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Biodegradable mesostructured polymer membranes

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Abstract

The extracellular matrix (ECM) has a quasi-ordered reticular mesostructure with feature sizes on the order of tenths of to a few hundred nanometers. Approaches to preparing biodegradable synthetic scaffolds for engineered tissues that have the critical mesostructure to mimic ECM are few. Here we present a simple and general solvent evaporation-induced self-assembly (EISA) approach to preparing concentrically reticular mesostructured polyol-polyester membranes. The mesostructures were formed by a novel self-assembly process without covalent or electrostatic interactions, which yielded feature sizes matching those of ECM. The mesostructured materials were nonionic, hydrophilic, and water-permeable, and could be shaped into arbitrary geometries such as conformally-molded tubular sacs and micropatterned meshes. Importantly, the mesostructured polymers were biodegradable, and were used as ultrathin temporary substrates for engineering vascular tissue constructs.

Keywords

Biodegradable; mesostructured; membranes; tissue engineering

Mesostructured constructs are important for a range of potential applications including molecular detection, separation, environmental science, medicine, catalysis, and optics^{1–12}. Mesostructured organic materials have been achieved by selective etching of ordered block copolymer domains^{11,13,14}, self-assembly of peptide amphiphile (PA) nanofibers^{12,15}, self-assembly of resin precursors with block copolymers^{3,8,10}, and polymerization-induced microphase separation⁵. Of these, only self-assembled PA, produced via sophisticated chemical design and synthesis, created mesostructured polymeric constructs (*e.g.*, membrane, monolith)^{12,15} that can be both biodegradable and biocompatible^{16–21}. Facile synthesis of mesostructured polymers with established biomaterial compositions and properties is needed but is yet to be achieved¹⁶.

Solvent evaporation induced self-assembly (EISA) is a versatile means of producing two dimensional (2D-) and three dimensional (3D-) mesostructured films^{1–3,7,8,10,22,23}, and typically involves templating from surfactants or block copolymers. EISA permits control of the final structure by adjusting chemical and processing parameters (*e.g.*, initial sol composition, pH, aging time, partial vapor pressures, convection, temperature)^{2,7}.

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Author contributions:

B.T. and D.S.K. conceived the study. B.T., S. S., H. H. C. and R. T. performed and analysed the experiments. B.T. and D.S.K. wrote the manuscript. All authors have given approval to the final version of the manuscript.

Additionally, this technique does not require lithography²⁴ or external fields²⁵, and cheap, large-scale processes such as dip-coating can be used^{2,26}. It is a powerful strategy for creating highly structured multifunctional materials and devices¹, but so far has only been used with inorganic²⁶, hybrid inorganic-organic^{1,2} and nondegradable organic materials³.

The ability of mesostructured biodegradable and biocompatible polymers to mimic the structure of the extracellular matrix (ECM) holds great promise in regenerative medicine¹⁶. Given the advantages of using biomaterials that have been extensively evaluated, we have prepared mesostructured polyol-polyester membranes (MPPM) by EISA (Fig. 1a), by blending poly(lactide-co-glycolide) acid (PLGA) or polylactide (PLA) with triblock poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) (Poloxamer 407) in a 1:3~1:5 mass ratio in tetrahydrofuran (THF) (Fig. 1a, I). The solution was transferred onto planar or nonplanar substrates by dip-coating (Fig. 1a, II), followed by solvent evaporation at ambient conditions (25 °C, 30–70 % relative humidity), and humidified incubation (5 % CO₂, 95 % O₂, 37 °C) overnight (Fig. 1a, III) for solidification. The excess Poloxamer-rich phase was then removed by leaching in phosphate buffered saline solution (1 × PBS) (Fig. 1a, IV). Finally, the membranes were isolated from the substrate and rinsed with deionized water three times, and dried in air (Fig. 1a, V). Unless otherwise noted, membranes were prepared from PLGA with a L/G ratio of 50:50 (5050 DLG 7E) and Poloxamer 407.

