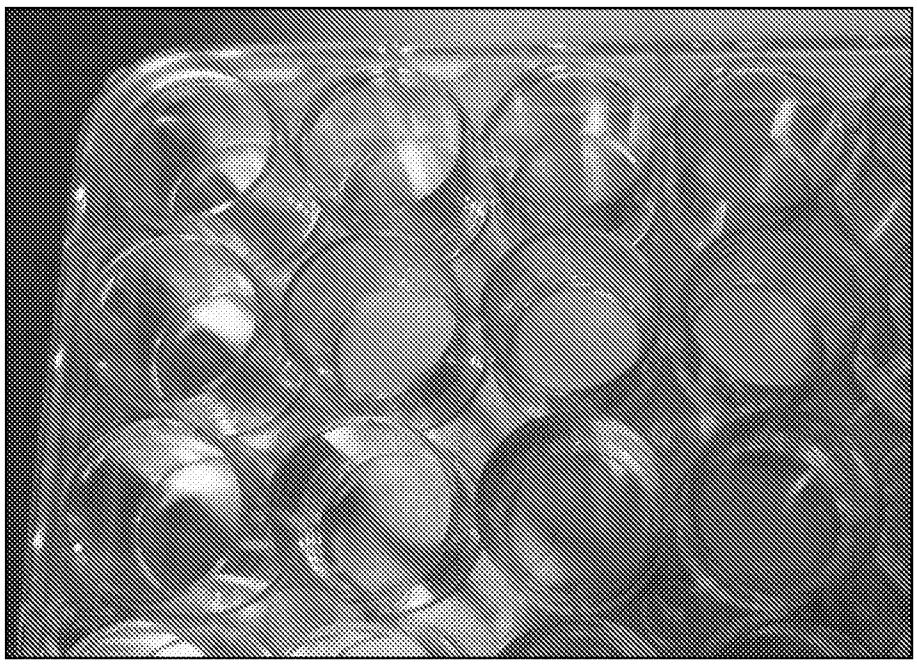


Fig. 15B

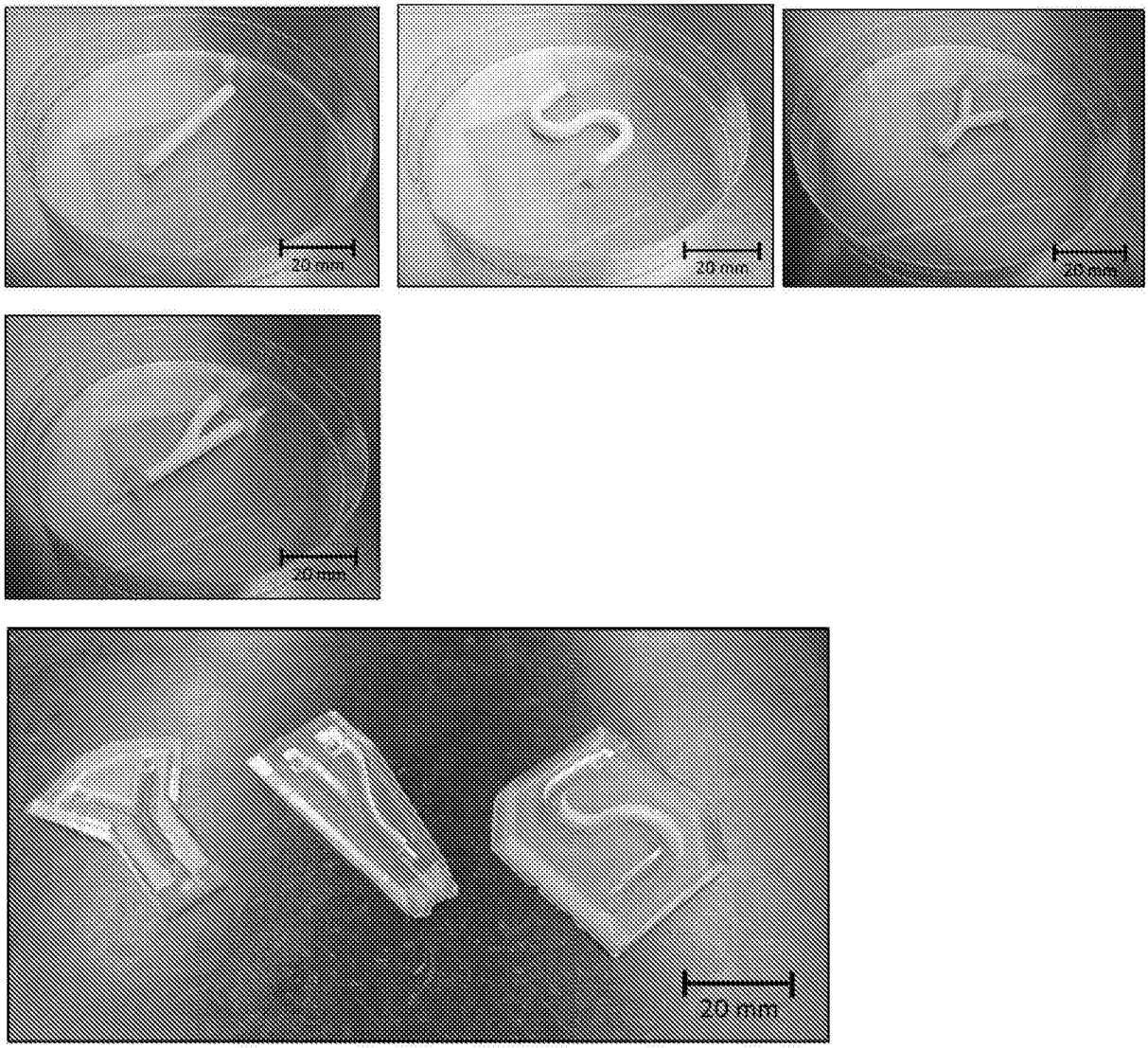


