Supplementary Information (SI) for Lab on a Chip.
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Supporting Information

Revealing Transport, Uptake and Damage of Polystyrene Microplastics using a Gut-Liver-on-a-Chip

Yushen Wang[1,2], Junlei Han[1,2], Wenteng Tang[1,2], Xiaolong Zhang[1,2], Jiemeng Ding[1,2], Zhipeng Xu[3], Wei Song[4], Xinyu Li[4*], and Li Wang[1,2*]

[1]School of Mechanical Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China

[2]Shandong Institute of Mechanical Design and Research, Jinan 250353, China

[3]Division of Clinical Medicine School of Medicine & Population Health University of Sheffield Medical School Beech Hill Road Sheffield S10 2RX

[4]Department of Oncology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, China

** Corresponding Authors: **Li Wang** (liwang@qlu.edu.cn) or*

***Xinyu Li** (lixinyu@sdfmu.edu.cn)*

Highlights:

- Development of a gut-liver-on-a-chip (GLOC) featuring biomimetic intestinal peristalsis and a dynamic hepatic flow environment.
- The process of translocation in the intestines and uptake in the liver of microplastics following oral ingestion was unveiled.
- Intestinal peristalsis was found to modulate the translocation rate of microplastics, while regulating hepatic cell injury.

Keywords: Gut-Liver-on-a-chip (GLOC), Microplastics, Intestinal transport, Liver uptake, oxidative stress

1. Experimental and Methods

1.1 Quantification of perfusion flow of culture medium

First, finite element analysis was conducted based on the designed dimensions to determine the theoretical flow rate of the culture medium. Simultaneously, to verify the accuracy of the peristaltic pump in low-speed perfusion, different flow rates were set, and continuous perfusion was conducted for 5 hours. During this process, we collected the medium discharged from the chip and measured its volume to determine the actual flow rate of the chip. We used a high-precision peristaltic pump and 10ml of culture medium, initiated the peristaltic pump, and filled the entire channel with the medium to eliminate the influence of internal factors (such as bubbles) on the experimental results. By setting the parameters of the peristaltic pump, the accuracy of perfusing the culture medium within the flow rate range of 100 μL/h to 1000 μL/h was calibrated, with perfusion time set at 5 hours. Finally, the actual perfusion flow rate of the culture medium was calculated by determining the volume of water collected.

1.2 Static cell culture

We employed static cultures of Caco-2 and HepaRG cells as controls, conducted in Transwell plates (Corning Incorporated, located in Lowell, Massachusetts). These plates contain porous polyester membrane inserts pre-coated with the same type of type I collagen used for the chip devices. Caco-2 and HepaRG cells were seeded at identical densities, with Caco-2 cells seeded on the upper layer and HepaRG cells on the bottom insert. The culture medium was changed every other day.

1.3 Impedance spectroscopy application in TEER detection

Impedance spectroscopy measurements were conducted using Autolab (PGSTAT302N, Herisau, Switzerland) every day. To minimize damage to the cells, we positioned two Ag/AgCl electrodes on the upper and lower layers of the porous membrane for detection. According to the equivalent circuit diagram of impedance measurements, the chip can be divided into four components. Firstly, there is the resistance of the culture medium inside the channel, followed by the vertical resistance allowed by the porous membrane for current to pass through. Lastly, the cell monolayer can be represented by the parallel combination of transmembrane resistance and cell capacitance. Employing the dual-electrode mode of electrochemical impedance spectroscopy, impedance of the non-battery device was measured with a 10 μA alternating current in

the frequency range of 1 MHz to 10 Hz to obtain the electrode resistance. Simultaneously, the optimal frequency range (100-10Hz) was selected based on the relationship between frequency and impedance, and the potential difference between the readout electrodes was recorded.

1.4 Removal of microbubbles in GLOC

During the process of injecting the culture medium into the chip, tiny bubbles are prone to form inside the medium due to the temperature difference between the incubator and the external environment. The presence of bubbles can significantly affect the growth status of the cells and is difficult to eliminate. To avoid this phenomenon, we placed the culture medium in a water bath and heated it to 37°C, which is the same temperature as the incubator, to release the dissolved oxygen in the medium in advance. Then, we placed a storage bottle in the middle of the infusion tube. The storage bottle contained culture medium at 37°C and was kept inside the incubator. Outside the incubator, pressure and fresh culture medium were provided using a peristaltic pump. Due to the pressure difference inside the storage bottle, the culture medium inside the bottle could be transported to the chip. This method not only effectively eliminates the presence of bubbles but also allows for convenient and rapid replacement of the culture medium.