Scanning electron microscopy (SEM) of a ~ 2 μm thick membrane after final drying showed smooth surfaces (Figs. 1b, 1c). The membrane was flexible and foldable, with a smallest bending radius of ~ 5 μm (Fig. 1b). The membrane could be peeled from an original glass substrate in water, float at a water-air interface, and be transferred onto another substrate (Fig. 1b, inset). The membrane surface featured reticular structures with fiber diameters of ~ 146 ± 11 nm (mean ± SD) (Fig. 1c) that were locally aligned, as indicated by the fast Fourier transform (FFT)²⁷ of the SEM image (Fig. 1c, inset). The average inter-fiber cavity width, ~ 310–350 nm, was comparable to the spacing between natural ECM nanofibers^{28,29}. The fiber diameter and cavity width were ~ 30–50 times larger than the corresponding feature sizes of mesostructured silica created by Poloxamer 407-mediated EISA²⁶. This observation suggests that the formation of quasi-ordered PLGA mesostructures is different from conventional lyotropic or thermotropic self-assembly, where the feature size is on the order of the amphiphilic chain length^{2,11,15}. The membrane had a uniform fibrous structure spanning the entire thickness (Fig. 1d) demonstrating 3D mesostructure. Nanofiber diameter was inversely related to the polyester lactide-to-glycolide (L/G) ratio (Fig. 1e); polylactide (*i.e.*, L/G = 100:0) yielded the minimum fiber diameter of ~ 60 nm. Hydrated membranes with different thicknesses could be axially stretched with failure strains of 20–28 % (Fig. 1f). The calculated tensile modulus of hydrated MPPMs was ~ 10–50 MPa, comparable to that of commercial polyglycolic acid yarns (Biomedical Structures LLC, Rhode Island)^{27,30} and articular cartilage²⁷.

Contact angles (Fig. 2a) of water on MPPMs, decreased from ~ 70° to below 20° in 12 s. The contact area between droplets and MPPMs did not change detectably during that period. Normalized droplet volume above the MPPMs (Fig. 2a, lower panel) also decreased over time; less than 10 % of the droplet was left above their surface after 12 s. These data demonstrate that the MPPM is hydrophilic and water permeable. A comparison of the compositions of nanometers-thin surfaces of MPPM, MPPM prepared without the leaching step, pure PLGA membrane, and pure Poloxamer 407 by carbon 1s X-ray photoelectron spectroscopy (XPS) (Fig. 2b) showed that MPPM was not pure PLGA; a C–O characteristic of Poloxamer 407 was identified at ~ 286.1 eV. Given the probing depth of XPS (<10 nm), the Poloxamer ‘brush’ was no more than a monolayer, as a thicker layer would yield a spectrum similar to that of pure Poloxamer 407 or the unleached MPPM. These data suggest that the MPPM is a polyester nanofibrous network with a surface layer of Poloxamer 407.

Multiple domains were observed in the MPPMs, with domain lateral dimensions of ~ 20–200 μm (Fig. 2c, left panel). The variability of domain size could be reduced by surface patterning^{31,32} or controlled thermal treatment¹⁵. Most domains had a concentric pattern, as revealed by both SEM and FFTs of four regions around the domain center (Fig. 2c, right panels 1–4). When crossing the domain boundary (dashed line in Fig. 2c, and Fig. 2d), nanofiber orientation changed while other structural parameters (*e.g.*, diameter, pattern morphology) remained unchanged.

We hypothesize that MPPM formation occurs as follows. Polyester and Poloxamer 407 undergo cooperative self-assembly to form phase-separated micro-domains (Fig. 2e, I). Poloxamer 407 molecules are anchored to PLGA domains, possibly by their hydrophobic poly(propylene glycol) (PPO) segments. Most Poloxamer 407 molecules are removed during the leaching step which yields an open, permeable framework (Fig. 2e, II). A monolayer of Poloxamer 407 is left on PLGA surfaces, with the hydrophilic PEO segments pointing outward (Fig. 2e, II). We hypothesize that Poloxamer 407-rich domains (light blue in Fig. 2e, I, upper image) can form locally ordered mesophases (red arrows, Fig. 2e, I) that guide the alignment of amorphous PLGA rich domains (dark blue in Fig. 2e, I and II, upper panels). The fact that the mesostructure feature size (*e.g.*, cavity size, solid phase thickness) was at least 30 times larger than that reported in mesostructured silica or resin^{3,8,9,26}, although the same structure-directing agent was used (*i.e.*, Poloxamer 407), shows that this structure-directing effect is long-range. This may be attributed to the fact that both polyol (*i.e.*, Poloxamer 407) and polyester (*i.e.*, PLGA) are nonionic. This is in contrast to conventional EISA² where structure-directing is at the length scales of individual micelles or polymer chains, *i.e.*, a shorter-range interaction^{2,7,26}. Multiple repeats of Poloxamer 407 aggregates (red arrows, Fig. 2e, I) might be involved in biphasic self-assembly, analogous to microscopic phase segregation in liquid-crystalline physical gels³³. Such long range templating explains the observed ECM-like feature size, which is sufficiently large to prevent coalescence of mesostructured nanofibers due to high internal Laplace pressures³⁴. Neither covalent nor electrostatic interactions were involved, which, together with the hydrolyzable backbones of PLGA and Poloxamer 407, contributed to the biodegradability (see below) of MPPM.