Fig. 16

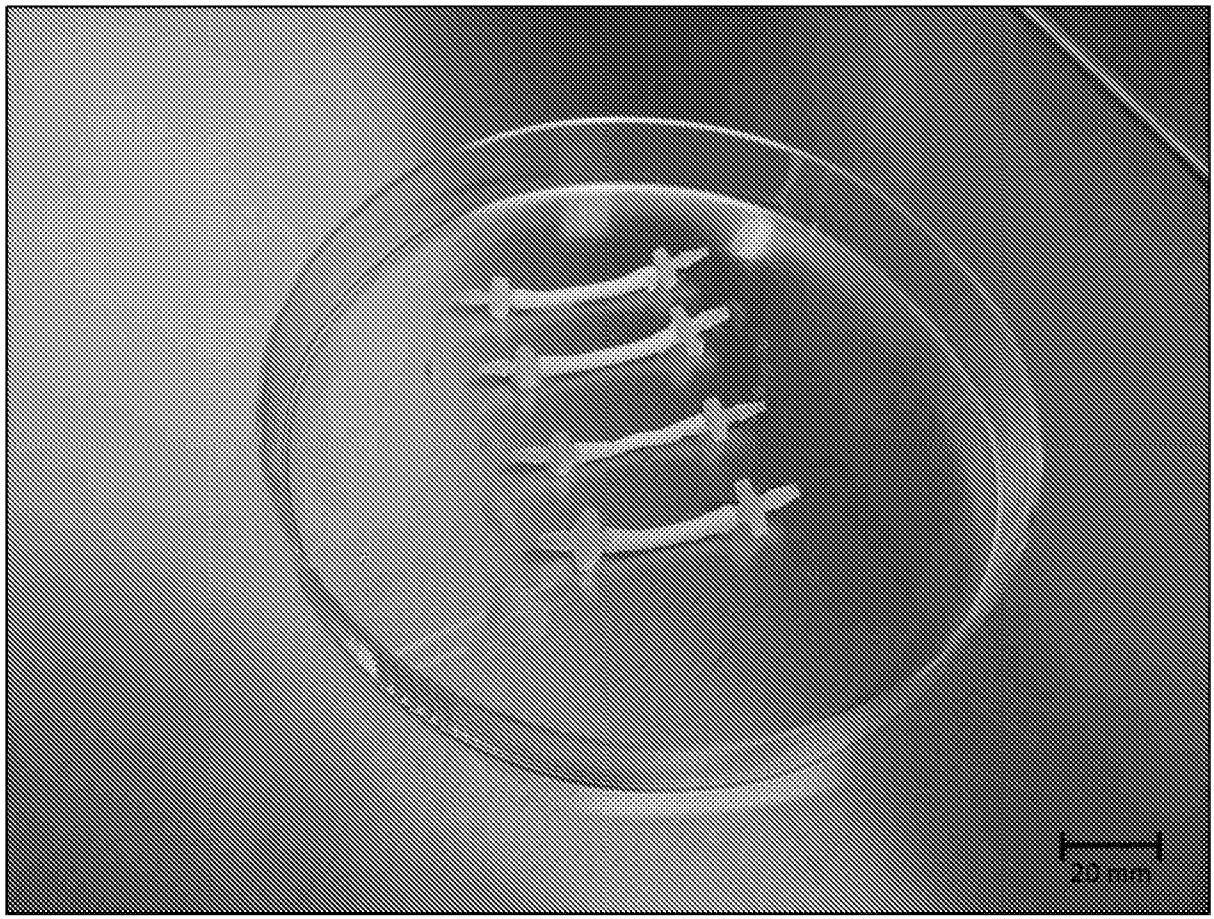


Fig. 17