2. Supporting Figures

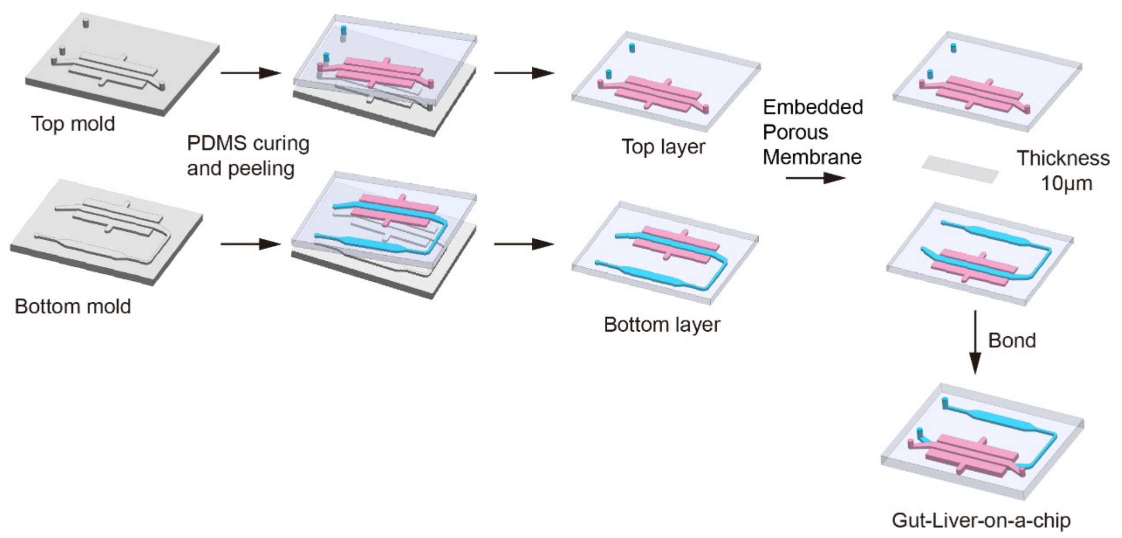

Fig. S1 The fabrication procedures of the gut-liver-on-a-chip.

