Supplementary Information

Mass-Producible Microporous Silicon Membranes for Specific Leukocyte Subset Isolation, Immunophenotyping, and Personalized Immunomodulatory Drug Screening *In Vitro*

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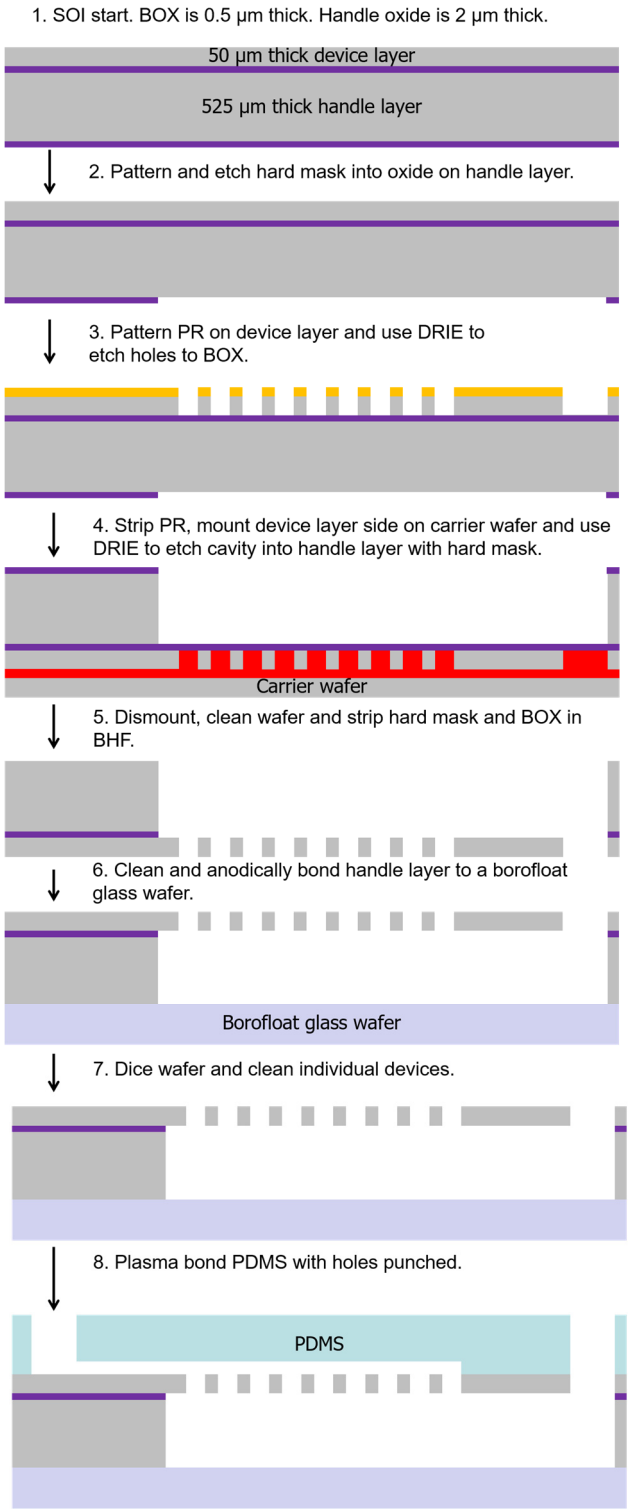


Fig. S1 Cross-sectional process flow for the fabrication of the SiMIPA device. Dark purple indicates thermal SiO_2, gold represents photoresist (PR), and red represents Crystal Bond 555. Drawings are not to scale.