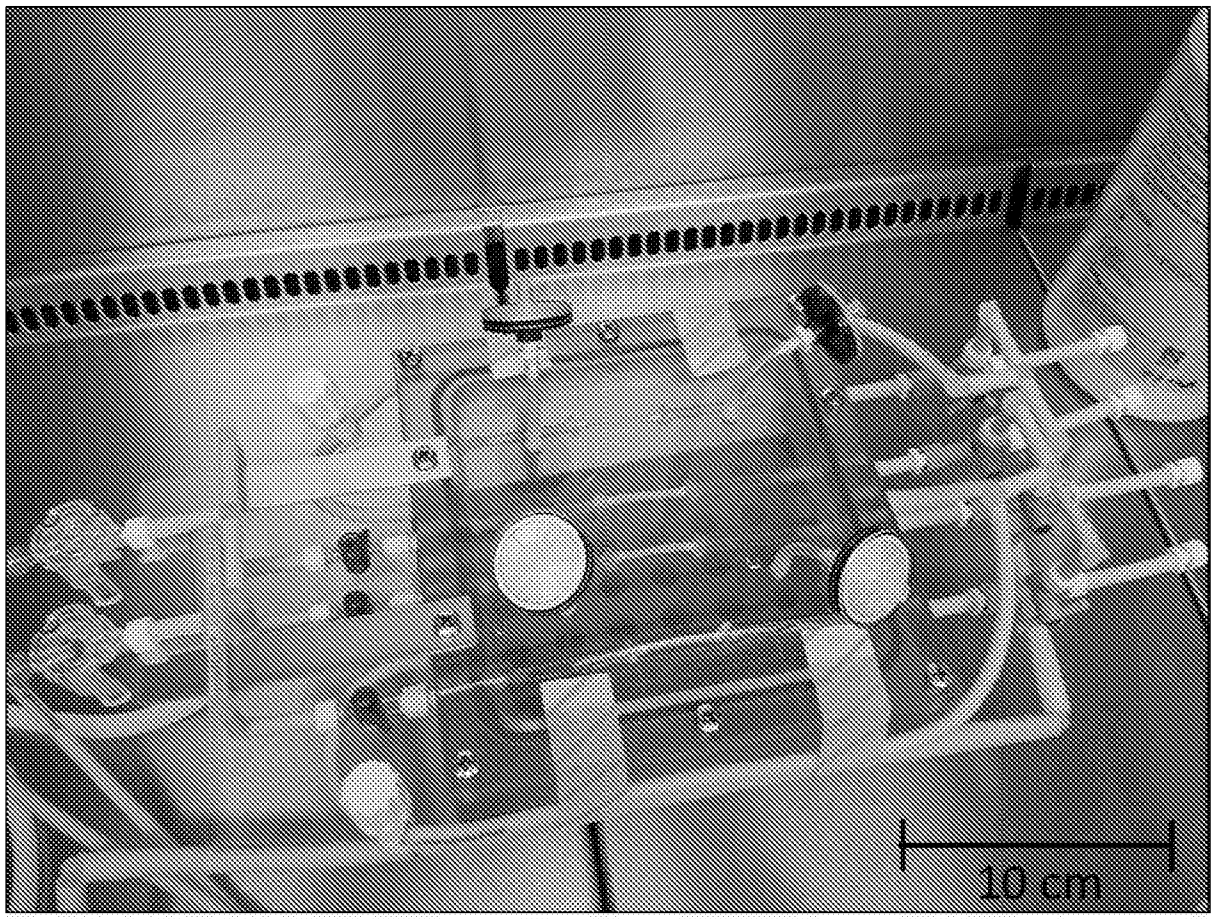


Fig. 18

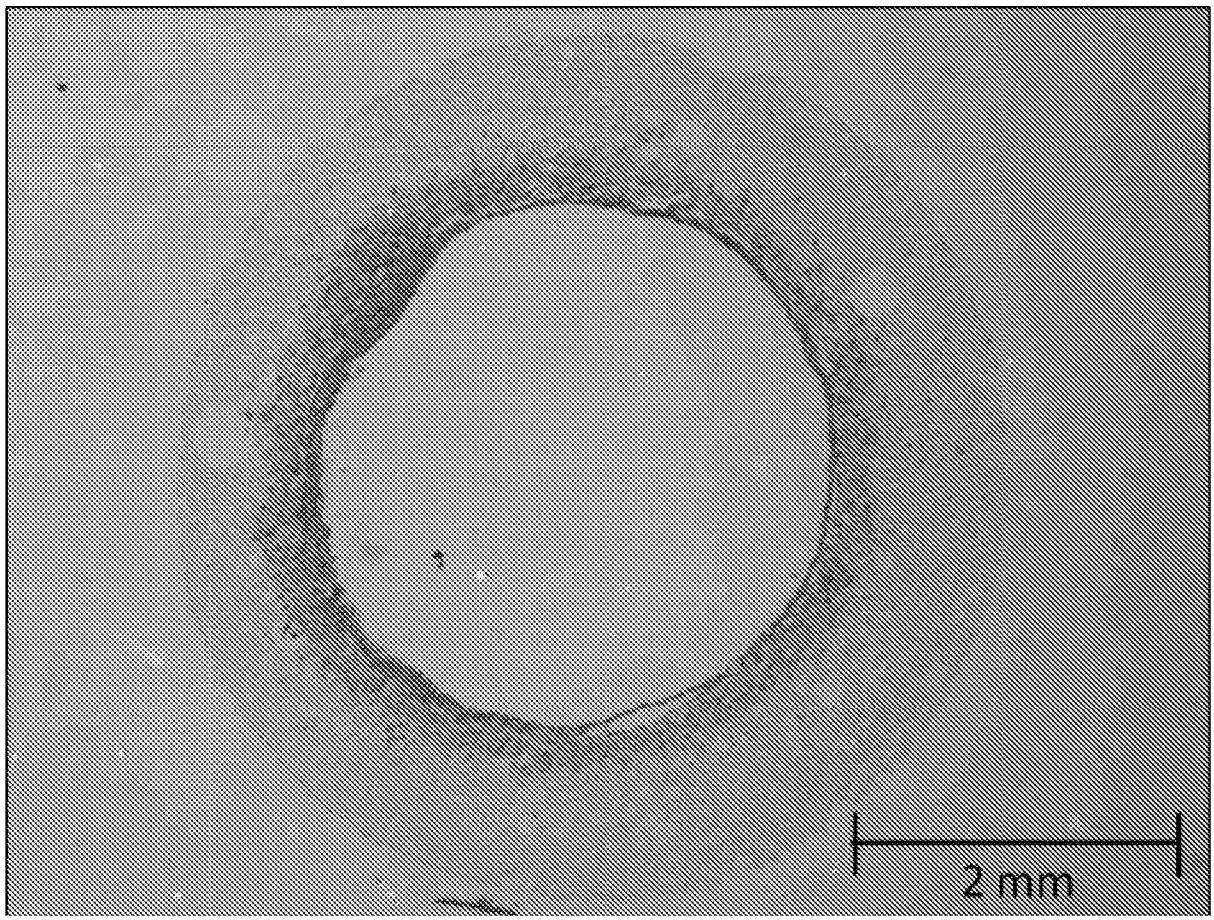


Fig. 19A