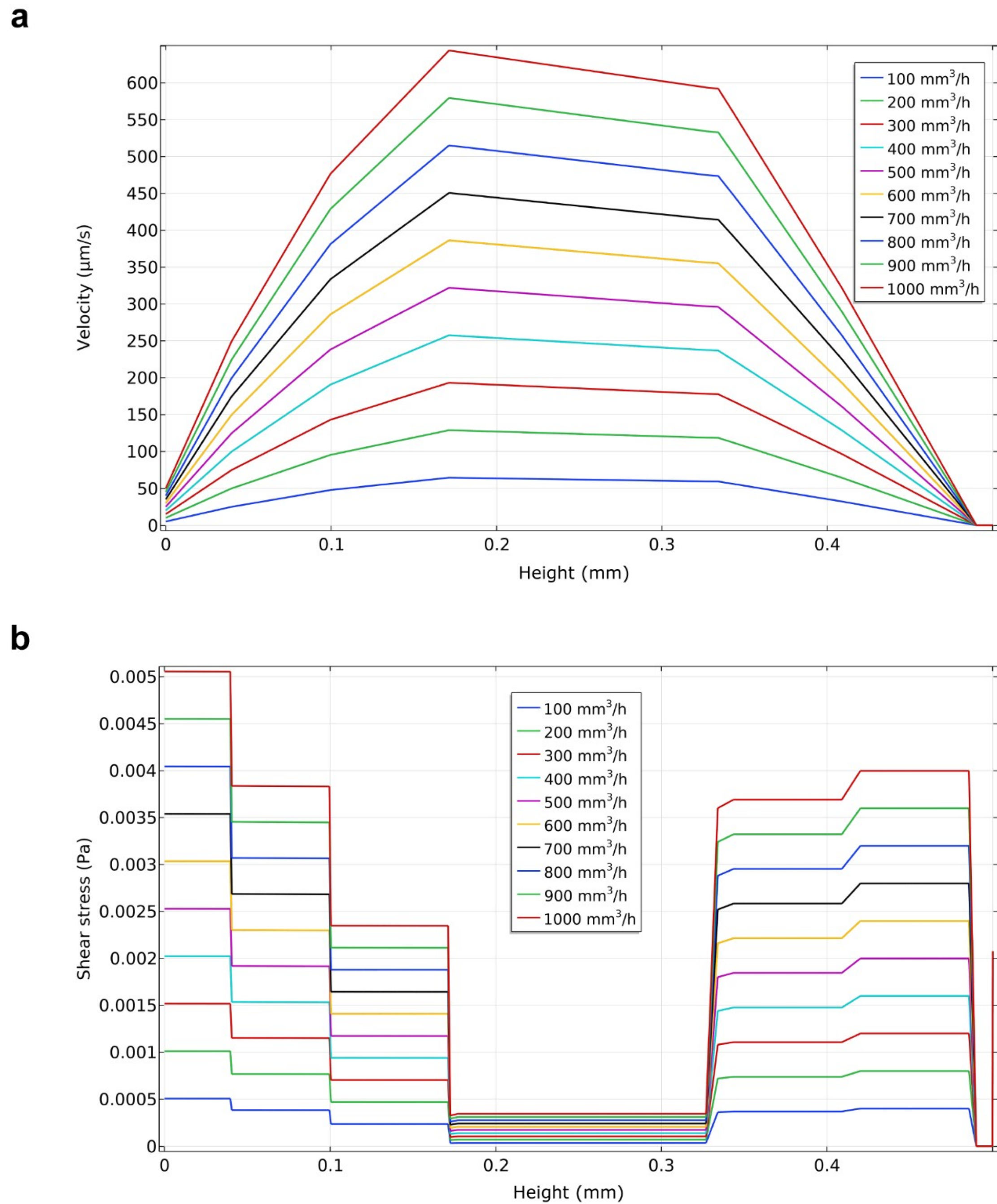

Fig. S2 Simulation of flow Field and shear stress in gut chamber. a, Flow field distribution under different flow rates of culture medium, with the x-axis representing the height of the upper channel. **b,** The distribution of shear stress corresponding to different flow rates of the culture medium.

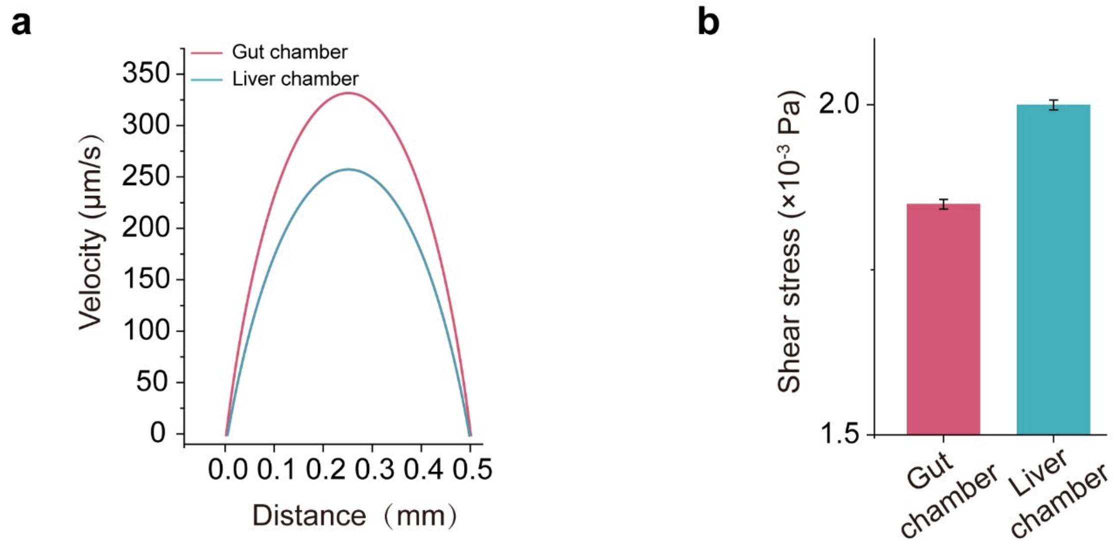

Fig. S3 Comparison of the linear velocity and corresponding shear stress between the intestinal and liver chamber. a, Comparison of linear velocities between the intestinal and liver chamber at a flow rate of 500 μL/h. The average linear velocities in the intestinal and liver chamber are 165.05 μm/s and 128.52 μm/s, respectively. **b,** Comparison of shear stress between the intestinal and liver chamber at a flow rate of 500 μL/h. The shear stress in the intestinal and liver chamber are 0.002 Pa and 0.0018 Pa, respectively.