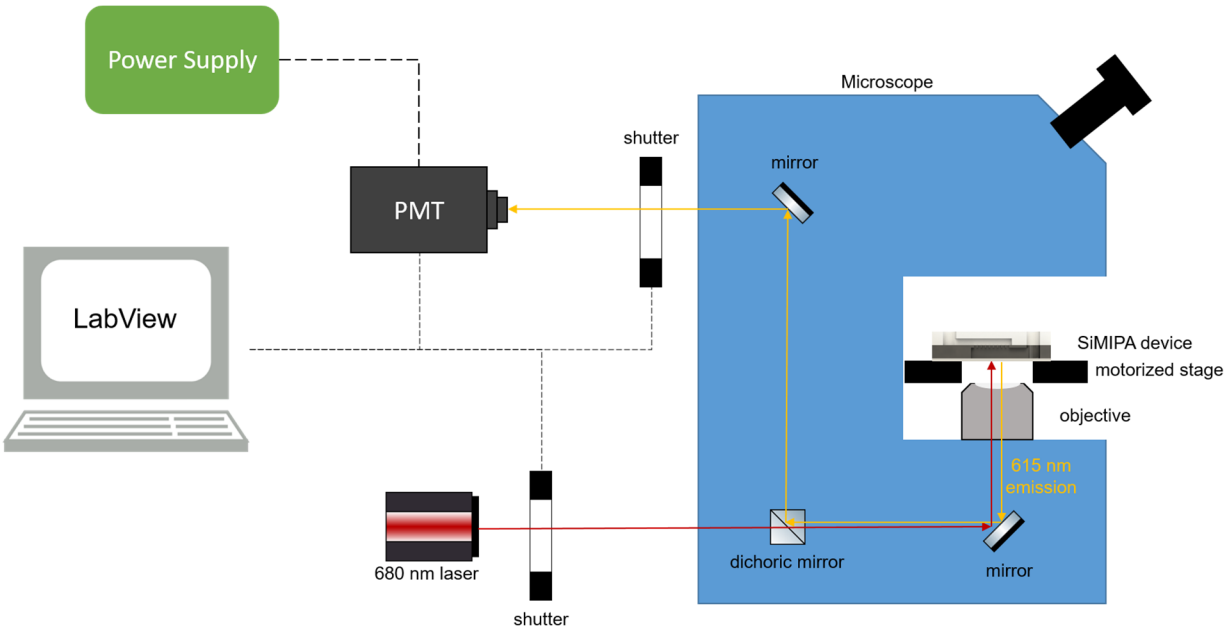


Fig. S2 Schematic of the optical setup for the quantification of secreted IL-2 in the SiMIPA using the human IL-2 AlphaLISA immunoassay. The computer with LabView is used to control both the shutters and the gain on the PMT as well as read the signal from the PMT.

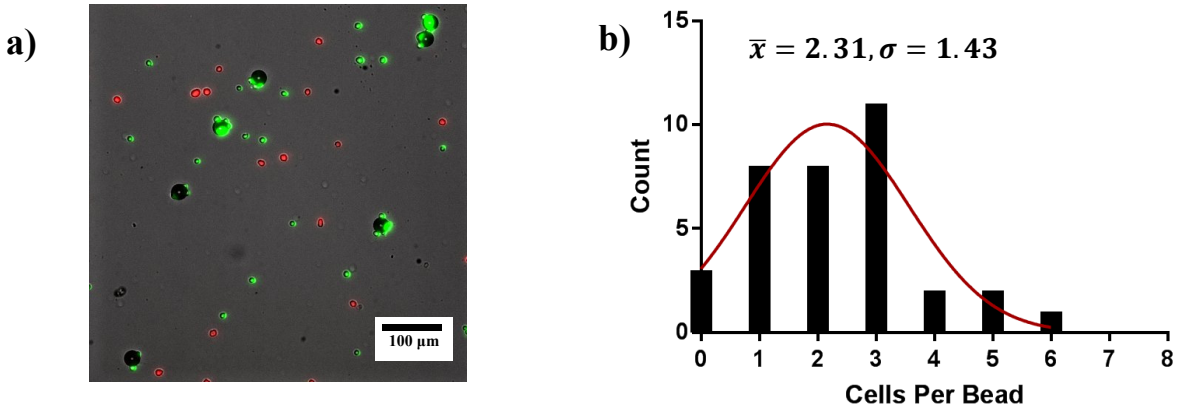


Fig. S3 Chacterization of selective capture of CD3+ Jurkat cells to anti-CD3 coated polystyrene beads from a typical experiment. (a) Composite brightfield and flouresence optical micrograph showing selective capture of CD3+ Jurkat cells (green) on anti-CD3+ coated polystyrene bead and THP-1 cells (red). (b) Histogram of average number of CD3+ Jurkat cells counted per anti-CD3 coated polystyrene bead.