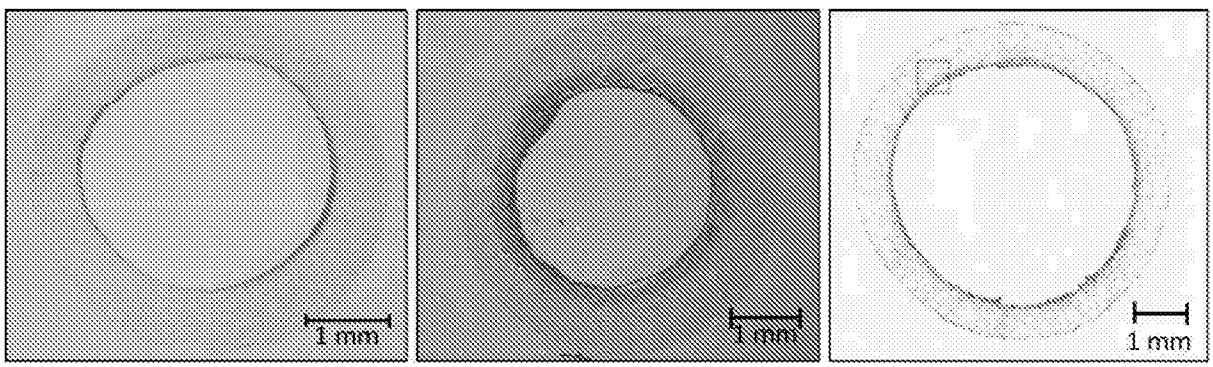


Fig. 19B

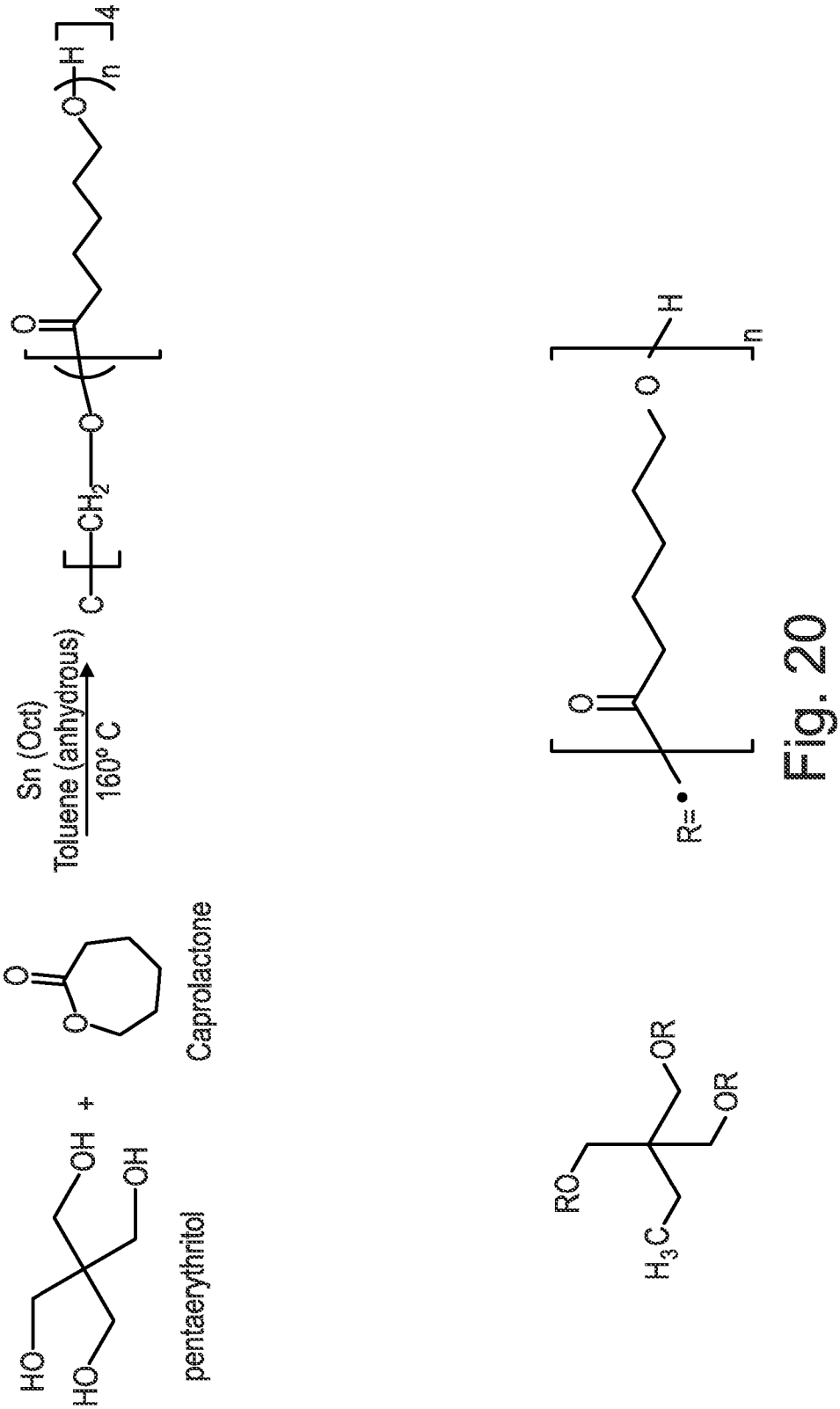
