Supplementary Information

Self-sufficient, low-cost microfluidic pumps utilising reinforced balloons

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Supplementary Information 1: Assembly process of the reinforced balloon pumps

**Step 1:**
Affix latex balloon to PCV tubing with clear adhesive tape

**Step 2:**
Attach stocking over balloon and affix with clear adhesive tape

**Step 3:**
Inflate the reinforced balloon to the desired pressure using a hand pump

**Figure S1.** The process of assembling of the reinforced balloon, consisting of three major steps:
Step 1: A latex balloon is affixed to a section of PVC tubing with removable adhesive tape.
Step 2: A stocking is then placed over the balloon and affixed to the PVC tubing.
Step 3: The assembled reinforced latex balloon is coupled with a digital pressure gauge and a manual hand pump via PVC tubing and a 3-way hose connector for inflation.
**Supplementary Information 2: Experimental setup**

**Figure S2.** Experimental setup of the reinforced balloon pump consisting of (i) a reinforced latex balloon, (ii) connected to a liquid filled syringe, (iii) using PVC tubing, (iv) with an optional 2-way valve, (v) interfaced with a PDMS based microfluidic structure, (vi) using Tygon® tubing.
**Supplementary Information 3:** Detailed geometry of microfluidic structures used in the manuscript

![Diagram of microfluidic structures](image)

**Figure S3.** Detailed geometry of the microfluidic structures used in our work, including (a-b) straight channels, (c) serpentine channel used for long-term experiments, (d) straight channel with U-shaped contraction used for hydrodynamic orbiting of cells, (e) Flow-focusing system with an integrated expanded channel used for dynamic exchange of solutions, and (f) U-shaped channel used for mechanobiology experiment.
Supplementary Information 4: Pressure-volume curve for various balloon configurations

Figure S4a. Inflation pressure-normalised volume curve ($P_{\text{inflation}}$ vs. $V/V_{\text{max}}$) obtained for the reference balloon. Images show the expansion of the reference balloon at various pressures. Error bars represent average ± standard deviation obtained in three sets of independent experiments.
Figure S4b. Inflation pressure-normalised volume curve ($P_{\text{inflation}}$ vs. $V/V_{\text{max}}$) obtained for the latex balloon reinforced with one layer of elastane fibres (B + 1R) using a 15-denier stocking. Images show the expansion of the reinforced balloon at various pressures. Error bars represent average±standard deviation obtained in three sets of independent experiments.
Appendix 1: Radius-inflation pressure curve of a latex balloon

The radius-inflation pressure curve of latex balloons is described using the spherical thin shell model \(^{1-4}\), as given below:

\[
P_{\text{inflation}} = P_{\text{balloon}} - P_{\infty} = \frac{2 \gamma t_o}{r_o} k(\lambda) \left( \lambda^{-1} - \lambda^{-7} \right)
\]

where, \(P_{\text{balloon}}\) and \(P_{\infty}\) are the internal balloon and ambient pressure, \(\gamma\) is the shear modulus of the latex, \(t_o\) and \(r_o\) are thickness and non-deformed radius of the balloon, \(\lambda = r/r_o\) is the stretch ratio of the balloon, and \(k(\lambda)\) is a dimensionless parameter, which according to the Mooney-Rivlin model \(^{5}\) can be calculated as \(k(\lambda) = 1 + \alpha \lambda^2\). The radius-inflation pressure curve of latex balloons has been investigated in detail in our previous work \(^{6}\).
Supplementary Information 5: Comparing the volume of the reference and reinforced balloons

Figure S5. Comparing the volume of the reference and reinforced latex balloons. (a) The reference (non-reinforced) latex balloon inflated to $P_{\text{inflation}} = 2.5$ kPa. (b) The latex balloon reinforced with three layers of elastane fibres (B + 3R) inflated to $P_{\text{inflation}} = 25$ kPa.
Supplementary Information 6: Variations of balloon inflation pressure and reinforcing coefficient versus the number of reinforcing layers

