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MDPI

Development of gelatin-based flexible three-dimensional capillary pattern microfabrication technology for analysis of collective cell migration

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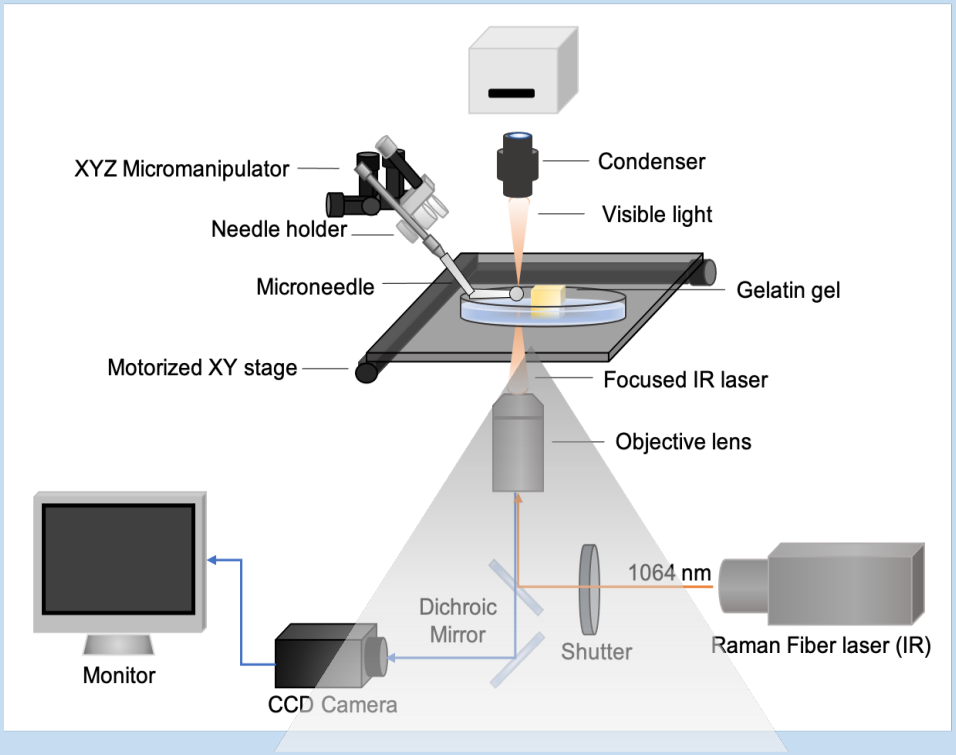
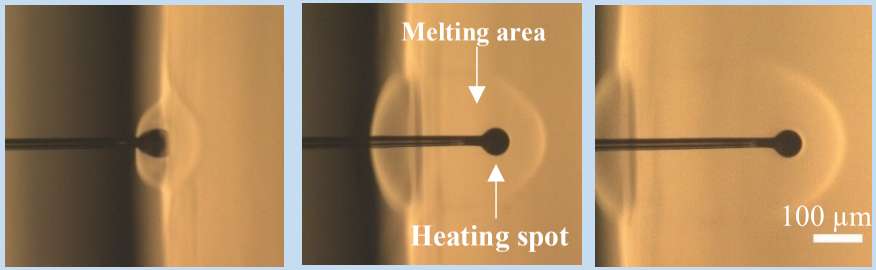
Graphical Abstract

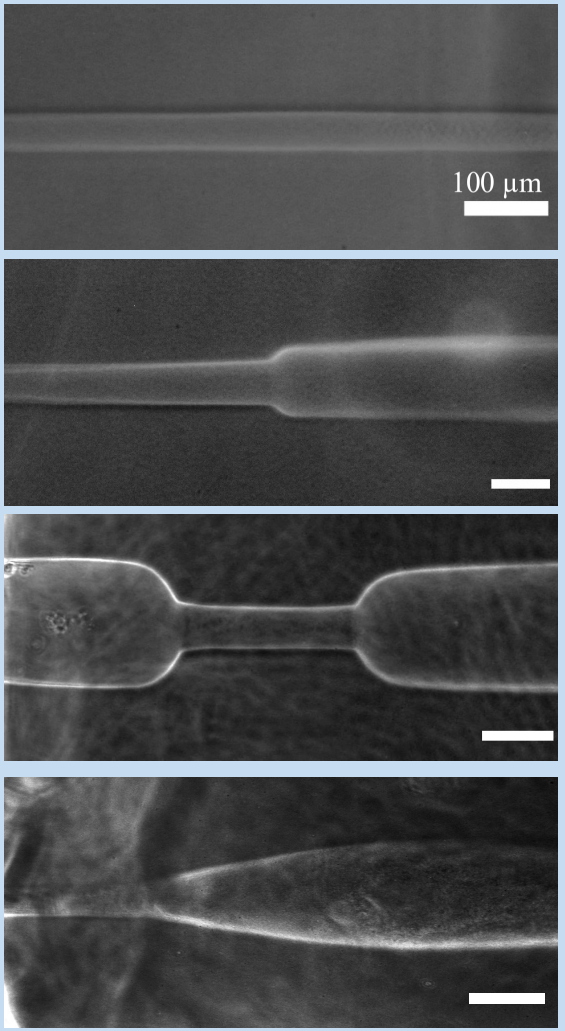
Title: Development of gelatin-based flexible three-dimensional capillary pattern microfabrication technology for analysis of collective cell migration