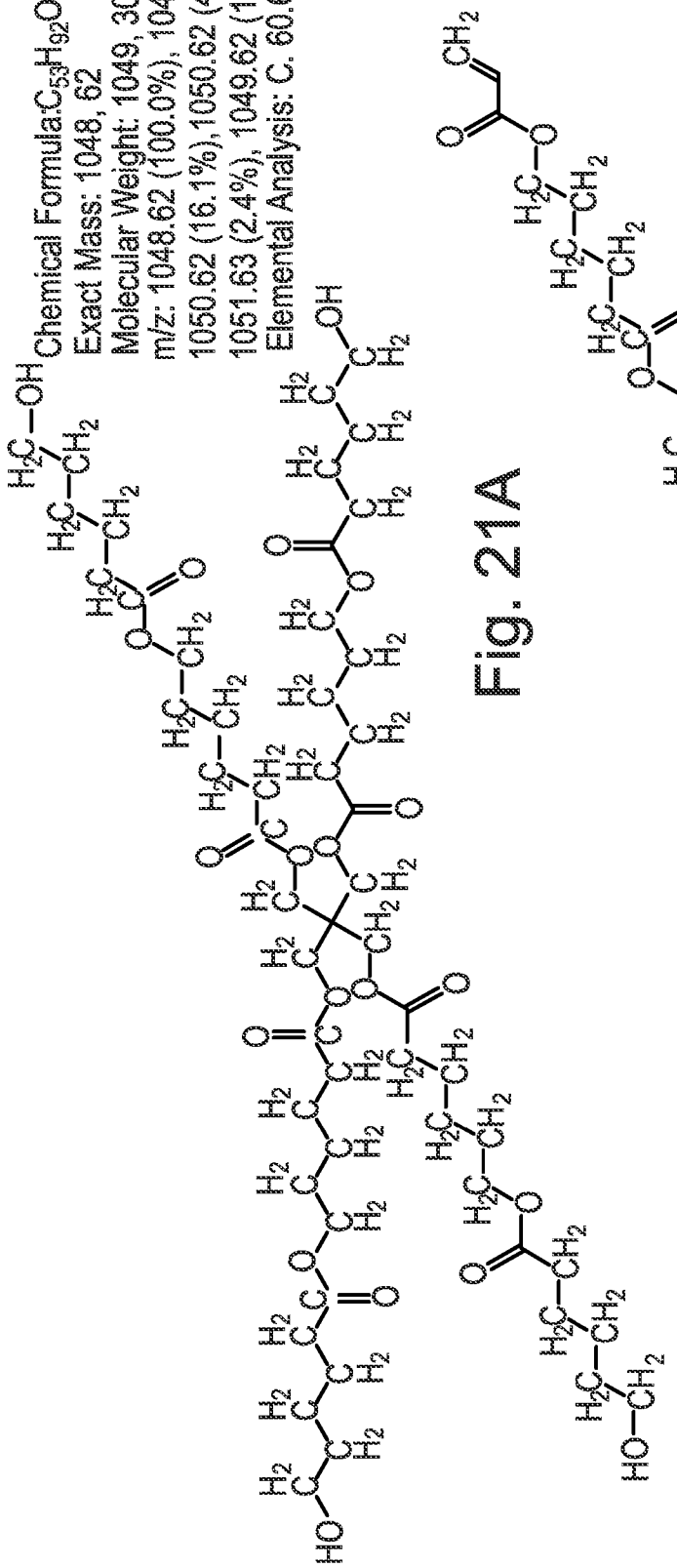
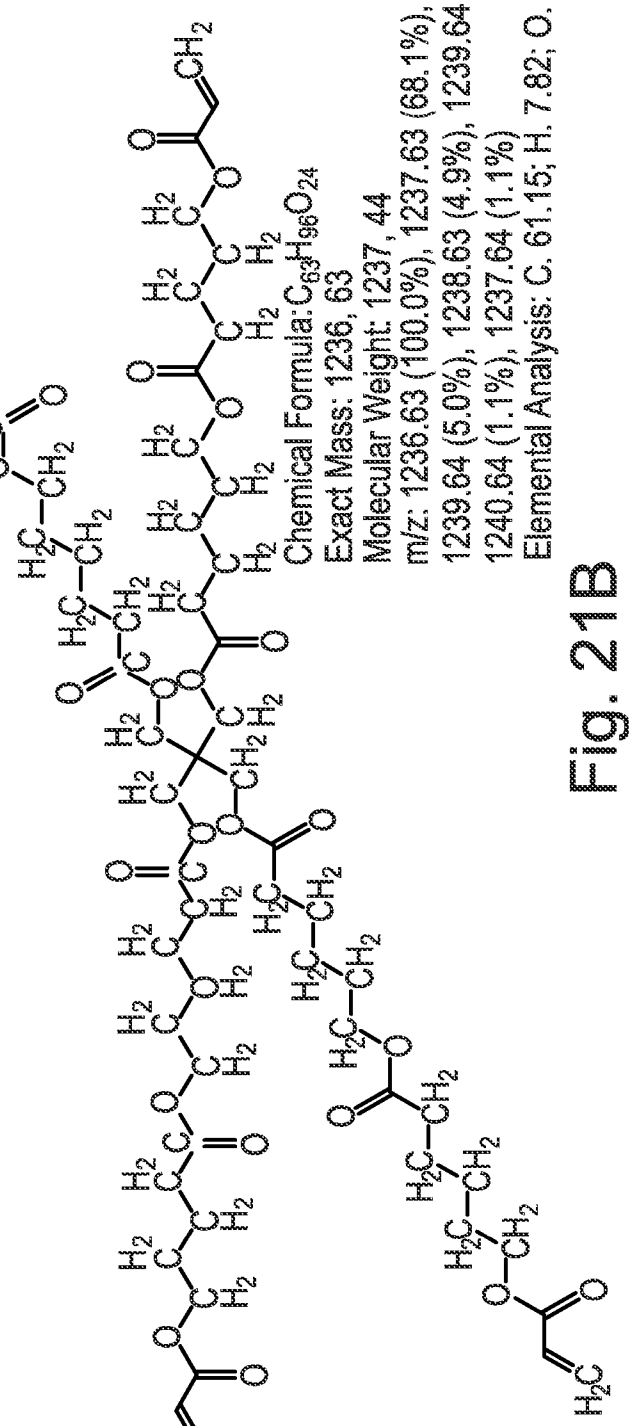