Figure S6. Variations of balloon inflation pressure and ‘reinforcing coefficient’ versus the number of reinforcing layers, obtained at \( V/V_{\text{max}} = 0.75 \). Error bars represent average±standard deviation obtained in three sets of independent experiments.
Supplementary Information 7: Pressure-volume curves of reinforced balloons obtained with various stocking brands

Figure S7. Pressure-normalised volume ($P_{\text{inflation}}$ vs. $V/V_{\text{max}}$) curves of reinforced balloons obtained with Big W 15-denier and Target 15-denier. Figure legend notation: B represents the latex balloon while R represents the reinforcing elastane layer. Error bars represent average ± standard deviation obtained in three sets of independent experiments.
**Supplementary Information 8:** Fatigue analysis of reinforced balloons over repeated inflations

**Figure S8a.** Variations of balloon inflation pressure versus the number of inflations ($P_{\text{inflation}}$ vs. $N_{\text{inflation}}$), obtained for the reinforced balloons using three layers of elastane fibres (B + 3R) and compression socks (B + Rcs). Error bars represent average±standard deviation obtained in five sets of independent experiments.
Figure S8b. Inflation pressure-normalised volume curve ($P_{\text{inflation}}$ vs. $V/V_{\max}$) obtained for the latex balloon reinforced with one layer of compression socks ($B + 1R_{cs}$). Images show the expansion of the reinforced balloon at various pressures. Error bars represent average±standard deviation obtained in three sets of independent experiments.
Figure S8c. Pressure-normalised volume ($P_{\text{inflation}}$ vs. $V/V_{\text{max}}$) curves of reinforced balloons obtained with stockings and compression socks. Figure legend notation: B represents the latex balloon while R represents the reinforcing elastane layer. Error bars represent average ± standard deviation obtained in three sets of independent experiments.
Supporting information 9: Pumping viscous liquids with the reinforced balloon pump

Figure S9. Liquid flow rate versus liquid viscosity ratio ($Q$ vs. $\mu/\mu_{\text{water}}$). Viscosity is changed by using various volumetric ratios of glycerol to water. Liquid is driven through a straight microfluidic channel using reference and reinforced latex balloons. Error bars correspond to average ± standard deviation values obtained in three sets of independent experiments.
Appendix 2: Flow rate of liquid through the microfluidic system operated by a pressure pump

The liquid flow rate of the liquid through the microfluidic system operated by a pressure pump is set by the balloon inflation pressure and the viscous pressure drop along the system, as described below:

\[ P_{inflation} = P_{balloon} - P_{\infty} = \Delta P_{tubes} + \Delta P_{channel} = \frac{128 \mu Q L_{tube}}{\pi D_{tube}^4} + \frac{k \mu Q L_{channel}}{W_{channel} H_{channel}^3} \]  

(2)

where, \( P_{balloon} \) and \( P_{\infty} \) are the internal balloon and ambient pressure, \( \mu \) is the liquid dynamic viscosity, \( Q \) is the liquid flow rate, \( L_{tube} \) and \( D_{tube} \) are the length and internal diameter of interconnecting Tygon® tubes at the inlet and outlet, \( k \) is a dimensionless parameter which depends on the aspect ratio of the microfluidic channel, and \( L_{channel}, W_{channel} \) and \( H_{channel} \) are the length, width and height of the microfluidic channel, respectively.
Supporting information 10: Instability patterns at the interface of two miscible liquids with a large viscosity contrast

Figure S10. Instability patterns at the interface of water and glycerol due to a large viscosity contrast. ($\mu_{\text{glycerol}} = 1078.2$ mPa.s, $\mu_{\text{water}} = 0.935$ mPa.s) (a) Schematics of the microfluidic T-junction structure. (b) Laminar flow observed when operating the system with a pair of reference balloon pumps at $P_{\text{inflation}} = 2.5$ kPa. (c) Instability patterns when operating the system with a pair of reinforced balloons (B + 2R) at $P_{\text{inflation}} = 15$ kPa.
Supplementary Information 11: Increasing the generation rate of droplets using reinforced balloon pumps

