ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Man vs. machine: Automated bioink mixing device improves reliability and reproducibility of bioprinting results compared to human operators

Supplementary File

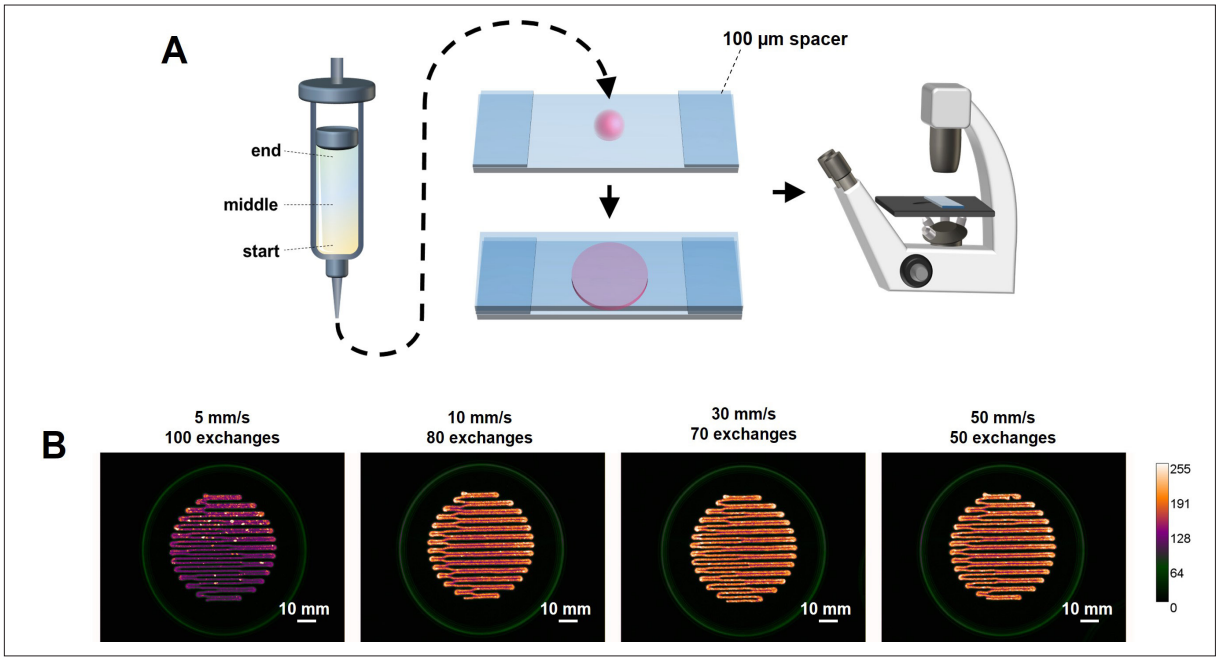

Figure S1. (A) Schematic illustration of cell distribution evaluation of bioink from three different positions. Following the mixing process, a sample of 0.5 mL was taken from the indicated position and placed onto a glass slide. The sample was then compressed into a thin layer by applying pressure to a cover slide propped up by two 100-µm-thick spacers. The resulting bioink layer was observed under a fluorescence microscope to assess the distribution of cells within the bioink. (B) The bioinks of the whole cartridge were extruded as zigzag model on a petri dish to assess the bioink homogeneity.