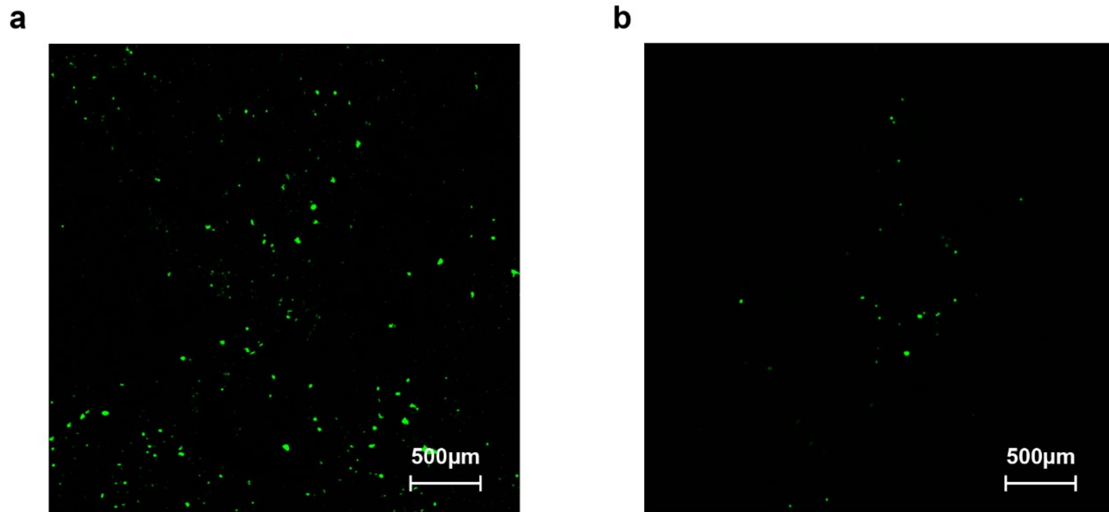

Fig. S4 Comparison of microplastic adsorption by PDMS before and after modification. To verify the effect of PDMS surface modification on microplastic adsorption, we compared the accumulation of fluorescent microparticles before (a) and after (b) modification. The results showed that oxygen plasma treatment and ECM coating effectively reduced microplastic adsorption.

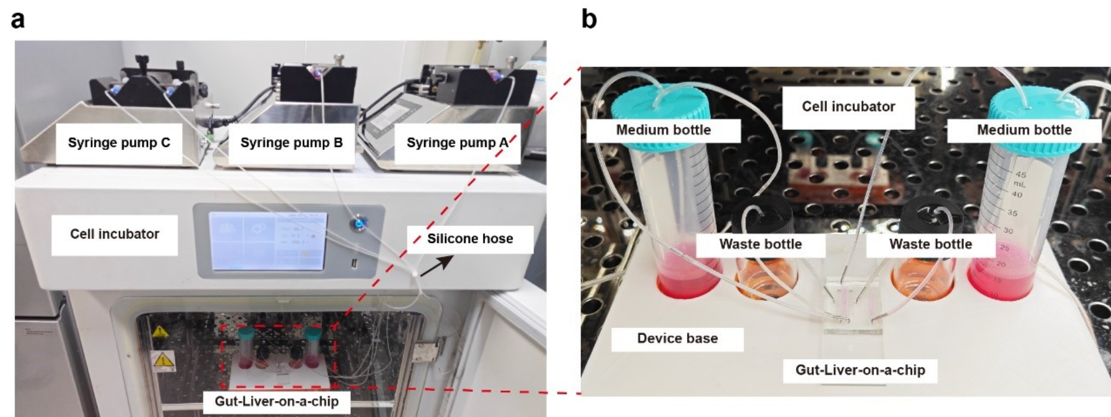

Fig. S5 A photograph showing the proposed GLOC with its culturing component. (a) Photograph of the entire system. (b) Close-up view highlighting the piping connections of the GLOC.

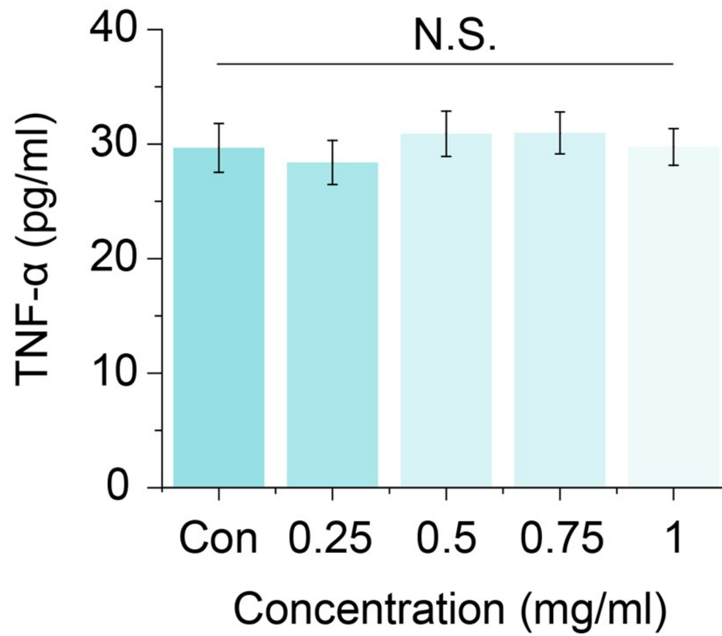

Fig. S6 Release of the pro-inflammatory cytokine TNF-α in a single Caco-2 model. To evaluate the effect of MPs on TNF-α release in Caco-2 cells, five different concentrations of MPs (control, 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, and 1 mg/ml) were applied to Caco-2. The results showed that MPs at concentrations ranging from 0 to 1 mg/ml did not exhibit toxic effects on Caco-2.