ENGINEERING MOUNTAIN FOLDS IN CELL ORIGAMI

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ABSTRACT

We report a method to create mountain folds, protruding ridges, in cell origami, a polymer structure folding technique driven by cell traction force (CTF). Formerly, cell origami was based on valley folds, indented creases, only [2]. We created mountain folds adjacent with valley folds. We present designs for self-folding structures as well as for physically stimulated structures. The adhered cells hold the microstructure in position after self-folding or stimulus, when without cells the structure unfolds again. Our method increases structure complexity achievable by cell origami and possibly by other micro-origami techniques that utilize an one-directional folding force.

KEYWORDS: micro-origami, three-dimensional, cell handling, microstructure

INTRODUCTION

Micro-origami, folding planar highly resolved shapes into three-dimensional structures inspired by the Japanese folding art origami, achieves high throughput with high resolution [1]. For biological applications like drug delivery or tissue supportive structures, biocompatibility is highly required. Cell origami combines biocompatible materials and self-driven folding that harnesses the CTF of adhered cells. Because CTF is one-directional, previously reported structures were entirely formed by valley folds [2]. In this paper, we establish mountain folds in cell origami. Only few MEMS fabrication technologies have been reported to enable valley and mountain folds within the same microstructure [1, 2, 4]. To maintain the simple fabrication technique and biocompatibility of cell origami, we propose an indirect fabrication method: A mountain exists when two valleys are adjacent to each other (Fig. 1 a). Adhered cells fold valley folds by CTF [2]. A row of four parylene microstructures (microplates) with adhered cells forms several valley folds and at least one mountain fold (Fig. 1 b). After cell adhesion, cells are supportively stimulated by a push with a glass capillary. Physical stimulus and CTF form a microstructure of a mountain fold embedded in valley folds (Fig. 1 c).

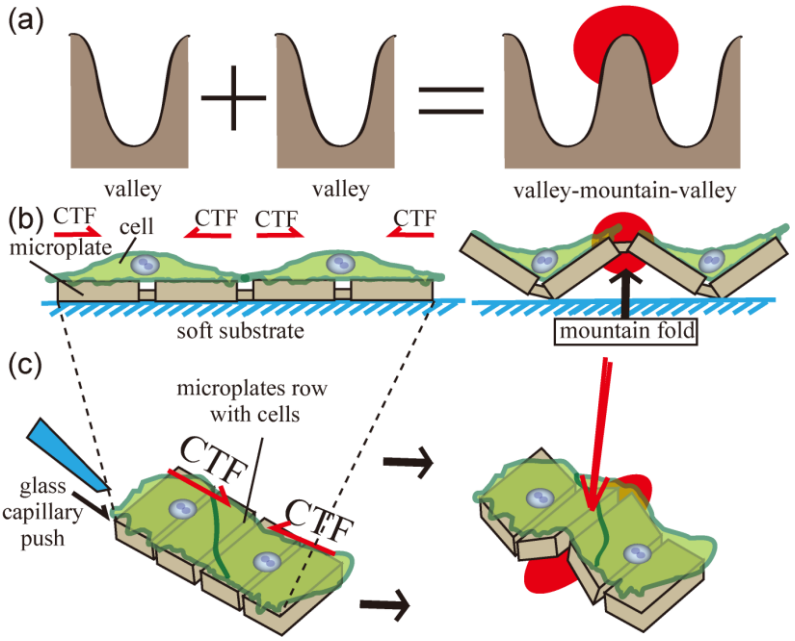

Figure 1: Formation principle for a mountain fold (MF) in cell origami. Embedded into neighbored valley folds, a MF (red mark) arises due to cell traction force (CTF) and supportive physical stimulus with a glass capillary.

IMPLEMENTATION

To implement the idea of a row of four microplates forming a mountain fold in the center, we investigated a number of microstructure designs in trial experiments. The most promising design for self-folding mountain folds is depicted in Fig. 2 a. The main obstacle is a folding of three adjacent valley folds as reported in [2]. To prevent this undesired behavior and enhance mountain fold formation, we focused on designs with supportive side structures. These act as fixation points and hold the structure in place. Additionally, when folding to a valley fold, side structures possibly act supportive towards a central mountain fold structure (Fig. 2).

The difference in the design for self-folding and stimulated folding is the length of connective bars. Short bars enhance side structure support towards a central mountain fold and more connectivity between cells that cover the entire structure (Fig. 2 a). With a distance of 70 µm between the central row of microplates and microplate side structures acting as fixation points, cells are separated. Hence, cell connectivity is significantly decreased to the area of the central row of microplates (Fig. 2 b). Slight differences in shape and attachment points of the bars seemed to be negligible.