There was great flexibility in the geometries in which the MPPM could be prepared (Fig. 3). A centimeter-scale round-ended tubular sac was formed by coating the inner surface of a glass test tube with THF solution containing PLGA and Poloxamer 407, followed by drying, leaching and membrane isolation from the test tube (Fig. 3a, I). SEM imaging of the edge of the sac (Fig. 3a, II) showed surface topography similar to that of a flat membrane (Fig. 1). To demonstrate the ability to generate hierarchical porous scaffolds, we prepared macroporous-mesostructured 3D constructs, using ~ 20 μm SiO_2 spheres as the template (Fig. 3b) during EISA. Nanofibrous topography was preserved on the macropore surfaces. MPPMs could also be micropatterned by deposition on a silicon oxide surface micropatterned with (heptadecafluoro)-1,1,2,2-tetrahydrodecyldimethyl-chlorosilane (Fig. 3c, inset); the fluoro-silane-modified square regions repelled the polymer coating in THF solution. The boundary between nanofiber coated region and uncoated region was sharp (Fig. 3c), but nanofiber orientation did not appear to correlate with the micropattern. The MPPM could also be used as a structural support for mesh-like nanowire nanoelectronics³⁵ (Fig. 3d), enabling their facile folding and rolling for potential applications in degradable and flexible electronics³⁶.

MPPM degraded in $1 \times \text{PBS}$ over a period of ~ 3 months (Fig. 4a). The degradation kinetics was comparable to that previously reported for nanostructured PLGA with the same L/G ratio and similar PLGA molecular weight^{21,37}. The *in vitro* cytotoxicity of the MPPM (Fig. 4b) was evaluated in the neuroendocrine cell line PC12, human umbilical vein endothelial

cells (HUVEC) and human aortic smooth muscle cells (HASMC) after MPPM modification with cyclic arginineglycine-aspartate (cRGD) peptides (see Supplementary Information) to enhance cell attachment¹⁶. Membranes without cRGD modifications showed poor initial cell attachment. Cell viability on RGD-modified MPPMs was similar to that in gelatin/fibronectin-coated 24-well plates over 12 days, as measured by a metabolic activity assay (MTS) (Fig. 4b). The results suggested that the MPPMs could be suitable for cell studies, such as for engineered tissue cultures.

In vivo biocompatibility of MPPM discs was assessed after subcutaneous implantation in rats for up to 1 month (Figs. 4c and 4d, n = 4 in each group). A mild inflammatory infiltrate (Fig. 4c, stars) encircled the implant cavity at day 7, consistent with results from pure PLGA implants³⁷. The inflammatory reaction decreased substantially by 1 month after implantation (Fig. 4d) when MPPMs were degraded by > 70 % (Fig. 4a). Sham (i.e., no MPPM) surgical procedures showed less inflammation than those with MPPMs at both time points. These results suggest that the biocompatibility of PLGA/Poloxamer membranes was comparable to that of PLGA alone³⁷.

Cyclic RGD-modified MPPM (Fig. 4e, I) were used to develop engineered vascular constructs (Figs. 4e–h). HASMC were cultured on ~ 1 μ m thick MPPMs, with sodium ascorbate added to the media to promote deposition of natural ECM³⁸ (Fig. 4e, II). Two days after cell seeding, the MPPM were rolled into multi-layered 3D tubular structures (Fig. 4e, III), and matured for at least 2 months to allow for thickening of the tissue layer and polymer degradation (Fig. 4e, IV and V). Cell viability on the surface of the construct was ~95 % (Fig. 4f). Hematoxylin & eosin and Masson's trichrome stained sections (Figs. 4g, h) revealed smooth muscle tissue ~ 200 μ m thick, with elongated cells and collagenous nanofibers (blue in Fig. 4h). These results showed that the MPPMs are biodegradable and biocompatible, and suggest their potential as low-cost and versatile synthetic ECM constructs for engineered tissues.

