Electronic Supplementary Information for

3D Hierarchical Scaffolds Enabled by a Post-Patternable, Reconfigurable, and Biocompatible 2D Vitrimer Film for Tissue Engineering Applications

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Experimental Section

Materials

Dicarboxyl terminated poly(acrylonitrile-co-butadiene) (PAB) (average $M_n \sim 3,800$ g mol$^{-1}$, acrylonitrile 8-12 wt%, carboxyl end group 2.4 wt%, product and CAS numbers are 418870 and 68891-46-3, respectively) and zinc acetate was purchased from Sigma-Aldrich. Octaglycidyl functionalized polyhedral oligomeric silsesquioxane (POSS) was supplied by Hybrid Plastics. Chloroform (CHCl$_3$, 99.5 %) purchased from Dae Jung was used as received. Microgrooved nickel molds (dimension; 80, 40, and 10 µm in a groove width with a constant depth of 5 µm) were provided by SJ company in South Korea.

Preparation of the vitrimer film (PAB-POSS vitrimer)

A vitrimer film named as PAB-POSS vitrimer was prepared as follows. 5 g of PAB and zinc acetate (10 mol% to carboxyl group of PAB) were introduced into a 50 mL round-bottom flask with a magnetic stirring bar and heated at 130 °C under vacuum. The reaction proceeded for 2 h until no acetic acid gas was emitted and the zinc acetate was completely solubilized. 2.16 g of the compound (1.02 mmol acid + 0.11 mmol zinc acetate) was mixed with POSS (0.19 g, 1.13 mmol epoxy) using minimal amount of CHCl$_3$ at 50 °C for 1 h. The solution was poured into a silicon mold (ca. 60 mm (L) × 60 mm (W) × 10 mm (T)) and stayed overnight until the solvent was completely
evaporated. A 500 µm thick vitrimer film could be obtained after a crosslinking reaction at 150 °C under vacuum for 20 h (gel fraction: 96.2 %).

Fabrication of the micro-patterned PAB-POSS vitrimer surfaces and 3D structures

Micro-patterned PAB-POSS vitrimers with groove widths of 80, 40, and 10 µm, respectively, were prepared by pressing the corresponding microgrooved nickel molds onto the PAB-POSS vitrimer under vacuum at 180 °C for 6 h. 3D structures could be constructed by shaping the vitrimer film into the desired 3D geometry, and then placing them in the vacuum oven at 180 °C for 3 h. A tubular structure with micro-patterned outer surface was particularly prepared from the PAB-POSS vitrimer with 10 µm groove width.

Cell culture

C2C12 muscle cells (ATCC® CRL-1772™) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, NY) supplemented with 10 % fetal bovine serum (FBS, Gibco BRL) and 1 % (v/v) penicillin/streptomycin (Gibco BRL).

Cytotoxicity test

Biocompatibility of the PAB-POSS vitrimer was determined by EZ-Cytox cell viability assay kit (Daeil Labservice, Korea) according to the manufacturer’s protocol (n = 3 per group). 100 µL of the cell suspension was dispensed in a 24-well plate and pre-incubated for 24 h in a humidified incubator (at 37 °C, 5 % CO₂). The circle-shaped
sample (10 mm (D)) loaded inserts were immersed in each well and incubated for 24 and 48 h, respectively. A glass substrate of the same size was used as a positive control in this test. After replenishing with fresh medium, CCK-8 solution was added into each well of the 24 well plate, and the cells were incubated for 2 h. The cell viability was evaluated using a colorimetric test based on formazan dye generated by intracellular dehydrogenases, which is directly proportional to the number of the viable cells. In brief, 10 uL of EZ-Cytox solution was added to each well of the plate and incubated for 3 h in the incubator. The absorbance was read at 450 nm using a microplate reader (Bio-Tek, PowerWaveX340).

**Cell alignment**

C2C12 cells were seeded on the PAB-POSS vitrimer, and the micro-patterned PAB-POSS vitrimers at a density of $5.3 \times 10^4$ cells/cm$^2$, and cultured for 24 h. To obtain field-emission scanning electron microscopy (FE-SEM) images, the cells on the substrates were fixed according to following protocol. Briefly, the cells rinsed with phosphate-buffered saline (PBS) were immersed in 3 % glutaraldehyde for 10 min at room temperature, followed by dehydration with ethanol. Ethanol was replaced by hexamethyldisilazane, and it was completely evaporated before analysis. The cell alignment was quantitatively analyzed using ImageJ software (National Institute of Health, Bethesda, MD, USA).

**Instrumentation and characterization techniques**
Infrared (IR) spectra were recorded on a Nicolet 6700 spectrophotometer (Thermo Scientific, USA) using attenuated total reflectance (ATR) equipment (FT-IR/ATR). The thermal transition behaviors of PAB-POSS vitrimer and PAB were examined by differential scanning calorimetry (DSC) using TA Instruments DSC-Q1000 under a nitrogen atmosphere. Dried samples with a typical mass of 3–7 mg were encapsulated in sealed aluminum pans. The samples were quenched to –80 °C for 5 min and maintained at this temperature for 5 min, then heated to 250 °C at a heating rate of 10 °C/min. The glass transition temperature ($T_g$) was taken as the point of inflection of the plot of the change in heat capacity with temperature in the DSC thermograms.