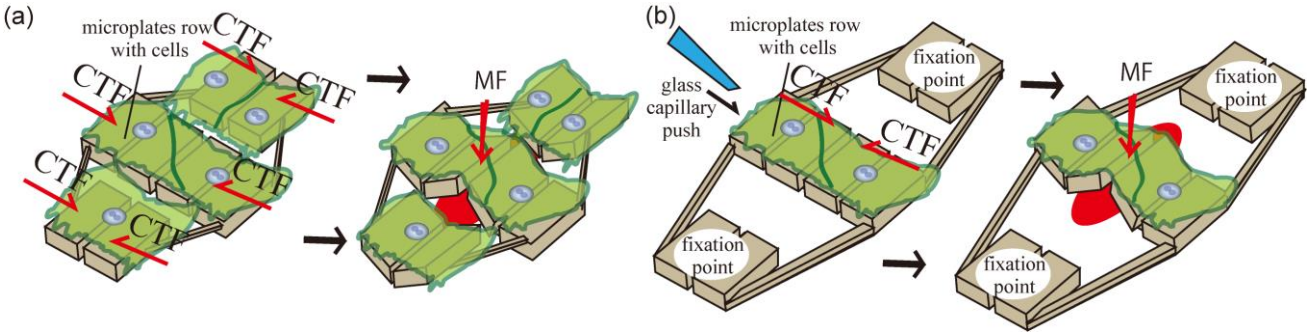

Figure 2: Implementation for a) self-folding and b) stimulated folding of mountain folds (MFs, read mark).

FABRICATION PROCESS

A single microplate has the dimensions of 23×50 µm^2 and forms with its neighbored microplate a 50×50 µm^2 area. Connective bars are 5 µm wide and used to space microplates 70 µm or less apart (Fig. 2). As sacrificial layer below the microstructures, we spin-coated temperature sensitive gelatin (2-5%, bovine/porcine, Sigma) and occasionally photoresist (S1818G, Shipley). 3 µm-thick parylene C (DPX-C, Specialty Coating Systems) was coated by chemical vapor deposition. Microplates were patterned by conventional photolithography. Hinges and occasionally bars were etched deeper separately by repetition of the fabrication steps. 2-methacryloyloxyethyl phosphoryl-choline (MPC) polymer, a phosphoryl-choline based lipid [5], was coated to prevent cell adhesion between microstructures. 10% rhodamine-tagged fibronectin (FNR01-A, Cytoskeleton, Inc.) and fibronectin (FN) (BT-226S, Biomedical Technologies, Inc.) with a total FN concentration of 50 µg/mL were printed by micro contact printing [6] to promote cell adhesion. NIH/3T3 cells were seeded at low cell concentration and cultured for two days in DMEM medium (D5796, Sigma-Aldrich) at 37 °C with 5% CO_2. Cell staining was performed after fixation with 4% para-formaldehyde fixative (Muto Pure Chemicals co., ltd.) several hours post experiment and consecutive blocking with 1% BSA (bovine serum albumin, A7906-50G, Sigma-Aldrich) in PBS buffer (ten times diluted Dulbecco's phosphate buffered saline 10×, D1408, Sigma-Aldrich). Cellular actin fibers were stained with Alexa 488 phalloidin A12379 (Invitrogen). Confocal images were obtained with a Zeiss inverted confocal microscope LSM780 and image processing was performed with ZEN software.

RESULTS AND DISCUSSION

With cells adhered, mountain folds were formed and fixated for microscopic observation. We observed a couple of self-folding mountain folds within the course of two-day cell culturing (not shown). However, the details of the folding mechanism remain completely obscure. For higher yield and reproducibility, we focused on supportive physical stimulation with a glass capillary. The structure was stimulated at three different points (Fig. 3 a); pushing in the center from the side generated mountain folds. Without adhered cells, structures relaxed after stimulation (Fig. 3 b, c).

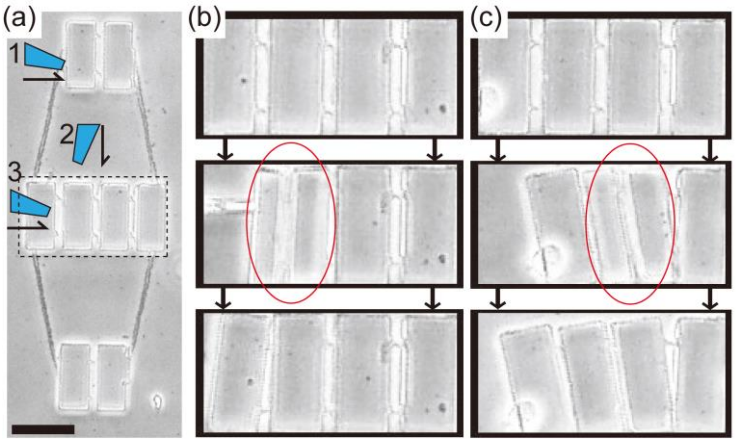

Figure 3: Physical stimulation with a glass capillary. a) Indication of physical stimulation. Stimulus 3 resulted in MF formation. b,c) MF formation (red circles) without cells is not permanent. Top-to-bottom flow: before, during stimulus, after.