Sn (Oct)
Toluene (anhydrous)
160° C
Caprolactone
pentaerythritol
R=
RO
OR
OR
H_3C

Fig. 20



Chemical Formula: $C_{53}H_{92}O_{20}$
Exact Mass: 1048.62
Molecular Weight: 1049.30
m/z: 1048.62 (100.0%), 1049.62 (57.3%), 1050.62 (16.1%), 1050.62 (4.1%), 1051.63 (3.0%), 1051.63 (2.4%), 1049.62 (1.1%)
Elemental Analysis: C, 60.67; H, 8.84; O, 30.49

Fig. 21A



Chemical Formula: $C_{63}H_{96}O_{24}$
Exact Mass: 1236.63
Molecular Weight: 1237.44
m/z: 1236.63 (100.0%), 1237.63 (68.1%), 1238.64 (22.8%), 1239.64 (5.0%), 1238.63 (4.9%), 1239.64 (3.4%), 1240.64 (1.1%), 1237.64 (1.1%)
Elemental Analysis: C, 61.15; H, 7.82; O, 31.03

Fig. 21B

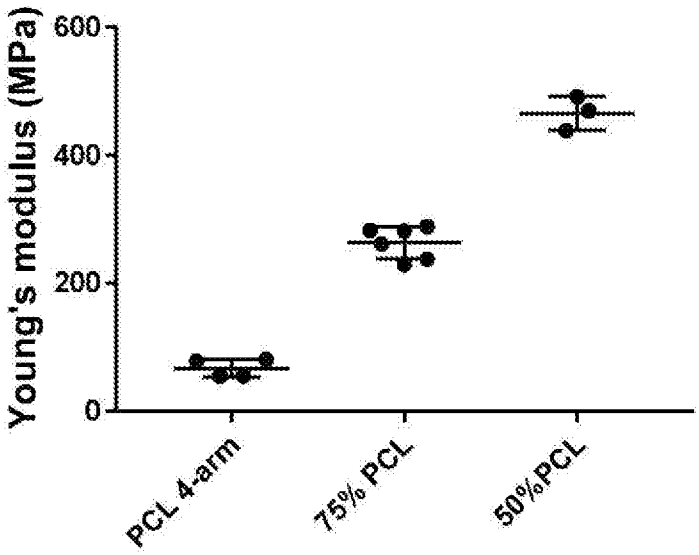