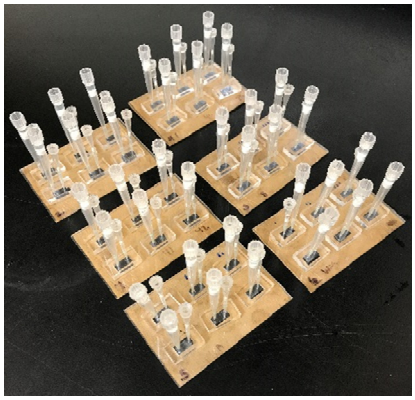


Fig. S4 SiMIPA devices on acrylic slides used during imaging and AlphaLISA measurement. The acrylic slides (brown) have rectangular holes cut in them where the SiMIPA devices sit so the transparent (glass) side of the SiMIPA device can be exposed to the laser used in the custom AlphaLISA setup (Fig. S2). Pipette tips are present in the PDMS inlet and outlet of each device. Pipette tips are present in the PDMS inlet and outlet of each device.

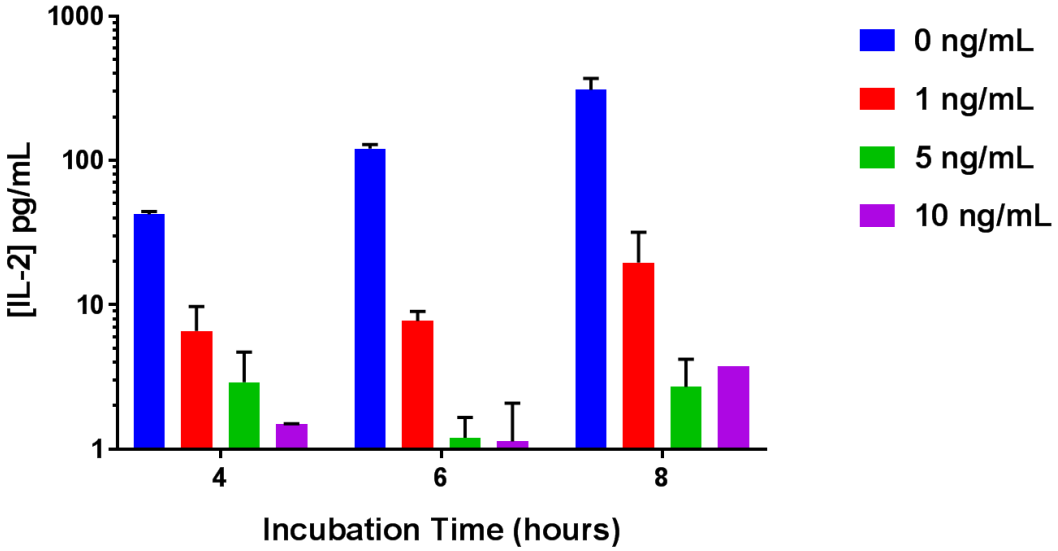


Fig. S5 ELISA quantification of IL-2 concentration in cell culture supernatant after incubation with PMA/Ionomycin and Tacrolimus. Each bar represents the average of 3 measurements. Error bars are one standard deviation above the mean.

Measuring IL-2 secreted from Jurkat cells under the influence of tacrolimus using ELISA

Jurkat cells were taken from culture, centrifuged at 1200 RPM for 5 minutes, and resuspended at $1x10^6$ cells/mL in fresh medium. This suspension was split in to five new 25 cm^2 culture flasks (Sigma). The final volume in each flask was 7 mL. After transfer to the culture flasks, a mixture of PMA and Ionomycin (Sigma) was added to four of the five flasks for a final concentration of

100/1000 ng/mL PMA/Ionomycin. Immediately after adding the PMA/Ionomycin, tacrolimus (Sigma), was added to the flask for a final concentration of either 1, 5, or 10 ng/mL. One flask was used as a control and no PMA/Ionomycin or tacrolimus was added. All flasks were then transferred to a CO_2 cell culture incubator (Thermo Scientific) and allowed to incubate. At each sample interval (4, 6, or 8 hours), the flasks were removed from the incubator and a 600 uL sample was taken from each flask, then immediately placed back in the incubator. The samples were centrifuged at 1200 RPM at 4ºC for 10 mins. The sample supernatants were then collected and stored at -80 ºC. To quantify the IL-2 present in the cell culture supernatant samples, the samples were first thawed and assayed using a commercially available ELISA kit (ThermoFisher, 88-7025-22) and plate reader (BioTek, SynergyH1).