Technology

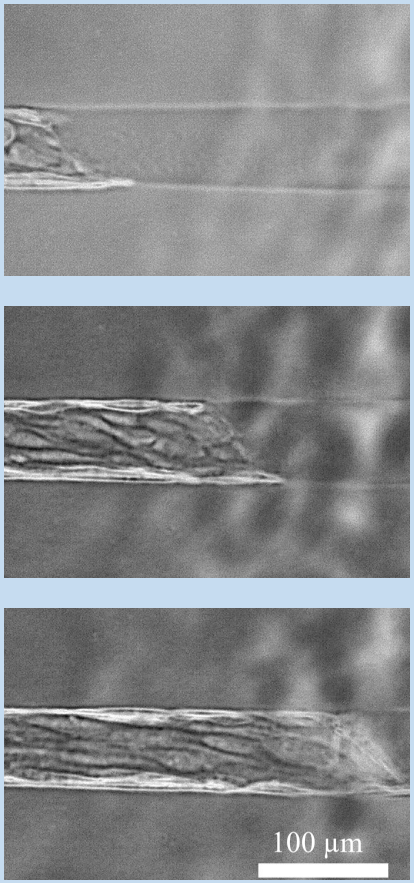




Structure



Culture



Abstract

The collective cell migration is thought to be a dynamic and interactive behavior of cell cohorts which is essential for diverse physiological developments in living organisms. Recent studies revealed that topographical properties of the environment regulate the migration modes of cell cohorts, such as diffusion versus contraction relaxation transport and the appearance of vortices in larger available space. However, conventional in vitro assays fail to observe the change in cells behavior in response to the structural changes. Here, we have developed a method to fabricate the flexible three-dimensional structures of capillary microtunnels to examine the behavior of vascular endothelial cells (ECs). The microtunnels with altering diameters were formed inside gelatin-gel by spot heating a portion of gelatin by irradiating the μm-sized absorption at the tip of the microneedle with a focused permeable 1064 nm infrared laser. In contrast to the 3D straight topographical constraint, which exhibited width dependence migration velocity, leading ECs altered its migration velocity accordingly to the change in supply of the cells behind the leading ECs, caused by the progression through the diameter altering structure. Our findings provide insights into the collective migration properties in 3D confinement structures as fluid-like behavior with conservation of cell numbers.

Keywords: Three-dimensional culturing environment, collective cell migration, microfabrication technology, vascular endothelial cells, fluid-like behavior

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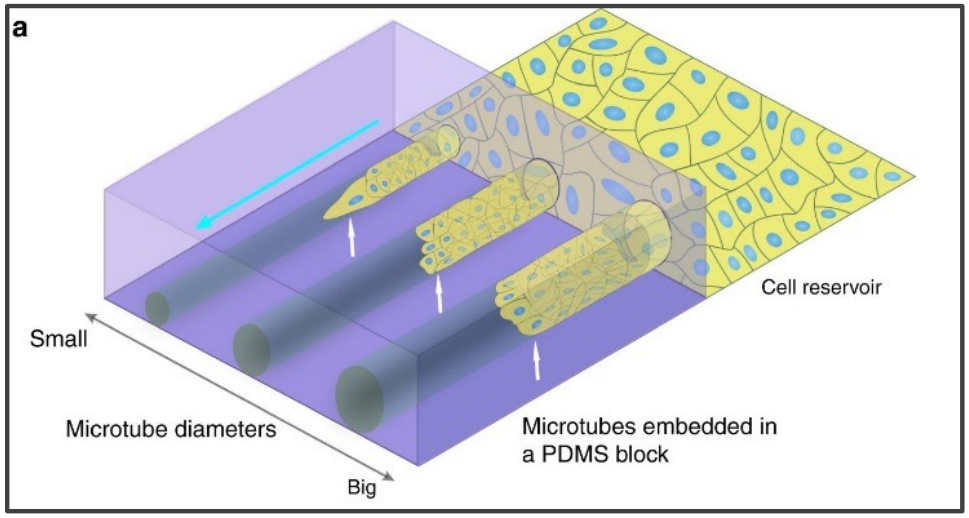
Introduction