Fig. 22A

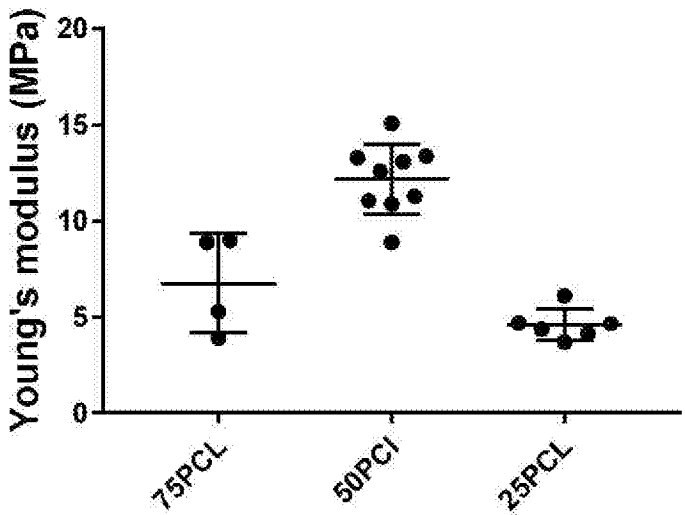


Fig. 22B

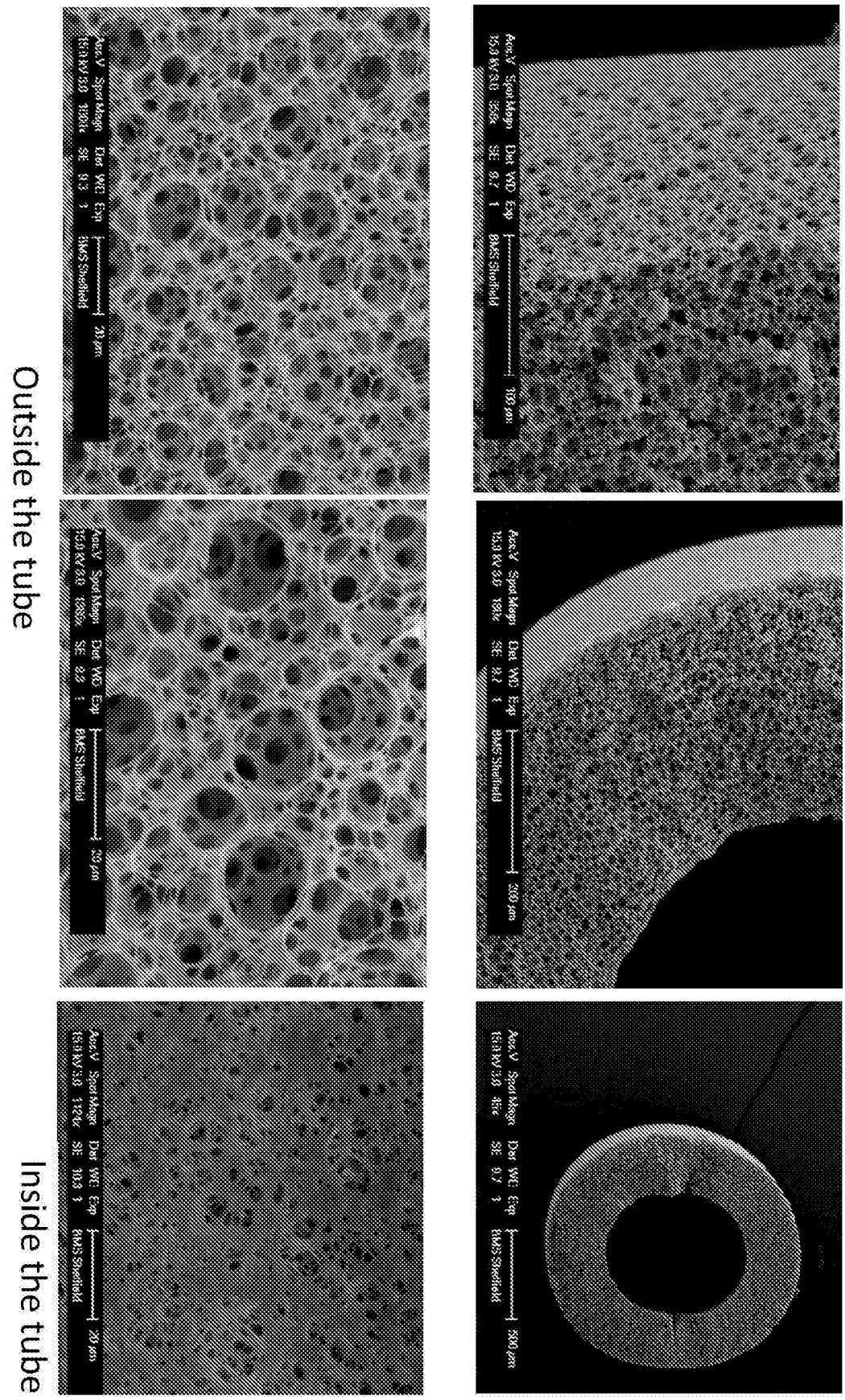


Fig. 23

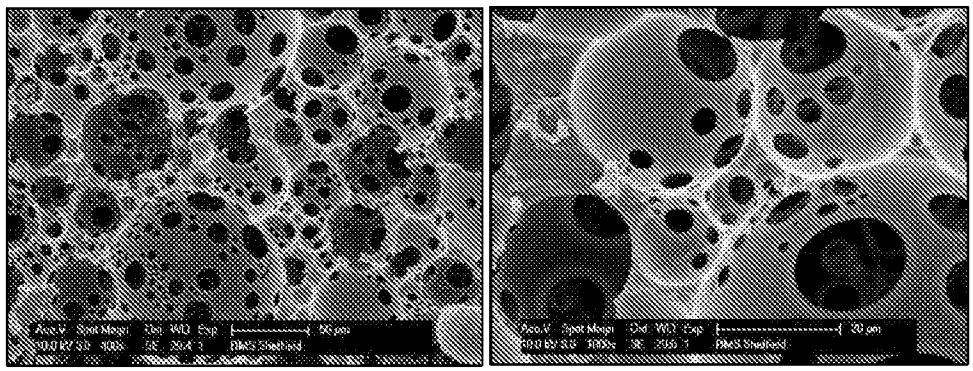


Fig. 24

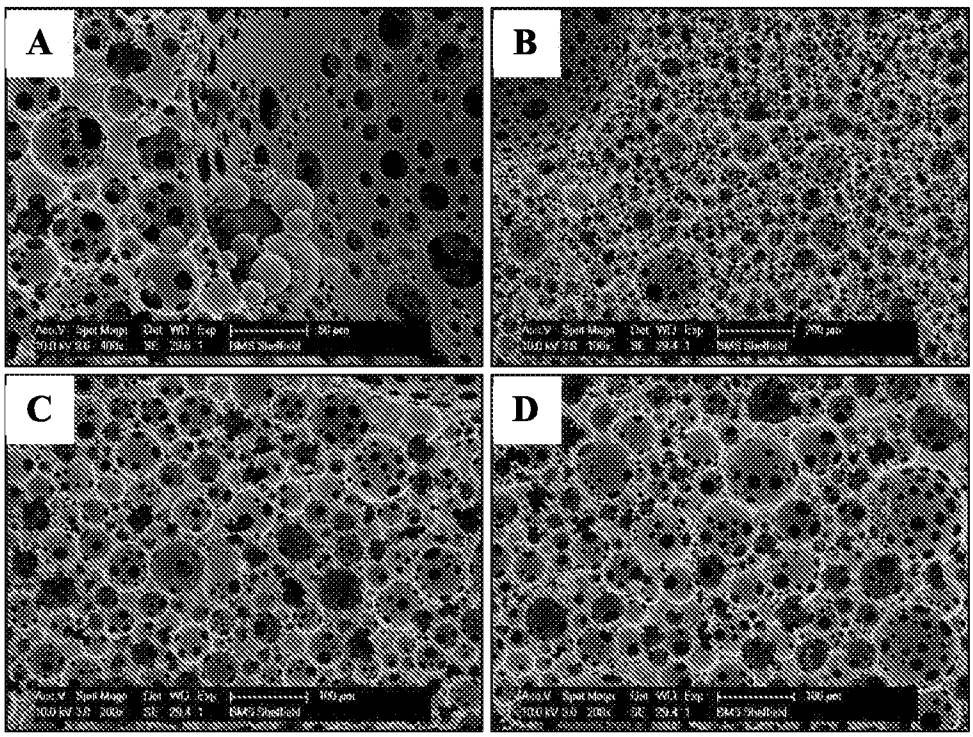


Fig. 25

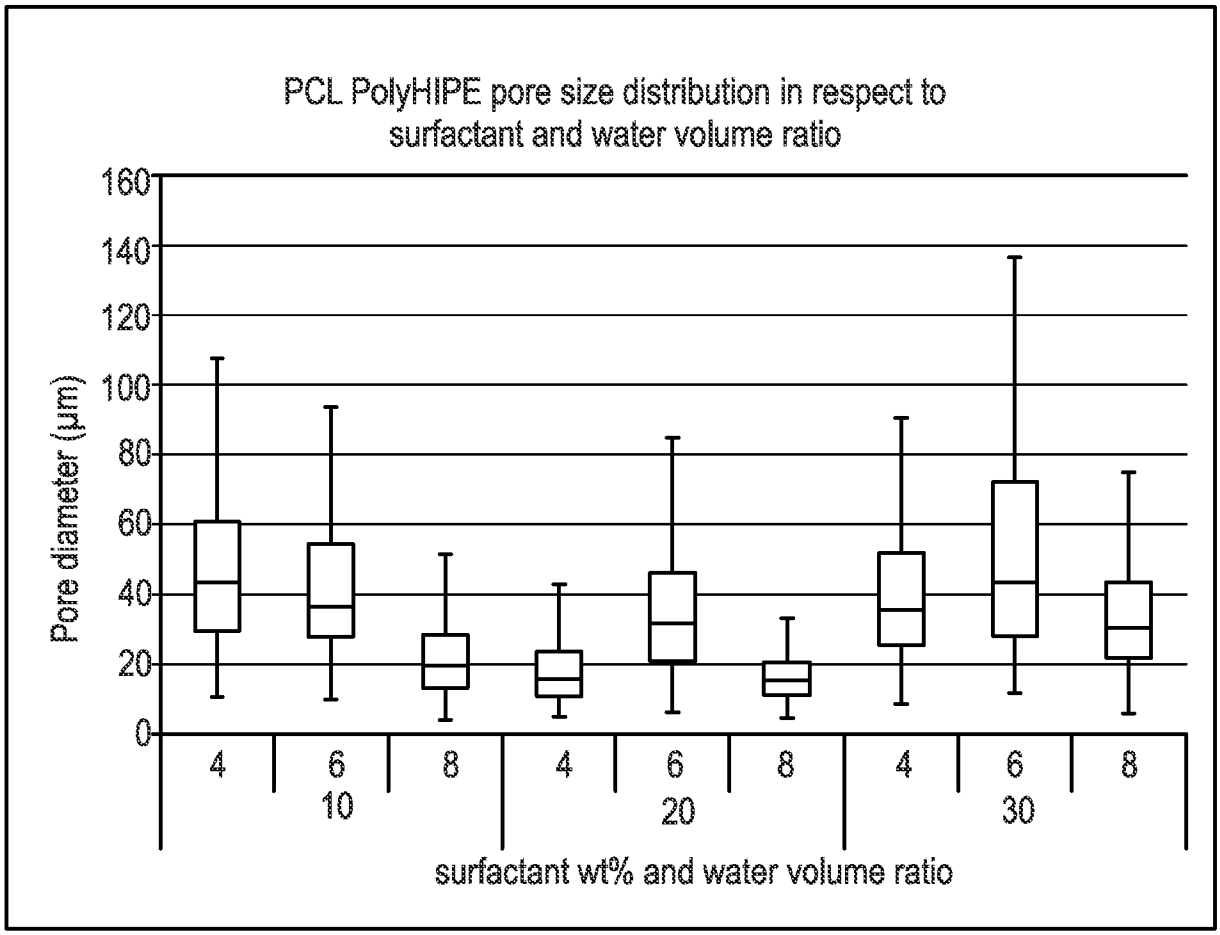

PCL PolyHIPE pore size distribution in respect to surfactant and water volume ratio
Pore diameter (µm)
160
140
120
100
80
60
40
20
0
4
6
8
4
6
8
4
6
8
10
20
30
surfactant wt% and water volume ratio

Fig. 26

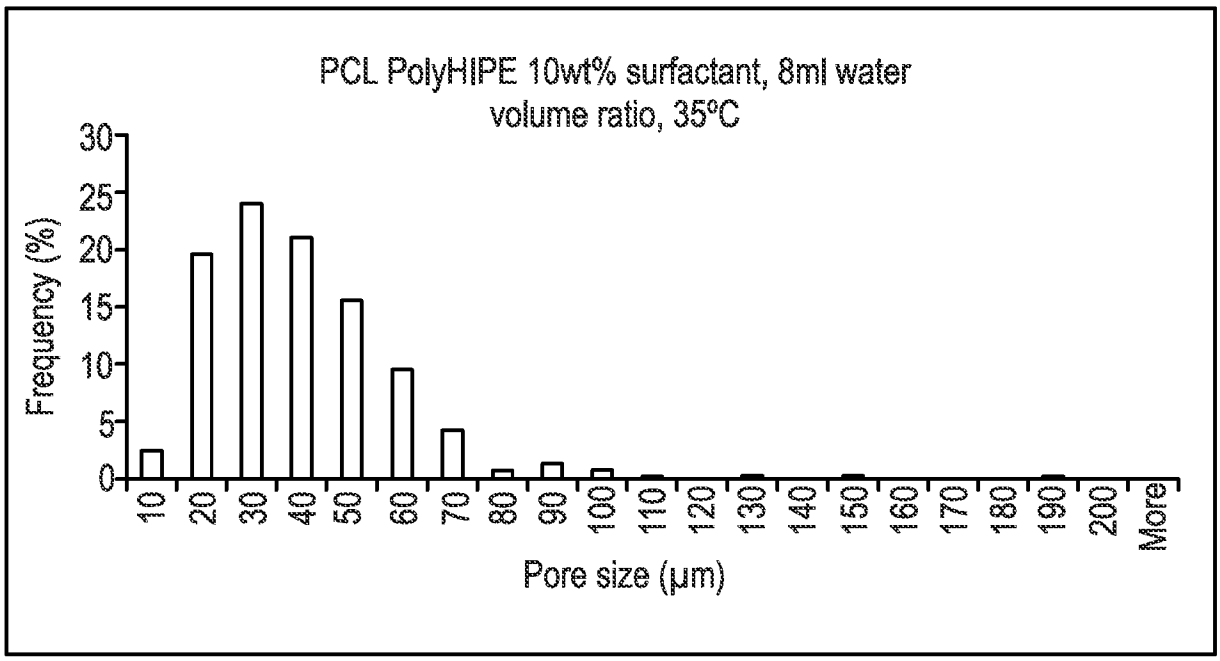

PCL PolyHIPE 10wt% surfactant, 8ml water volume ratio, 35°C
Frequency (%)
30
25
20
15
10
5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
More
Pore size (µm)

Fig. 27

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/051261

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/18 A61L27/56
ADD.

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)
A61L

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YULIA LUMELSKY ET AL: "A degradable, porous, emulsion-templated polyacrylate", JOURNAL OF POLYMER SCIENCE, PART A: POLYMER CHEMISTRY, vol. 47, no. 24, 15 December 2009 (2009-12-15), pages 7043-7053, XP55616301, US ISSN: 0887-624X, DOI: 10.1002/pola.23744 the whole document -----	1-28

☐ 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
29 August 2019	05/09/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fort, Marianne