The ELISA results are displayed in Fig. S5. The 0 ng/mL Tacrolimus condition increased at each time point. The 1 ng/mL Tacrolimus condition showed suppressed IL-2 secretion compared to 0 ng/mL, and the concentration also continued to increase over time. Higher concentrations of Tacrolimus greatly suppressed IL-2 secretion compared to both 0 ng/mL and 1 ng/mL. For the 5 ng/mL and 10 ng/mL, the IL-2 concentration can be assumed to be zero, as the rated limit of detection of the ELISA kit used was 2 ng/mL. Trypan blue viability stains showed that the viability was >99% for all Tacrolimus concentrations (data not shown). Thus, it can be inferred that concentrations of Tacrolimus in cell culture 5 ng/mL and above will completely suppress the cell's ability to produce IL-2 in response to stimulation by PMA/Ionomycin.

Finite element modeling of the deflection of the PDMS MIPA membrane

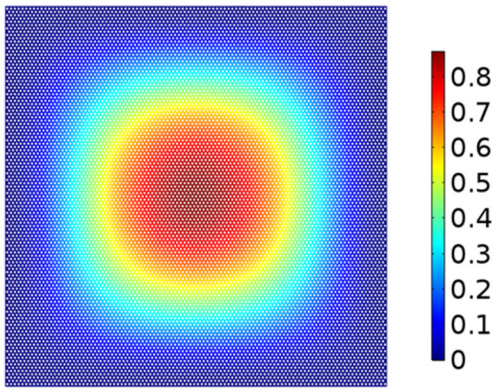
COMSOL was also used to model the 3 x 3 x 0.01 mm^3 PDMS MIPA membrane that was previously used[1,2]. The pore diameter and lattice were identical to the SiMIPA, but the PDMS membranes were drawn with the support posts used previously (60 μm diameter circles in a square lattice with 200 μm center-to-center spacing) as well as without support posts. Applied stresses were modified for the 0.01 mm thick membrane using Equation 1. Fig. S6a shows the deflection of the PDMS membrane without support posts at a flow rate of 10 μL/s and Fig. S6b shows a plot of the maximum deflection modeled at various flow rates. At only 10 μL/s, the PDMS membrane without support posts deflects 873 mm! Since the chamber is only 0.10 mm tall in MIPA device, there is a definite need for support posts to prevent the membrane from collapsing to the floor of the chamber. Fig. S6c shows the calculated von Mises stress on the PMDS membrane without posts at a flow rate of 10 μL/s and Fig. S6d shows a plot of the maximum von Mises stress at various flow rates. From Fig. S6c, it is clear that the stress pattern is the same as for the Si membrane. However, likely due to the thinner PDMS membrane, the maximum von Mises stress is ~6.3x larger for the same flow rates than on the Si membrane. For 10 μL/s flow rates, the maximum von Mises stress is ~23 MPa, which is an order of magnitude greater than the yield stress of PDMS (2.24 MPa). Thus, the PDMS membrane would certainly break at 10 μL/s flow rates. By extension, the PDMS membrane would also fail at larger flow rates since the stresses are even greater.

Fig. S6e shows the calculated deflection of the PDMS membrane with support posts under 2000 μL/s flow rate and Fig. S6f shows the maximum deflection at various flow rates. The deflection pattern shown in Fig. S6e is representative of the deflection pattern at all flow rates. The maximum deflection occurs between the support posts. At 2000 μL/s flow rate, the deflection is ~6.7 mm, which means the PDMS membrane would aggressively collapse to the bottom of the chamber. However, at 20 μL/s, the maximum deflection was only ~67 μm, which means the membrane would not collapse to the bottom of the chamber. At 100 μL/s the maximum deflection

was calculated to be ~ 334 μm, which indicates that the membrane would have collapsed to the bottom of the chamber. Fig. S6g is a plot of the calculated von Mises stress on the PDMS membrane with support posts at a flow rate of 2000 μL/s and Fig. S6f is a plot of the maximum von Mises stress at various flow rates. The maximum von Mises stresses are located at the support posts and are ~71x less and ~ 11x less than for the free standing PDMS and Si membranes, respectively. Thus, the support posts are quite effective at reducing the stress on the membrane. The regression analysis shows that the yield stress of PDMS would be eclipsed at ~80 μL/s.

