Printing Parameters Affect Key Properties of 4D Printed SMPs
Supplemental Methods

2.1 Sample Preparation

2.1.1 *Filament Fabrication*

Using a custom-built melt-spinner, SMP pellets were extruded to make 1.75 mm diameter filament (Figure 2). The pellets were heated in the barrel of the melt spinner at 200˚C, followed by extrusion out of a die. The filament was then collected and stored in a desiccator until use.

2.1.2 *Raster Printed Samples*

Preliminary printing was conducted to determine the range of printing parameters that produced samples of sufficient quality, which we defined as a lack of bubbles, gaps, or defects in the object upon visual inspection[1]. Based on the preliminary printing evaluation, the following printing parameters were chosen for the study. The printing temperatures used were 215˚C and 225˚C, with an intentional separation of ten degrees to prevent an overlap in printing temperature due to over- or undershot nozzle heating. The extrusion multipliers used were 0.95 and 1.0. To provide volumetric flow rates that could be employed on other printers that might use different means of controlling the relationship between flow and print speed, the volumetric flow rates are calculated as follows. For the filament diameter of 0.2 mm and printing speed of 3600 mm/min:

$$\text{Volume/min} = \pi r^2 \times 3600 \text{ mm/min} = \pi(0.1)^2 \times 3600 = 36 \text{ mm}^3\text{/min}$$

$$= 0.6 \text{ mm}^3\text{/sec (@ multiplier = 1)}$$

$$= 0.57 \text{ mm}^3\text{/sec (@ multiplier = 0.95)}$$

Samples were printed with fiber orientations of 0˚, 45˚, and 90˚. Every layer of the dogbone was printed at the same angle and orientation, with no alternation of laminate orientation between layers, with the orientation codes for the fiber orientation angles for the samples being:

90˚ samples: [90]

45˚ samples: [45]

0˚ samples: [0]

The infill was set to 100%, and each layer in a sample was printed using the same parameters. The print bed was set to 25˚C and the printing speed was held constant at 3600 mm/s. All dogbones were printed in batches of three samples (with the same parameters) in the same place on the print bed in case of non-uniform heating. The samples were left to cool on the print bed before removal.

2.1.3 *Hot-Pressed Samples*

Before punching the control samples, filament from the melt-spinner was placed between two sheets of Teflon with a 0.60 mm spacer and inserted between two heated plates of a benchtop hydraulic press (Carver 3851-0, USA) at 215˚C or 225˚C. The filament was pressed stepwise at 0.25 tons per 5 minutes to eliminate air bubbles, then held at 1 ton until cool.

2.2 Material Characterization

Fixing and recovery ratios were calculated after preforming a 1WSMC using DMA. The 1WSMC is a thermomechanical cycling of an SMP[2], wherein the SMP is heated above its T_{trans} to a rubbery state, then deformed under stress to a predetermined strain. The force is held constant as the SMP is cooled back below T_{trans} to fix the polymer chains. The sample is unloaded, and the temperature is increased above T_{trans}, triggering the release of the stored strain energy as the SMP recovers[2]. The strains measured before and after deformation, after unloading, and after recovery can be used to determine the fixing and recovery ratios.

2.3 Cytocompatibility Assay

All SMP and control samples were sterilized in ethanol for 1 hour and dried for 24 hours, then rinsed in sterile PBS and conditioned with Basal Medium Eagle (BME) with 10% fetal bovine serum for 1 hour to help with cell attachment. C3H10T1/2 murine fibroblasts (ATCC) were cultured as previously described[3]. Cells were then droplet seeded 15,000 cells/mL onto all samples and incubated at 37℃ for 48 hours.

2.4 Statistical Analysis

One-way ANOVA was performed with Tukey's HSD for multiple comparison testing, and a two-way ANOVA was performed for comparisons with multiple variables. Temperature, multiplier, and fiber

orientation data were analyzed as categorical factors. Means were considered statistically different for $p < 0.05$. Bartlett's test or F-test was used to determine equal variance. Comparisons between fixing ratios and between recovery ratios were made within each printed and punched group to determine the effect of printing parameters, within each hot-pressed group to determine the effect of temperature, and between the printed and punched groups to determine the effect of raster-printed edges.

1. Gordeev, E. G., Galushko, A. S. & Ananikov, V. P. Improvement of quality of 3D printed objects by elimination of microscopic structural defects in fused deposition modeling. (2018).
2. Mather, P. T., Luo, X. & Rousseau, I. A. Shape Memory Polymer Research. *Annu. Rev. Mater. Res.* **39**, 445–471 (2009).
3. Davis, K. A., Luo, X., Mather, P. T. & Henderson, J. H. Shape Memory Polymers for Active Cell Culture. *J. Vis. Exp.* 2–6 (2011) doi:10.3791/2903.