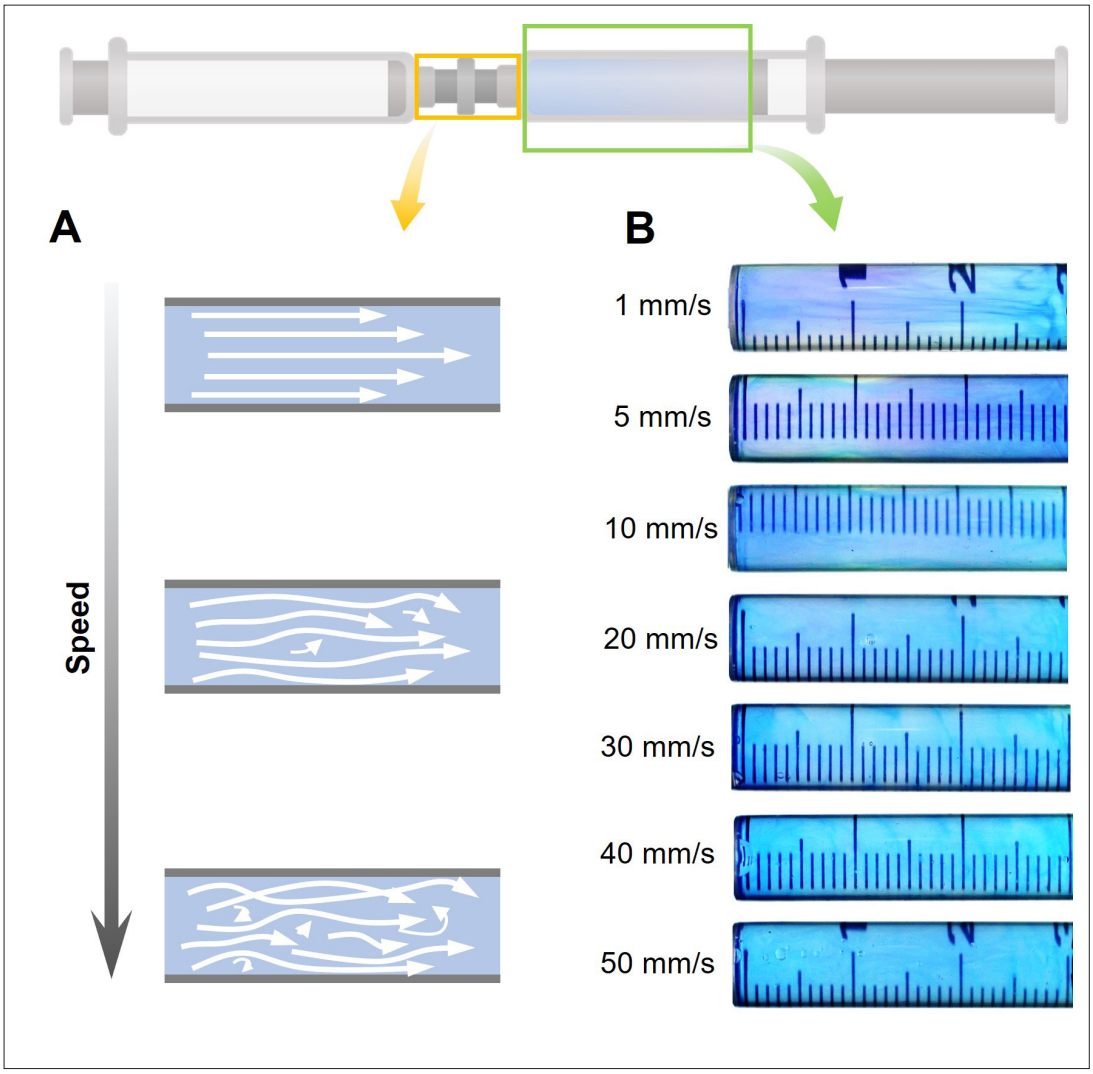

Figure S2. Flow behavior of the hydrogel during mixing influenced by mixing speed. (A) Schematic of the flow pattern of laminar flow and turbulent flow in theory. (B) Photos of mixtures in the syringes show the component mixing status: The experiment was performed using 6% alginate hydrogel and 0.001% methylene blue solution on the automated bioink mixing device for 10 mixing exchanges at different mixing speeds.