While the simulation data presented in Fig. S6 is contrary to claims made previously that the PDMS MIPA could withstand flow rates up to 333 μL/s without deformation or rupture[1,2], our group's experience with the PDMS MIPA limited flow rates to 0.42 μL/s with a syringe pump and manually pipetted no more than 10 μL at a time to prevent the PDMS membrane from either collapsing to the bottom chamber or ripping due to shear stress. Thus, the simulation data supports our group's observations of the poor mechanical stability of the PDMS membrane and emphasizes the need for more the more robust SiMIPA membrane which did not fail at flow rates of ~2000 μL/s in the simulations or experimentally.

(a)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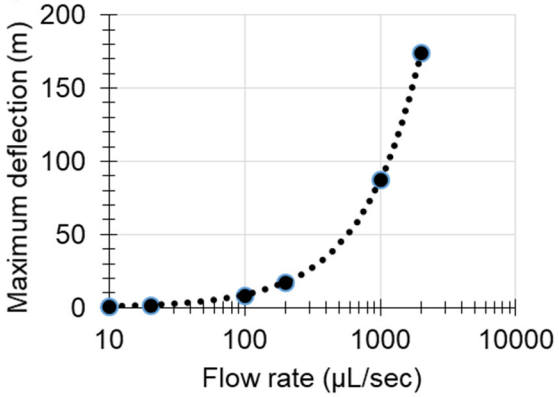
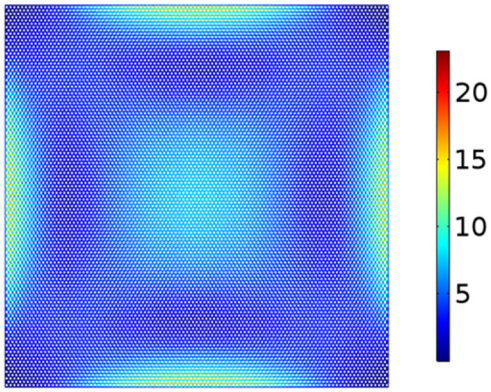
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

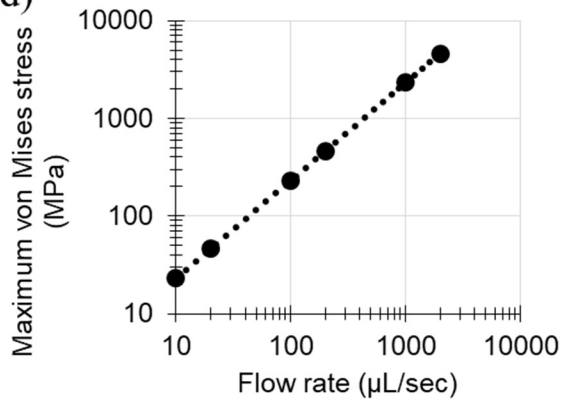
(b)


Maximum deflection (m)
200
150
100
50
0
10
100
1000
10000
Flow rate (µL/sec)

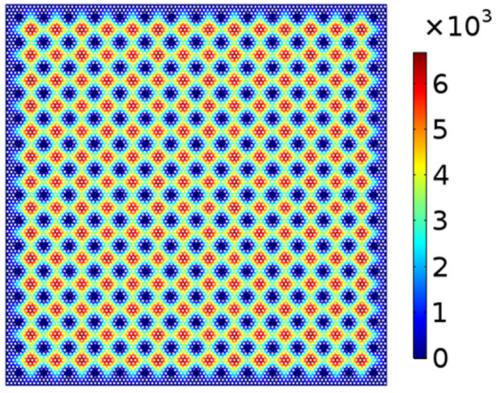
(c)


Surface: von Mises stress (MPa)
20
15
10
5

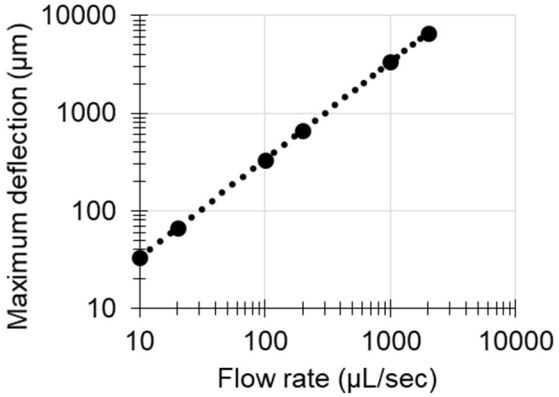
(d)