Figure S11. Increasing the generation rate of droplets through a flow focusing droplet generation system by implementing reinforced balloons pumps. Mineral oil (RTM8 oil, Sigma-Aldrich) with a viscosity of 10.37 mPa.s was used as the oil phase and water coloured with blue dye at a volumetric ratio of 20:1 with a viscosity of 0.93 mPa.s was used as the water phase: (a-b) Droplet generation using reference latex balloons operating at \( P_{\text{oil}} = 2.5 \) kPa and various \( P_{\text{water}}/P_{\text{oil}} \) ratios. (a'-b') Droplet generation using reinforced latex balloons operating at \( P_{\text{oil}} = 25 \) kPa and various \( P_{\text{water}}/P_{\text{oil}} \) ratios. (c) Droplet generation rate versus water to oil pressure ratio (\( N_{\text{droplet}} \) vs. \( P_{\text{water}}/P_{\text{oil}} \)) obtained by reference and reinforced latex balloons. Error bars represent average±standard deviation obtained in four sets of independent experiments.
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<th>Flow rate range</th>
<th>Flow stability over time</th>
<th>Fabrication or maintenance requirement</th>
<th>Liquid sensitive to liquid properties</th>
<th>Temperature sensitive</th>
<th>Bio-compatible</th>
<th>External power requirement</th>
<th>Supporting equipment requirement</th>
<th>Cost</th>
<th>Suitability for point-of-care applications</th>
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Notes:

i. Low flow rates are defined as $Q < 1 \text{ mL/min}$. Medium flow rates are defined as $1 < Q < 10 \text{ mL/min}$. High flow rates are defined as $Q > 10 \text{ mL/min}$.

ii. Rapid here refers to dynamic response times in the order of milliseconds.

iii. Being sensitive to liquid properties such as viscosity (pressure pumps), electrical conductivity (electrohydrodynamic pumps), pH (electroosmotic, surface tension driven pumps).

iv. Peristaltic pumps are particularly suitable for recirculation of liquid for long-term experiments.

v. Commercially available miniaturized piezoelectric pumps are also available, as presented in (33).

vi. In this work (18), commercially available miniaturized diaphragm pumps have been utilized.

vii. The pumps referred to here are made of gallium based liquid metal alloys, which can be placed onto the desired locations of the system using a pipette.

viii. These pumps release gallium ions to the solution.

ix. Pressure vs temperature curves are presented in Figure 2d.

x. Flow rate vs liquid viscosity curves are presented in Figure S9.

xi. Pressure vs time curves are presented in Figure 2a.
**Supplementary Information 12:** Variations of flow rate versus balloon inflation pressure through the microfluidic channel with a U-shaped contraction

**Figure S12.** Variations of flow rate versus balloon inflation pressure ($Q$ vs. $P_{\text{inflation}}$) through the microfluidic channel with a U-shaped contraction used for the hydrodynamic manipulation of human monocytes. Error bars represent average ± standard deviation obtained in five sets of independent experiments.
Supplementary Information 13: Microscopic imaging of large human monocytes

Figure S13. Microscopic images revealing the large size contrast between normal THP-1 monocytes ($d_{\text{cell}} = 12.5 \pm 1.4 \ \mu m$) and large monocytes ($d_{\text{cell}} = 40 \pm 3.6 \ \mu m$).
**Supplementary Information 14:** Lagrangian particle tracking model for the trajectory of human monocytes downstream of the U-shaped contraction

**Figure S14.** Flow streamlines at the downstream of the U-shaped contraction obtained by the Lagrangian particle tracking model. (a) Trajectory of normal THP-1 human monocytes ($d_{cell} = 12.5 \, \mu m$). (b) Trajectory of large human monocytes ($d_{cell} = 40 \, \mu m$).
**Supplementary Information 15:** Characterisation of capturing performance of the vortex at various cell concentrations