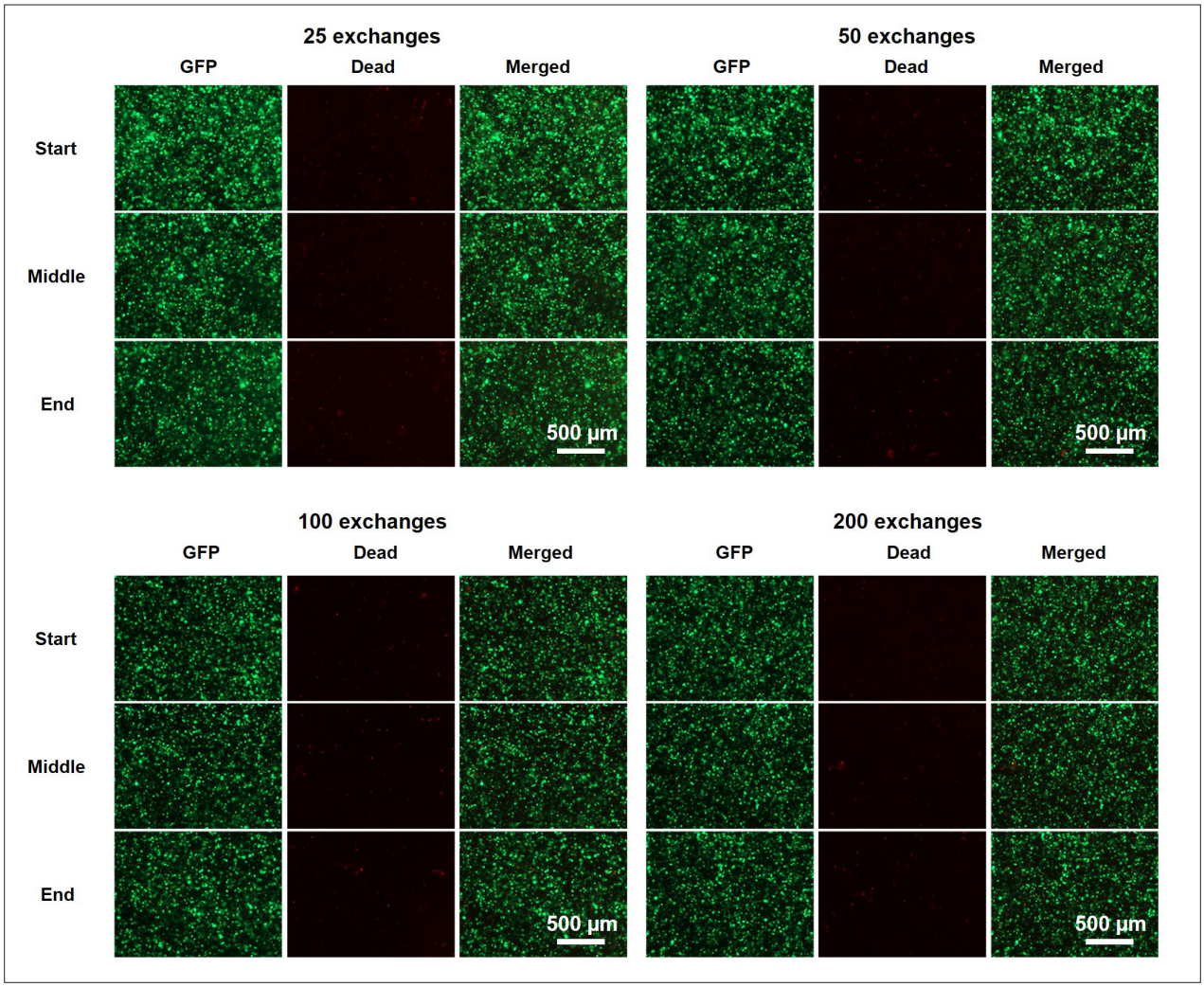

Figure S3. Cell status after mixing different numbers of exchanges. HEK293-GFP cell suspensions were mixed with 6% alginate hydrogel for different number of exchanges varying from 25 to 200 exchanges: Dead cells were stained with ethidium homodimer-1 (red) 1 day after bioprinting, and the green signal indicates GFP expression by living HEK293-GFP cells.