Maximum von Mises stress (MPa)
10000
1000
100
10
10
100
1000
10000
Flow rate (µL/sec)

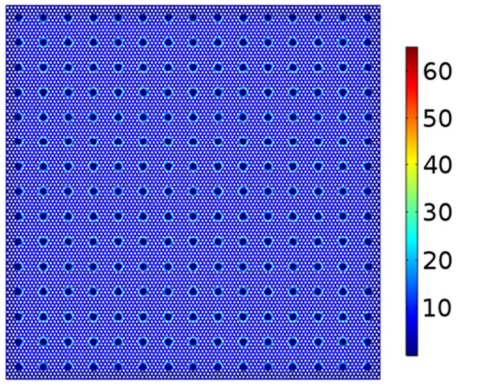
(e)


Surface: Total displacement (µm)
×10^3
6
5
4
3
2
1
0

(f)


Maximum deflection (µm)
10000
1000
100
10
10
100
1000
10000
Flow rate (µL/sec)

(g)


Surface: von Mises stress (MPa)
60
50
40
30
20
10

(h)

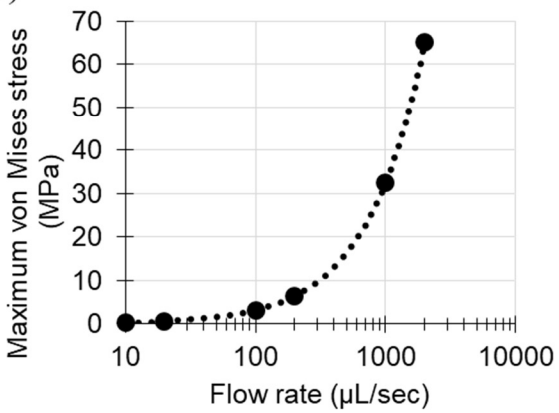

Maximum von Mises stress (MPa)
70
60
50
40
30
20
10
0
10
100
1000
10000
Flow rate (µL/sec)

Fig. S6 Finite element modeling of the deflection of the 3 x 3 x 0.01 mm^3 PDMS microporous membrane without (a-d) and with (e-h) support posts under fluid flow. Both membranes have 20 μm holes in a hexagonal lattice with a center-to-center spacing equal to 30 μm and the 3 x 0.01 mm^2 sides are all fixed in the model. For the membrane with posts (e-h), the 60 μm diameter posts are arranged in a square lattices with a 200 μm center-to-center spacing and the surface of every post is fixed in the model. (a) Rendering from COMSOL showing the deflection of a PDMS membrane under a 10 μL/sec flow rate. (b) A plot of the simulated deflection of the PDMS membrane under different flow rates. The linear regression line has $R^2 = 1$. (c) Rendering from COMSOL showing the von Mises stress on a PDMS membrane under a 10 μL/sec flow rate. (d) A plot of the simulated maximum von Mises stress observed on the PDMS membrane under different flow rates. The linear regression line has $R^2 = 1$. (e) Rendering from COMSOL showing the deflection of a PDMS membrane with support posts under a 2000 μL/sec flow rate. (f) A plot of the simulated deflection of the PDMS membrane with support posts under different flow rates. The linear regression line has $R^2 = 1$. (g) Rendering from COMSOL showing the von Mises stress on a PDMS membrane with support posts under a 2000 μL/sec flow rate. (h) A plot of the simulated maximum von Mises stress observed on the PDMS membrane with support posts under different flow rates. The linear regression line has $R^2 = 1$.

References

1 N.-T. Huang, W. Chen, B.-R. Oh, T. T. Cornell, T. P. Shanley, J. Fu and K. Kurabayashi, *Lab Chip*, 2012, **12**, 4093.

2 W. Chen, N. T. Huang, B. Oh, R. H. W. Lam, R. Fan, T. T. Cornell, T. P. Shanley, K. Kurabayashi and J. Fu, *Adv. Healthc. Mater.*, 2013, **2**, 965–975.