The thermal stability of the vitrimer film was investigated by thermogravimetric analysis (TGA) using TA Instruments TGA Q-5000IR under a nitrogen atmosphere. 10 mg of the dried sample was heated to 700 °C at a heating rate of 10 °C/min. The mechanical properties of the PAB-POSS vitrimer were measured using a universal testing machine (UTM, Instron-5543, USA). Rectangular-shaped specimens (ca. 60 mm × 5 mm) were prepared using a cutting die, and the tensile properties of the films were measured at 25 °C under 40 % RH conditions with a gauge length and cross head speed of 20 mm and 10 mm/min, respectively. Cyclic tensile testing was performed on the same UTM using the same rectangular shaped film (gauge length = 20 mm) at a strain rate of 10 mm/min. Dynamic mechanical analysis (DMA) was performed on a TA Instruments DMA Q800 with attached cryo accessory using rectangular-shaped PAB-POSS vitrimer film (ca. 30 mm × 5 mm). DMA was conducted in the film tension mode with a 2.5 Hz frequency, 0.1 % strain, and 0.1 N axial force. The specimens were first cooled down from room temperature to –100 °C and then heated to 60 °C at a
constant rate of 5 °C/min in a nitrogen atmosphere. Cross-linking density \( (v_e)^{\text{SI}} \) of the PAB-POSS vitrimer was calculated as

\[
v_e = \frac{E'}{3RT}
\]  

(S1)

where \( E' \), \( R \), and \( T \) are the storage modulus, universal gas constant, and absolute temperature in the rubbery region (ca. 298.15 K), respectively. Stress relaxation experiments of the circle-shaped vitrimer film (ca. 8 mm (D), 1 mm (T)) was carried out at specified temperature (180–220 °C) using a rotational rheometer (ARES-G2, TA Instruments, USA). The sample was allowed to equilibrate at each temperature for 10 min, then subjected to a constant strain of 10 % until it had reached to the relaxation time \( (\tau^*)^{\text{SI}} \) which was defined as the time required for the stress relaxation modulus had relaxed to at least 37 % \((1/e)\) of its initial value. A constant normal force of 2.0 N was applied throughout the measurement to ensure a good contact of the material with the geometries. \( \tau^* \) was determined via the stress relaxation analysis at varying temperatures from 180 to 220 °C. These points were then plotted vs. 1000/T and fit to the Arrhenius relationship as follows

\[
\tau^*(t) = \tau_0 e^{E_a/RT}
\]  

(S2)

where \( \tau_0 \) is the characteristic relaxation time at infinite \( t \), \( E_a \) (kJ/mol) is the activation energy of the transesterification reaction, \( R \) is the universal gas constant and \( T \) is the absolute temperature at the experiment was performed.\(^{53}\) Freezing transition temperature \( (T_v) \) is defined as the point at which a vitrimer exhibits a viscosity of \( 10^{12} \) Pa s, also known as the liquid to solid transition viscosity \((\eta)\).\(^{54}\) Using Maxwell’s relation (Eq. S3) and \( E' \) (average storage modulus in the rubbery region) determined
from DMA, $\tau^*$ was determined to be ca. $1.3 \times 10^6$ s at $T_v$. The Arrhenius relationship from Eq. S2 was then extrapolated to $\tau^* = 1.3 \times 10^6$ to determine the $T_v$.$^83$

$$\eta = \frac{1}{3} E^* \tau^* \quad \text{(S3)}$$

The surface morphologies of the micropatterned PAB-POSS vitrimer was analyzed using FE-SEM (Carl Zeiss SUPRA, Germany). All the samples were coated with platinum under vacuum prior to the measurement. The cross-section of the micropatterns was obtained using focused ion beam (FIB) milling combined with scanning electron microscopy (SEM, Carl Zeiss AURIGA, Germany) and the depth of the micropattern was measured at a tilted stage.
FT-IR spectra of PAB-POSS vitrimer, PAB, and POSS

Fig. S1  FT-IR spectra of the PAB-POSS vitrimer, PAB, and POSS in the wavenumber ranges of (a) 950-800 cm\(^{-1}\) and (b) 1800-1600 cm\(^{-1}\), respectively.
Thermal properties of PAB-POSS

Fig. S2  (a) TGA thermogram of the PAB-POSS vitrimer and (b) DSC traces of PAB and PAB-POSS vitrimer.
Mechanical properties of PAB-POSS

Fig. S3  (a) A representative stress-strain curve and (b) cyclic stress-strain curves (strain limit = 50 %) of the PAB-POSS vitrimer.
Dynamic mechanical analysis of PAB-POSS vitrimer

**Fig. S4**  DMA curves of the PAB-POSS vitrimer.
Micropattern depths of PAB-POSS vitrimers

Fig. S5  Representative SEM images of the cross-sections of PAB-POSS vitrimers with (a) 80, (b) 40, and (c) 10 µm groove widths, respectively. The cross-sections were obtained by FIB milling technique and the depth of each sample was measured at a tilted stage.
Micropattern on the reconfigured 3D structure

**Fig. S6** A SEM image of the micropatterns with 10 µm groove width on the outer surface of the reconfigured tubular structure (scale bar = 20 µm).
Quantitative analysis of the C2C12 cell alignment on PAB-POSS vitrimers

**Fig. S7**  Quantitative analysis of the cell alignment on the (a) pristine PAB-POSS vitrimer, and micro-patterned PAB-POSS vitrimers with (b) 80, (c) 40, and (d) 10 µm groove widths, respectively.
References for electronic supplementary information