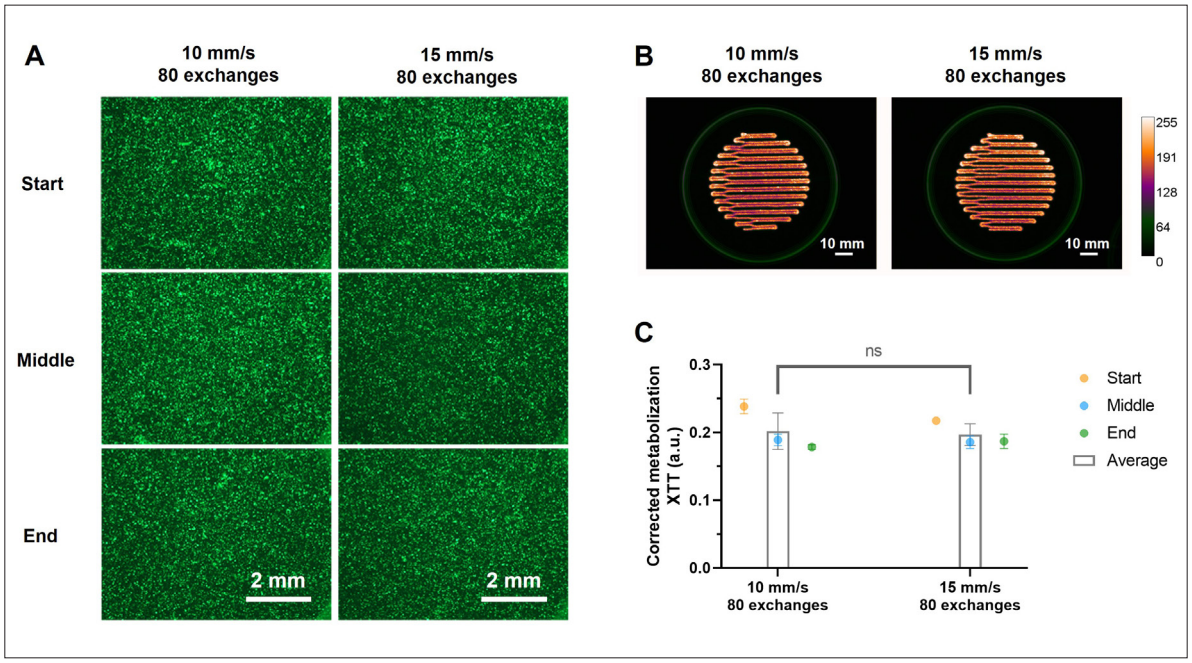

Figure S4. Comparison between bioink mixing at 10 mm/s 80 exchanges and 15 mm/s 80 exchanges. The results of cell distribution (A), extrusion of whole cartridge bioink (B), and cell viability (C) are shown.