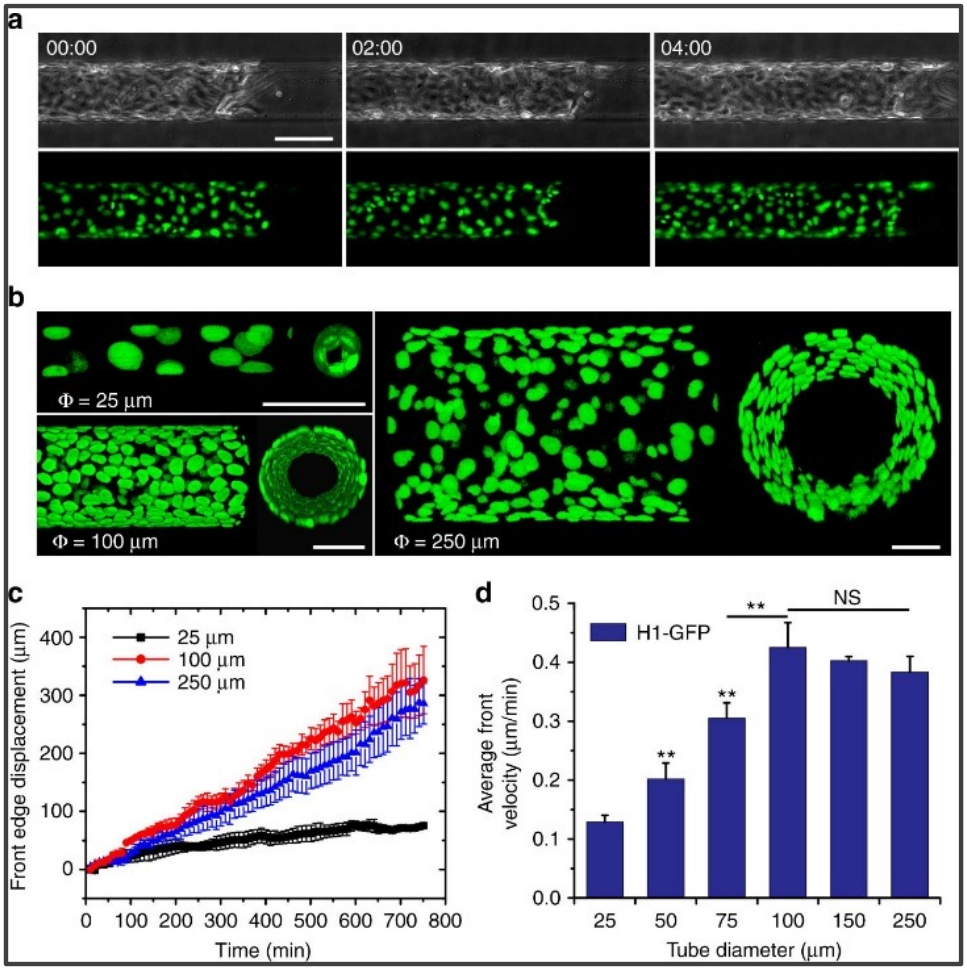


Fig. 1. Cell migration inside straight microtubes embedded in a PDMS block
(Xi, W., et al. Nat. Comm., 2017)

In recent studies, collective cell migration through simple 3D structure with correct apical-basal polarity has been observed

For the construction of a culturing environment which replicates a more in-vivo like situation, microfabrication technology that can flexibly change the structure is required

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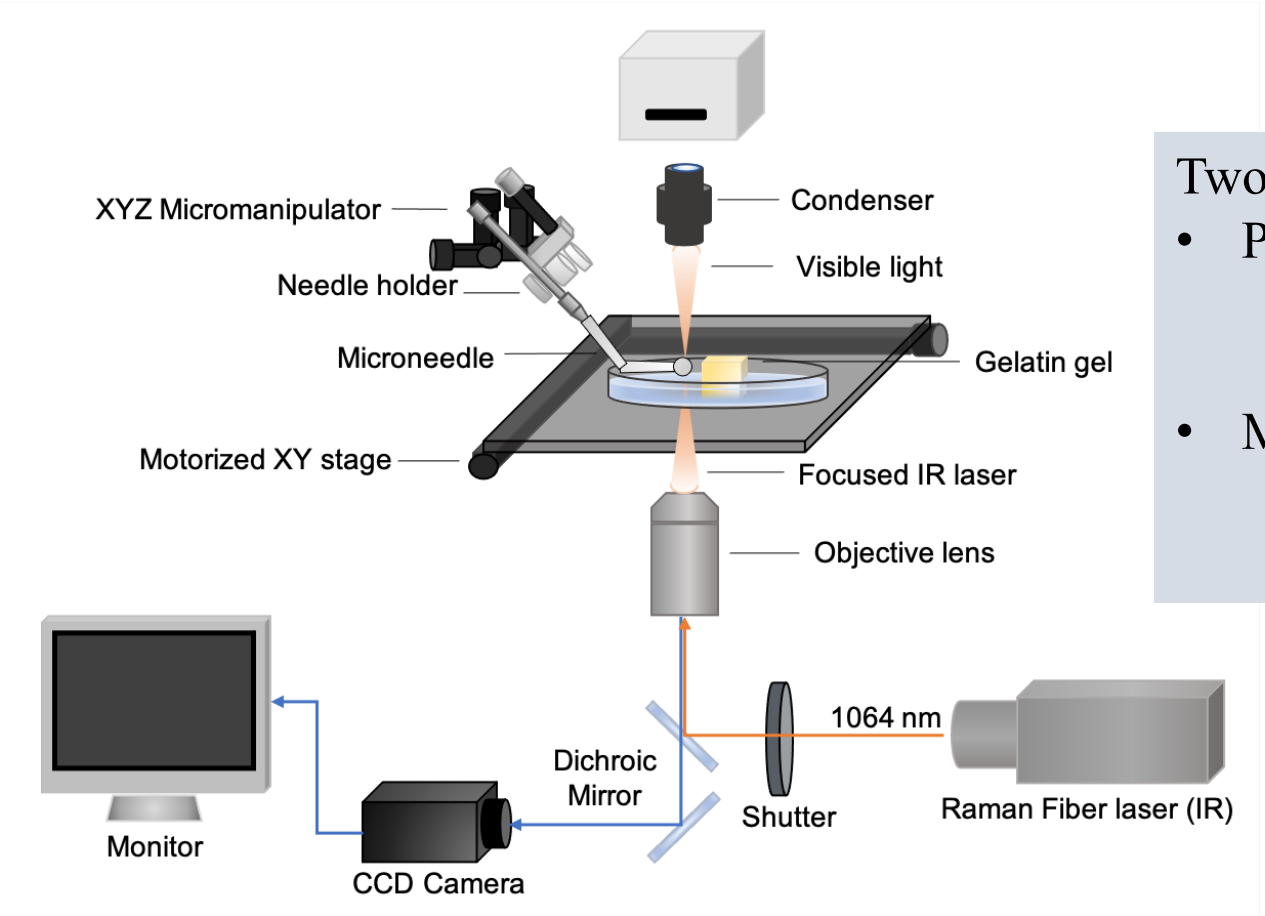
Results and Discussion

Developed Microfabrication Technology

Schematic diagram of the system set-up



Two main technologies

- Permeable 1064 infrared laser
  - ➢ For spot heating on the absorbance tip of the microneedle
- Motorized XY stage
  - ➢ For maneuvering the position of the gel with respect to the needle

Fig. 2. Developed microfabrication set-up

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Results and Discussion

Developed Microfabrication Technology

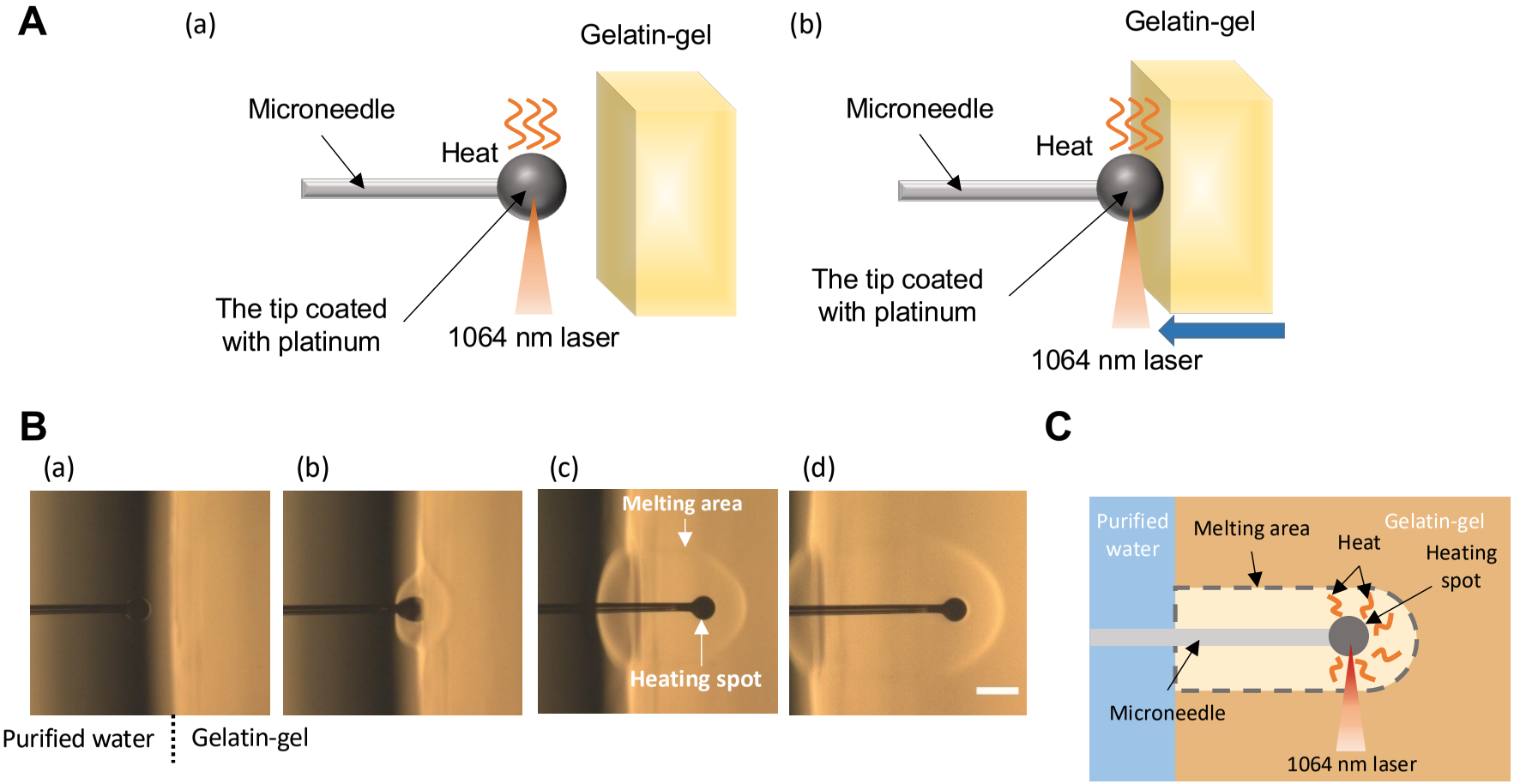

Fig. 3. (A) Illustration of the process of gelatin microfabrication (B) A series of micrographs during the process of microtunnel formation (C) Illustration representing the process of generating a tunnel

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Results and Discussion

Relationship between tunnel diameter and laser power

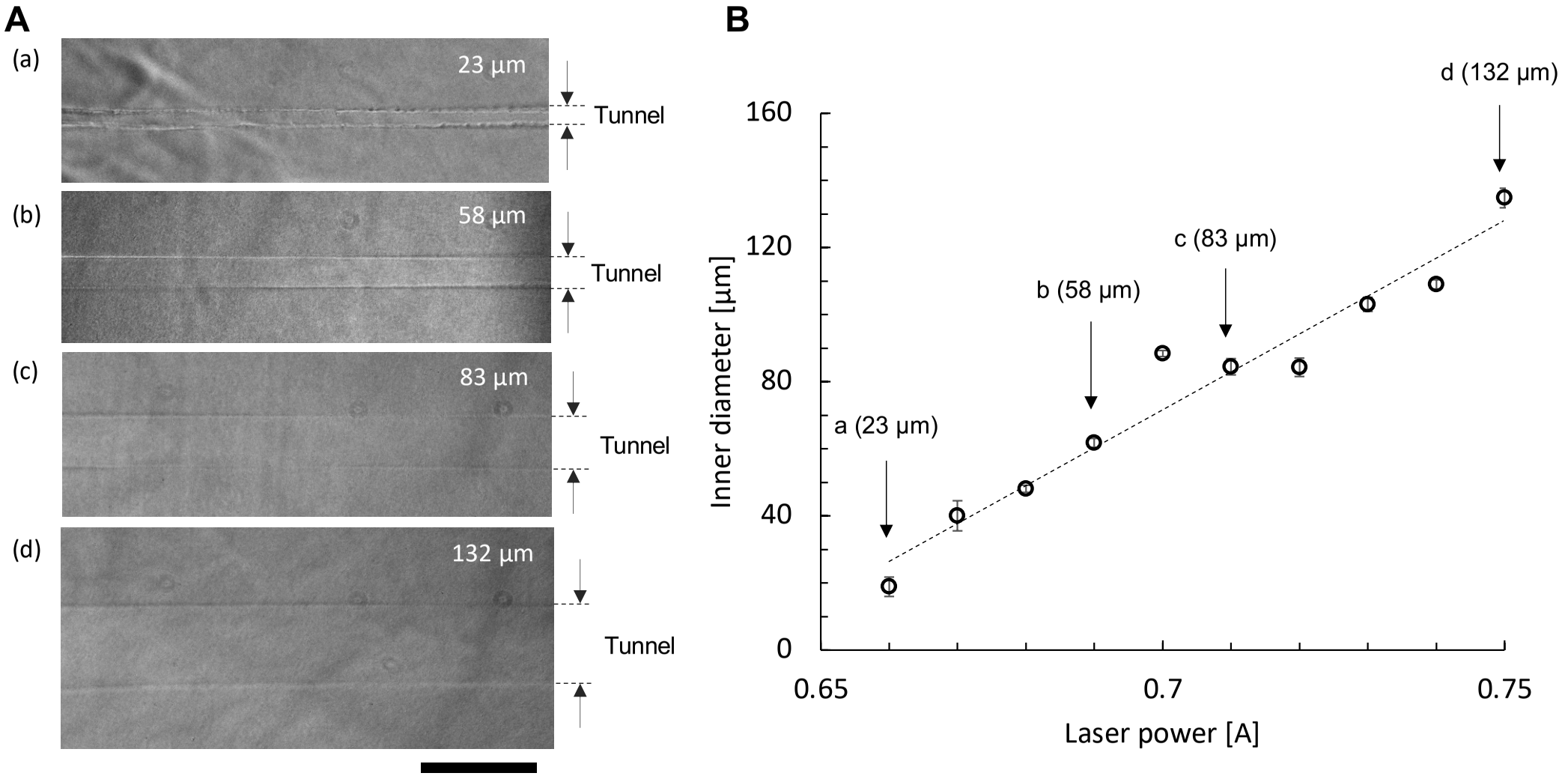

Fig. 4. (A) Phase contrast images of microfabricated tunnels (B) Correlation demonstrating the laser intensity and the tunnel inner diameter

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Results and Discussion

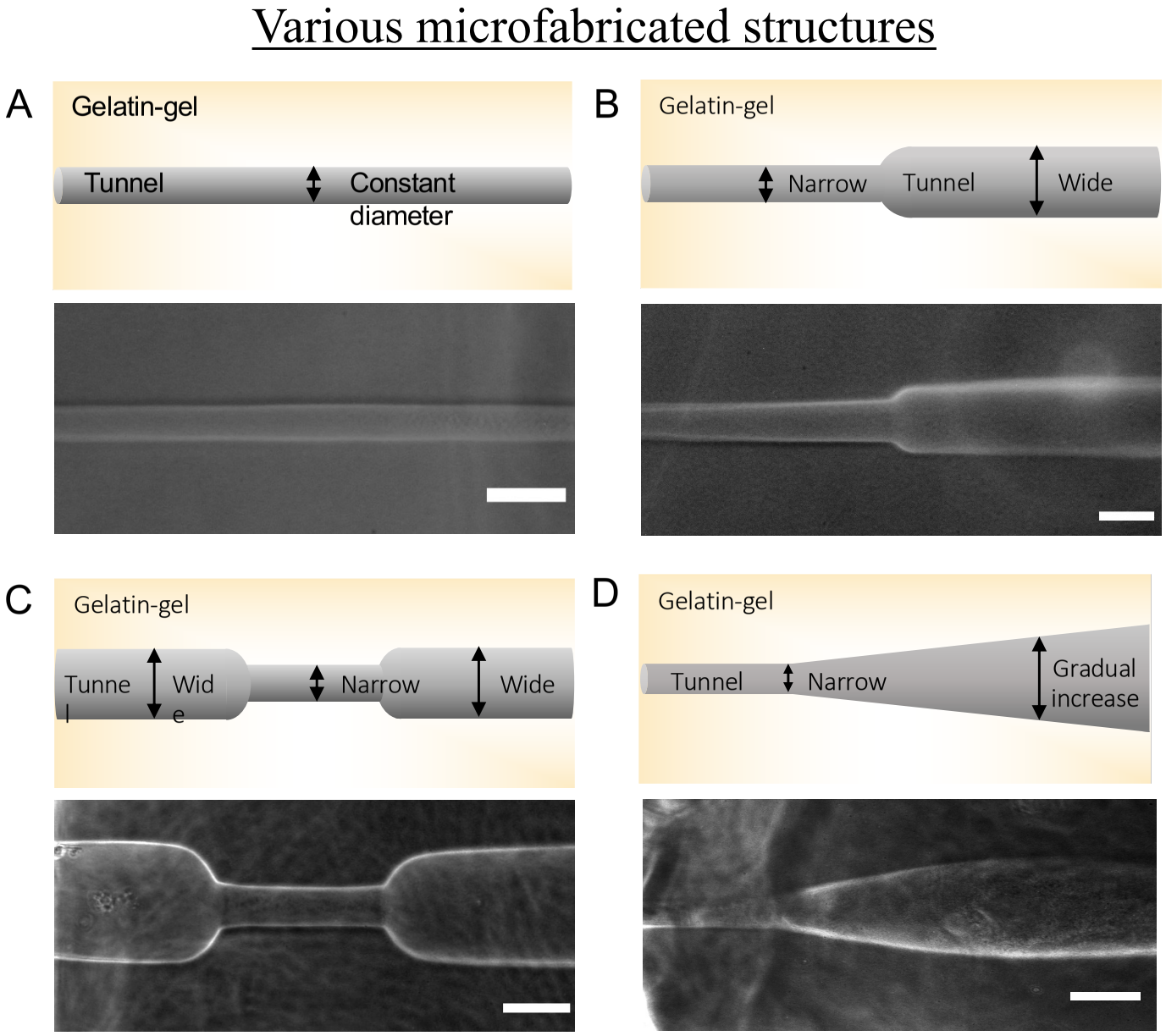

Fig. 5. Examples of various microtunnel structures (A)Straight (B)Narrow to wide (C)Wide-narrow-wide (D) Gradually narrow-wide

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Results and Discussion

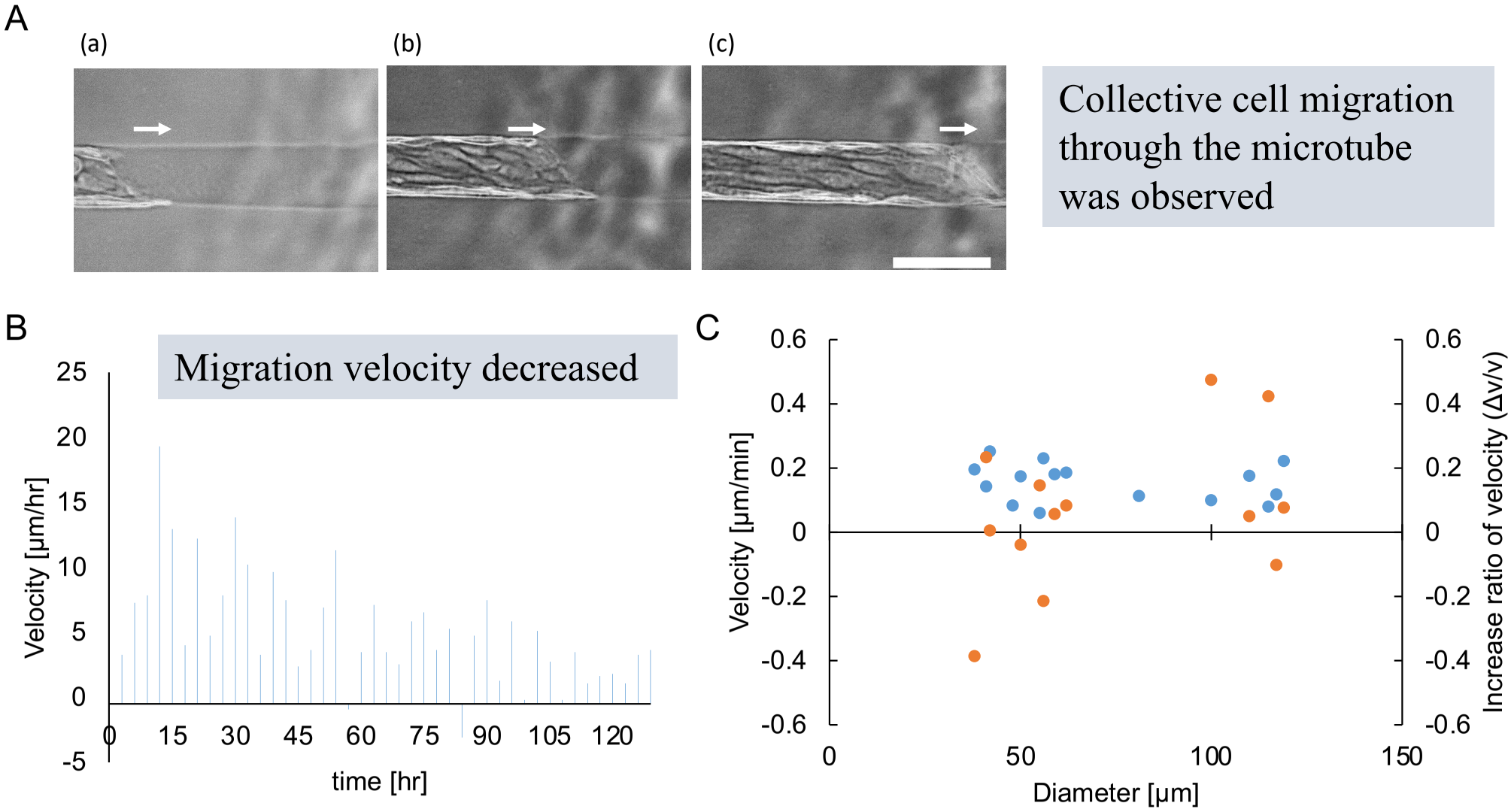

Fig. 6. (A) The bright field images of the collective cell migration into the 50 μm straight capillary microtunnel (B) Initial collective cell migration velocity and the change of migration velocity at the 100 μm length point (C)

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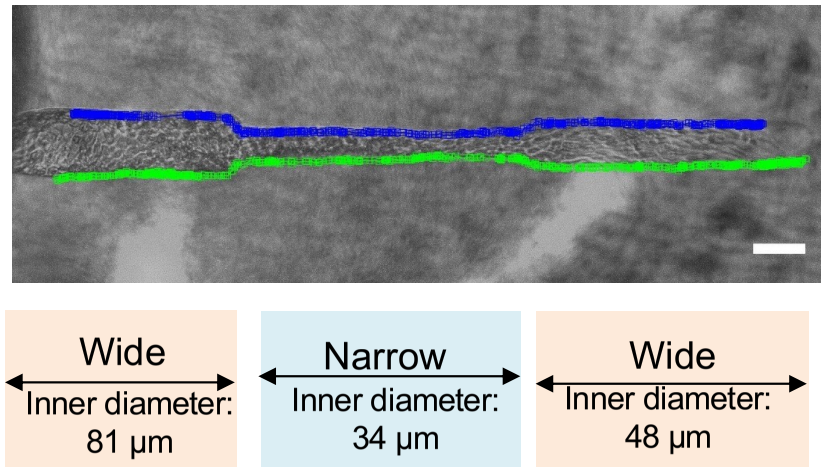
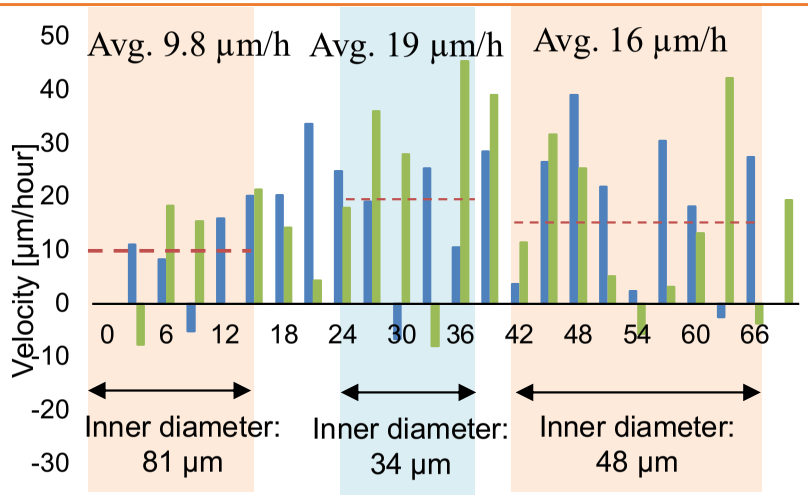
Results and Discussion

Collective cell migration inside diameter changing structures

An example demonstration of the technology for forming the migration environment





Velocity increased and then decreased

Fig. 7. Obtained time-lapse imaging of the collective cell migration on the inner peripheral surface of the generated three-dimensional microtunnel and the associated velocity analysis of the cell tracking on the image slices.

- Succeeded in the observation of collective cell migration inside varying diameter structure
- Change in microtubular structure was accompanied by a change in migration velocity
- For wide to narrow to wide, velocity increased as the tunnel constricted, and then decreased after the tunnel widened

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Conclusion

1) We have developed a gelatin-gel microfabrication technology for the formation of flexible three-dimensional structures.

2) Various structures on which cells can migrate inside was formed for the observation of cellular dynamic properties.

3) We have succeeded in the observation of changes in cell dynamics, particularly, the cell sheet velocity in response to structural changes.

Currently in submission process for review in a journal

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Acknowledgments

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