We observed a high number of mountain fold microstructures formed by stimulation (Fig. 4). The underlying folding angle of over 100 ° appears to be dependent on the hinge thickness. Interestingly, the observed hinge thickness is larger than the maximal reported hinge thickness for folding [2]. Due to physical stimulation, the limit of hinge thickness dependent on CTF was overcome. The hinge thickness is now limited by material properties; high hinge stiffness causes detachment of the entire structure from the sacrificial layer and substrate before structure formation can be achieved.

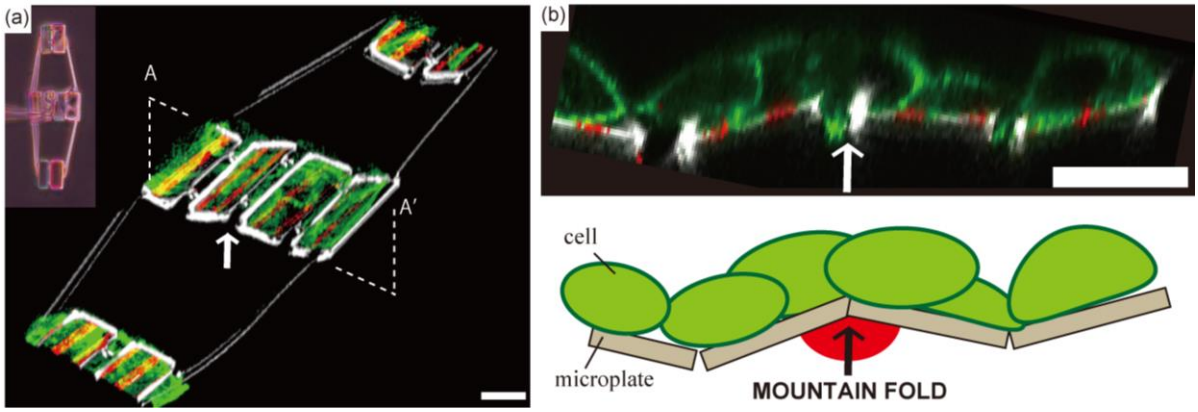

Figure 4: Reconstructed confocal fluorescence image of parylene structure (white, false-colored autofluorescence) with fibronectin pattern (red) and cellular actin (green). a) Three-dimensional reconstruction. Inset shows light microscopic image during stimulation. A to A` cross section is enlarged in b): Cross section with its corresponding schematic drawing. Red mark and arrows indicate the mountain fold in the center of the structure. Scale bars represent 20 µm.

The yield of mountain fold formation by stimulation was very high, approx. 85 %. However, the position of the mountain fold within the structure varied and a central mountain fold was achieved in approx. 30 % of all cases. Preliminary data of a more compact design – a row of four microplates with the first plate being significantly larger (100×100 µm^2) – shows that physical stimulation resulted in central mountain folds in approx. 57% of the stimulation cases, detachment of the microstructure and formation of three adjacent valley folds occurred in the remaining cases.

CONCLUSION

We introduced self-folded and physical stimulated cell origami structures of adjacent mountain and valley folds. We demonstrated that stimulation yields mountain folds from various designs. Due to supportive stimulation, microstructure size and adjacent valley folds can be designed quite deliberately. Thereby, our mountain fold fabrication method increases the possible complexity of origami structures. Next, we want to create a stent-like tissue supportive structure.

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REFERENCES

[1] Scott T. Brittain, Olivier J. A. Schueller, Hongkai Wu, Sue Whitesides, and George M. Whitesides, "Microorigami: Fabrication of Small, Three-Dimensional, Metallic Structures," *J. Phys. Chem. B*, vol. 105, pp. 347-350, 2001

[2] Kaori Kuribayashi-Shigetomi, Hiroaki Onoe, Shoji Takeuchi, "Cell Origami: Self-Folding of Three-Dimensional Cell-Laden Microstructures Driven by Cell Traction Force." *PLoS ONE* vol. 7, no. 12: e51085, pp. 1-8, 2012

[3] Pablo O. Vaccaro, Kazuyoshi Kubota, Thomas Fleischmann, S. Saravanan, Tahito Aida, "Valley-fold and mountain-fold in the micro-origami technique," *Microelectronics Journal* vol. 34, pp. 447–449, 2003

[4] Mustapha Jamal, Aasiyeh M. Zarafshar, and David H. Gracias, "Differentially photo-crosslinked polymers enable selfassembling microfluidics, " *Nature Communications* vol. 2, no. 527, pp. 1-6, 2011

[5] Kazuhiko Ishihara, Hiroto Nomura, Takashi Mihara, Kimio Kurita, Yasuhiko Iwasaki, Nobuo Nakabayashi, "Why do phospholipid polymers reduce protein adsorption?," *J. Biomed. Mater. Res.*, vol. 39, pp. 323-330,1998

[6] Anne C. von Philipsborn, Susanne Lang, André Bernard, Jürgen Loeschinger, Christian David, Dirk Lehnert, Martin Bastmeyer, Friedrich Bonhoeffer, "Microcontact printing of axon guidance molecules for generation of graded patterns," *Nature Protocols* vol.1 no.3, pp. 1322-1328, 2006

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