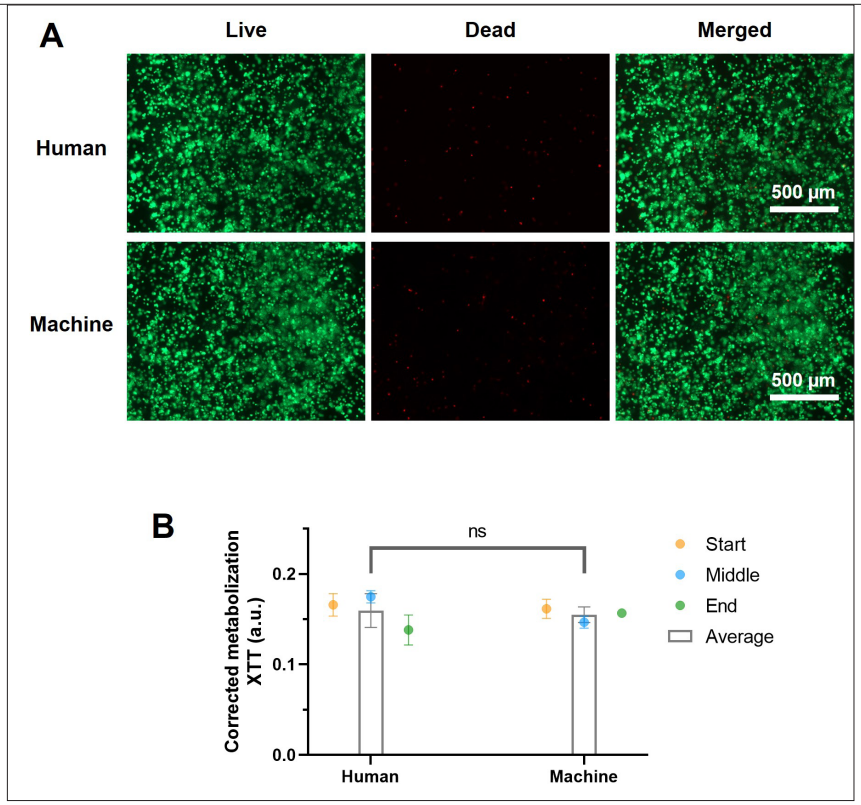

Figure S5. HepaRG cell viability after mixing process. The HepaRG cells were mixed with 6% alginate hydrogel by an experienced human operator or the automated machine (15 mm/s for 80 exchanges). The mixed bioinks were bioprinted in a 48-well plate, and cell viability was immediately characterized. (A) The HepaRG cells were stained with a live/dead staining kit. Living cells were stained green, while dead cells were stained red. (B) The HepaRG cell viability was also measured by XTT assay after mixing and bioprinting.

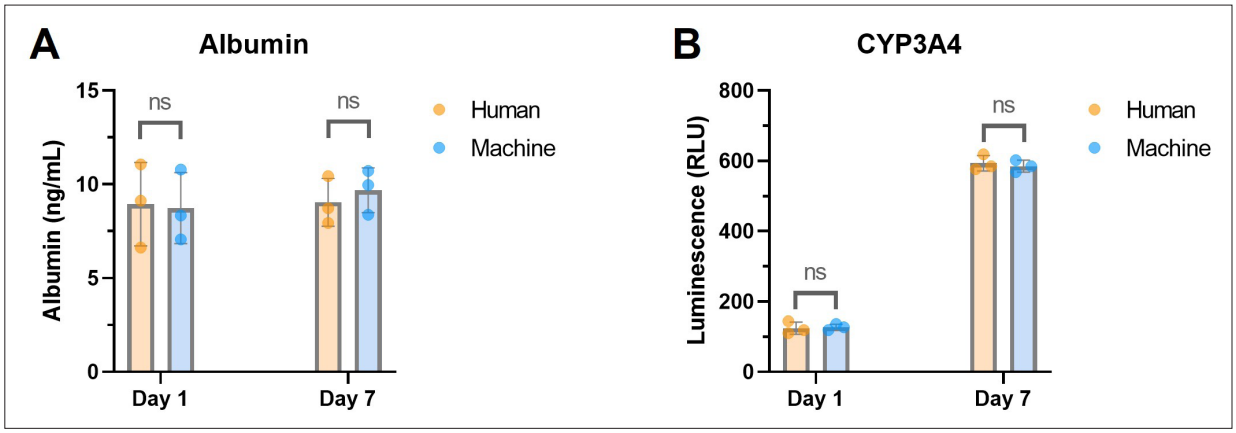

Figure S6. Evaluation of liver functions in HepaRG cells. The HepaRG cells were mixed with 6% alginate hydrogel by an experienced human operator or the automated machine (15 mm/s for 80 exchanges). The mixed bioinks were bioprinted in a 48-well plate and characterized after culture for 1 day and 7 days. (A) Secretion level of albumin in supernatant was measured using the enzyme-linked immunosorbent assay (ELISA; DY1455, Human Serum Albumin DuoSet ELISA, Bio-Techne, Minneapolis, MN, USA). (B) The CYP3A4 activity was detected using luminescent assay according to the manufacturer's instruction (V9001, P450-Glo™ Assays, Promega, Madison, WI, USA).

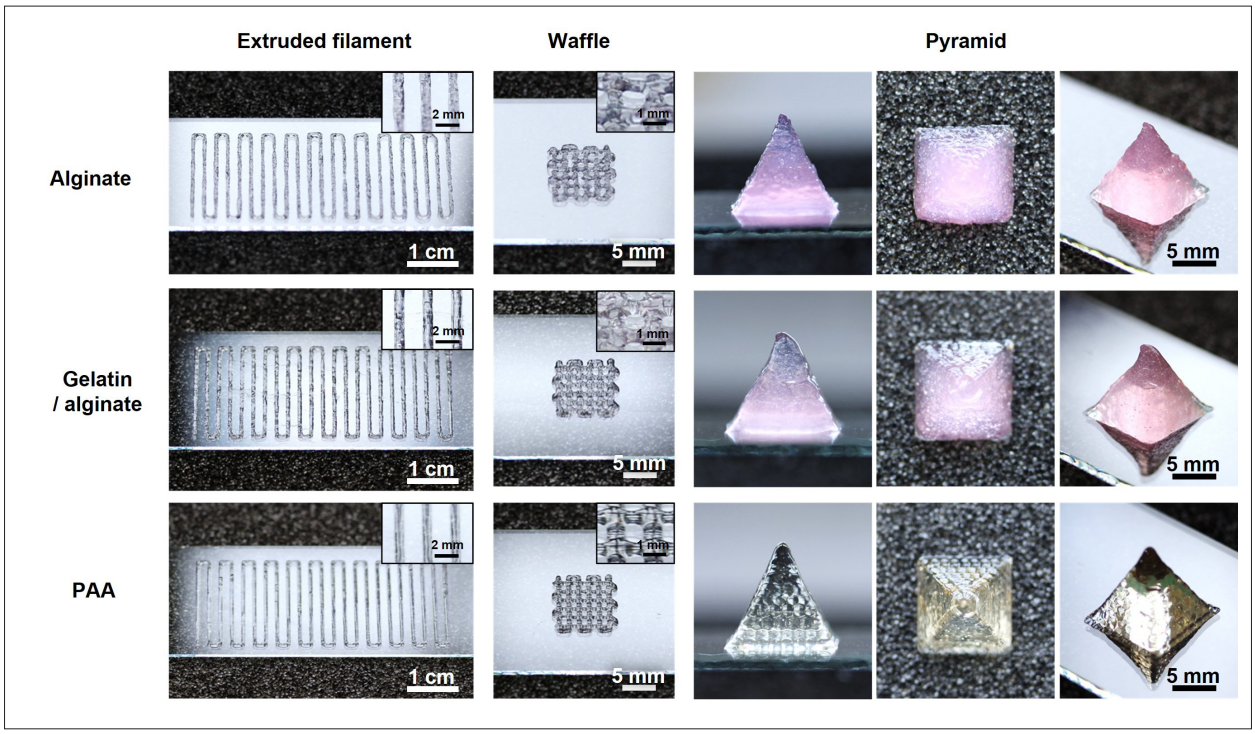

Figure S7. Printability of the bioinks after machine mixing. Three different bioinks (alginate, gelatin/alginate, and PAA) were printed in long filaments, waffle structures, and pyramid models after machine mixing. The insets show high-magnification images to allow for comparison of the quality of filament extrusion and waffle bioprinting.

Table S1. Mixing formula of the hydrogels

Ratio of MB solution volume to alginate hydrogel volume	0.001% MB solution	Alginate hydrogel volume	Alginate concentration	Total volume	Final alginate concentration
1 : 3	0.75 mL	2.25 mL	4.0%	3 mL	3%
1 : 2	1 mL	2 mL	4.5%		
1 : 1	1.5 mL	1.5 mL	6.0%		
2 : 1	2 mL	1 mL	9.0%		
3 : 1	2.25 mL	0.75 mL	12.0%		

Alginate hydrogels of various concentrations were used to test the mixing effect at different ratios of methylene blue (MB) solution volume to alginate hydrogel volume. A mixture with a volume of 3 mL and a final alginate concentration of 3% was obtained for all groups.

Supplementary video:

Video S1. Video of the mixing device.