In summary, we have developed mesostructured polyol-polyester membranes that are formed by a novel self-assembly via an EISA process. These membranes are composed of biomaterials that have been extensively evaluated in regenerative medicine and drug delivery, are biodegradable and water permeable, and show minimal cytotoxicity *in vitro* and *in vivo*. They may find broad applicability in a range of biomedical applications such as cell encapsulation and immunoisolation^{39,40}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Preparation and characterizations of mesostructured polyol-polyester membranes (MPPM)

a, Schematic of MPPM preparation. **b**, SEM image of a ~2 μm membrane with surface wrinkles. Inset, a photograph of a membrane transferred onto a glass slide; the dashed lines mark the membrane boundary. **c**, A SEM image highlighting the mesoscale surface topography. Inset is the fast Fourier transform (FFT). **d**, SEM image of a broken membrane edge, showing the 3D mesostructure. Dashed lines mark the edges of a membrane corner. **e**, Effect of L/G ratio on fiber diameter. Data are means ± SD, n=20. **f**, Membrane tensile characteristics. The membranes were 1 cm wide, and 12 μm (blue), 4 μm (black) or 2 μm (green) thick.

Figure 2. Characterization of the mesostructured membrane

a, Water contact angle experiments. Upper panel: side-view photographs recorded at 0 s (left), 5 s (middle) and 10 s (right) on a MPPM. Middle panel: time-lapse contact angle measurements on MPPMs. Lower panel, time-lapse water droplet volume changes calculated from contact angle measurements on MPPMs. Data are means $\pm$ SD, $n=6$. **b**, Carbon 1s XPS spectra of MPPM, unleached MPPM, pure PLGA and pure Poloxamer 407. **c**, SEM image showing the long-range concentric pattern and the domain boundary (dashed line). FFTs (right panels) were obtained from regions 1–4 in the SEM image. The arrow shows the center of the concentric domain. **d**, A SEM image highlighting the mesostructure at one domain boundary. **e**, A proposed mechanism. (I) the long-range structure-directing effect, and (II) the hydrophilic open framework after leaching.

Figure 3. Shaping and patterning of mesostructured polymer constructs

a, Photograph (I) and SEM (II) of a mesostructured sac. **b**, SEM of macroporous-mesostructured construct where the MPPM was created around a template of 20 μm SiO₂ microspheres. **c**, Mesostructured polymer mesh micropatterned on fluorosilane-modified rectangular domains. The boundary with the uncoated area is indicated with arrows. **d**, Photograph of mesostructured polymer membrane used as a support for nanoelectronic devices. The red dashed circle highlights one nanowire field effect transistor device. The transparent ribbons (red arrows) are SU-8 structures used to support and insulate device interconnects.

Figure 4. Mesostructured membranes are biodegradable, biocompatible and can be used in vascular construct engineering

a, Gravimetric weight loss as a function of time. Membranes were weighed after rinsing and drying at each time point. Data are mean $\pm$ SD, $n = 5$. **b**, Cell survival by MTS cytotoxicity assay. Cultures on gelatin/fibronectin coated 24-well plate were used as controls. Data are means $\pm$ SD, $n = 6$. **c**, Hematoxylin & eosin stained dermis and muscle sections immediately adjacent to mesostructured membranes 1 week after subcutaneous implantation. Scale bars: 500 μ m. **d**, Hematoxylin & eosin stained sections 1 month after implantation. Scale bars: 500 μ m. In **(c)** and **(d)**, arrows mark the MPPMs' locations. **e**, Schematic of the preparation of engineered vascular constructs from MPPM. Blue = MPPM; pink = cells. **f**, Cell survival

in an engineered vascular construct evaluated with a LIVE/DEAD[®] Viability/Cytotoxicity assay, 2 months after seeding. Green = live, red = dead. **g**, Hematoxylin & Eosin stained sections of an engineered vascular construct, 2 months after seeding. **h**, Masson's Trichrome stained sections of an engineered vascular construct, 2 months after seeding, highlighting the collagen matrix (blue).