**Figure S15.** The cell vortex saturation characteristics of our microfluidic system. (a) Variations of the maximum number of captured cells against cell concentration ($N_{\text{orbit}}$ vs. $C_{\text{cell}}$). (b) Variations of the vortex saturation period against cell concentration ($\Delta t_{\text{sat}}$ vs. $C_{\text{cell}}$). Error bars represent average ± standard deviation obtained in five sets of independent experiments.
**Supplementary Information 16:** Laminar flow of neighbouring red and blue streams at low pressures

**Figure S16a.** Laminar flow of miscible red and blue flow using a pair of reference balloons at \(P_{\text{inflation}} = 2.5 \text{ kPa}\) obtained by experiments: (a) Static conditions. (b) Expanded core flow by manually squeezing of the red balloon. (c) Narrowed core flow by manually squeezing of the blue balloon.
Figure S16b. Laminar flow of miscible red and blue flow using a pair of reference balloons at $P_{\text{inflation}} = 2.5$ kPa obtained by numerical simulations: (a) Reference mode. (b) Expanded core flow by increasing the inflation pressure of the red balloon. (c) Narrowed core flow by increasing the inflation pressure of the blue balloon.
**Supplementary Information 17:** Vortex formation at the expanded channel at high inflation pressures

![Diagram of vortex formation](image)

**Figure S17.** Flow streamlines obtained by numerical simulations revealing the formation of vortices at the expanded channel at high inflation pressures using reinforced balloons.
**Supplementary Information 18:** Dynamic pressure changes obtained by manually squeezing a reinforced balloon

**Figure S18.** Dynamic pressure changes of a reinforced balloon following manual squeezing.  
(a-b) A reinforced balloon before and after manual squeezing with a digital pressure gauge.  
(c) The dynamic pressure curves obtained by various squeezing patterns.
Supplementary Information 19: Concentration profiles of red and blue flows through the flow focusing systems

Figure S19a. Top-view concentration profile of red and blue flows through the flow focusing system obtained by numerical simulations at high inflation pressures: (a) Reference mode: $P_{\text{red}} = 15$ kPa, $P_{\text{blue}} = 17.5$ kPa. (b) Narrowed core flow: $P_{\text{red}} = 15$ kPa, $P_{\text{blue}} = 22.5$ kPa. (c) Expanded core flow: $P_{\text{red}} = 20$ kPa, $P_{\text{blue}} = 17.5$ kPa. With insets showing the cross sections of the channel prior to the expansion.
Figure S19b. Isometric-view concentration profile of red and blue flows through the flow focusing system obtained by numerical simulations at high inflation pressures: (a) Reference mode: $P_{\text{red}} = 15$ kPa, $P_{\text{blue}} = 17.5$ kPa. (b) Narrowed core flow: $P_{\text{red}} = 15$ kPa, $P_{\text{blue}} = 22.5$ kPa. (c) Expanded core flow: $P_{\text{red}} = 20$ kPa, $P_{\text{blue}} = 17.5$ kPa. With insets showing the cross sections of the channel prior to the expansion.
Supplementary Information 20: Rapid exchange of solutions enabled by the manual squeezing of a reinforced balloon

Figure S20. Rapid exchange of solutions within an expanded channel downstream of a flow focusing system enabled by the manual squeezing of a reinforced balloon. (a) The regions of interests (ROIs) for the vortices and core flow. (b) The normalised mean intensity of the red channel at the vortex and core ROIs extracted using ImageJ over time.
Supplementary Information 21: Investigating the mechanobiology of endothelial cells under shear stress using reinforced balloon pumps

Figure S22. Investigating the mechanobiology of HAECs under shear stress utilising a reinforced balloon pump: (a1-b1) Fluorescent micrograph of HAECs following 9 hours of stimulation under low (3 dyne/cm²) and high (12 dyne/cm²) shear stress conditions, (a2-b2) Coloured contours of stress fiber orientation angle.
References: