2013 IEEE/RSJ International Conference on
Intelligent Robots and Systems (IROS)
November 3-7, 2013. Tokyo, Japan

Controlled Patterning of Magnetic Hydrogel Microfibers under Magnetic Tweezers

Chengzhi Hu*, *Student Member, IEEE*, Masahiro Nakajima, Tao Yue, Yajing Shen, Toshio Fukuda, *Fellow, IEEE*, Fumihito Arai and Minoru Seki

***Abstract*—3D tailor-made biodegradable scaffold integrated with biological cells or molecules is of great importance for tissue engineering. This paper addresses an improved method for exploring magnetic tweezers in patterning and aligning magnetic hydrogel fiber to fabricate large-scale engineered cell-hydrogel constructs. Magnetic hydrogel fibers were fabricated based on microfluidic device. The fabricated hydrogel fiber is made of alginic acid sodium and with a diameter of 34 μm. Magnetic nanoparticles is added into the alginic acid sodium solution to append magnetic material inside the fibers. The magnetic material inside the hydrogel fiber is regulated by the microfluidic device. Magnetic tweezers system based on solenoid electromagnet is utilized to evaluate the magnetic response of the magnetic hydrogel fiber. Evaluation results show the hydrogel fiber can be maneuvered by the proposed system with a positioning resolution of sub-micro level. The cultivation results of hydrogel fiber with C2C12 cells shows the potential for real applications of the proposed method in tissue engineering.**

I. Introduction

MAGNETIC particles have been widely studied for the applications on bio-sensing, cell sorting, drug and gene targeting, cell enrichment, protein digestion, magnetic resonance imaging and cancer therapy [1-3]. The advantages lie in their controllability by using external magnetic fields and stability for bearing chemical or biological function. Magnetic force is used to assemble microparticles, also to retrieve the particles from previously built structures. In a previous study, magnetic hydrogel microparticles have been fabricated for microRNA detection and manipulated inside microwells [4].

Hydrogels are used as base material for many applications in regenerative medicine due to their unique biocompatibility, flexible methods of synthesis, range of constituents, and desirable physical characteristics. Mass transport of oxygen, essential nutrients, growth factors, metabolic waste and secretory products can be realized by diffusion and transportation across the hydrogel networks. Thus cell-seeded hydrogels hold dominant position in overcoming the challenging issues in tissue engineering, such as generation of vascularized tissues and mimicking the complex structure and architecture of biological tissues [5-8]. Hydrogels have been tailored to mimic natural tissues and employed to provide bulk and mechanical constitution to tissue constructs as engineered scaffold. Cells are cultured by adhering to or suspending within the 3D hydrogel constructs [9-11].

To generate microengineered hydrogels and related cell-encapsulated hydrogel complex, many techniques have been investigated such as emulsification, photolithography, micromolding and microfluidic synthesis [12-14]. A. Winkleman et al demonstrated the fabrication and manipulation of millimeter-scale spheres fabricated from ionotropic hydrogels that are cross-linked with paramagnetic metal ions. These ionotropic hydrogels introduce a force under a magnetic field gradient that has close relationship with the concentration of the paramagnetic cations cross-linking the polymer [15]. In an externally applied magnetic field, the paramagnetic hydrogel spheres were assembled into precisely patterned arrays or constrained its geometrical structures in the regions with highest magnetic field. These spheres can be separated from heterogeneous mixtures of diamagnetic materials using a simple bar magnet. [12, 16].

As for the hydrogel material, alginic acid sodium is water-soluble, nontoxic, hydrophilic, biocompatible and with a relatively low cost. The alginate gel can be formed in the presence of divalent cations, such as Ca^{2+}, Mg^{2+} ions, and so on, because the cations act as ionic bridges between L-guluronic acid residues on adjacent chain segments. Due to its ability to form gels under mild conditions with the divalent cations, alginate has been utilized as gelling agent for extruding wounds, dental impression, controlled drug delivery, and the encapsulation of living cells [17, 18].

As most of human tissue is composed of three kinds of cells, endothelial cell, smooth muscle cell, and collagen fiber, our strategy for tissue regeneration is encapsulating these cells inside hydrogel, and using cell-seeded hydrogel to fabricate magnetic hydrogel fiber. We use 3D magnetic tweezers to control and align the fibers to specific pattern. Then these well-organized magnetic hydrogel fibers are kept inside the incubator for tissue regeneration as illustrated in Fig. 1.

In this paper, we used fluidic chip and syringe connected with micro pipette tip to generate alginate hydrogel fibers. For the availability of control to the hydrogel fiber, MRI contrast media was employed to add magnetism inside the

This work was partially supported by MEXT KAKENHI Grant Number 23106006 and COE for Education and Research of Micro-Nano Mechatronics of Nagoya University Global COE Program. *Asterisk indicates corresponding author.*

C. Hu, M. Nakajima, T. Yue, Y. Shen, and T. Fukuda are with the Department of Micro-nano Systems Engineering, Nagoya University, Nagoya 464-8603, Japan (Corresponding email: huchengz@ gmail.com).

F. Arai is with Department of Mechanical Science and Engineering, Nagoya University, Nagoya 464-8603, Japan.

M. Seki is with Department of Applied Chemistry and Biotechnology, Chiba University, Chiba 263-8522, Japan.

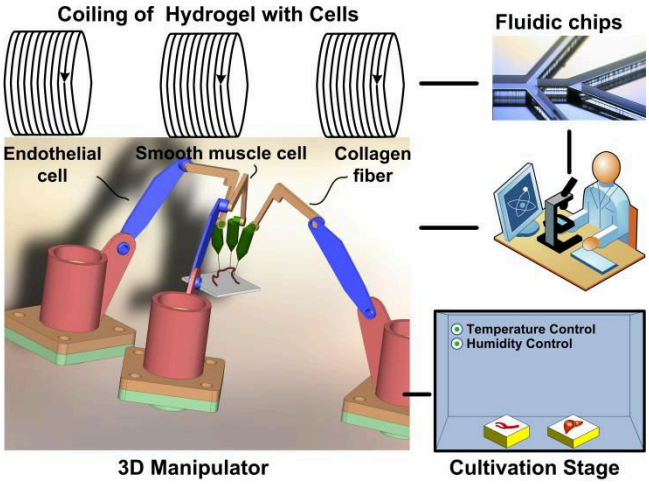

Fig.1. Illustration of tissue regeneration with magnetic hydrogel fiber.

microfluidic chips. Electromagnet based magnetic tweezers system was used to control the movement of hydrogel fibers. The cultivation results of hydrogel fiber with fibrous C2C12 cells showed the potential for real applications of the proposed method in tissue engineering.

II. Material and Method

A. *Preparation of the Fluidic Chips*

A micro fluidic device which can generate laminar flow is utilized for fabricating magnetic hydrogel fiber. 2% alginic acid sodium (MP Biomedicals, France) is used as hydrogel solution, 0.2% calcium chloride solution mixed with 10% dextran is used to solidify the hydrogel, and 10% Dextran solution (Dextran 200000, Wako Pure Chemical Industries, Ltd. Japan) is used as the buffer flow to separate the alginic acid sodium flow and calcium chloride solution. Magnetic resonance imaging (MRI) contrast media solution (Resovist Inj., Japan) is added into the alginic acid sodium solution to append magnetic material inside. The pattern of the flow channel is configured as shown in Fig.2(a). In this design, the width of channel for alginate solution is 100 µm, which is chose for generating a thickness of hydrogel fiber about 50 µm. The size for other channels and the configuration of the channels are shown in Fig.2 (b). We used the SU-8 3050 as the mold materials with the consideration that it is a high contrast, epoxy based photoresist designed for micromachining and other microelectronic applications, where a thick, chemically and thermally stable image is desired. The fabricated SU-8 model is observed under surface profiler as shown in Fig. 2 (b).

Microfluidic chip made of polydimethylsiloxane (PDMS) is a key component for many microfluidic and lab-on-chip applications. The fabrication procedure for PDMS (SILPOT 184 W/C, Dow Corning Toray) microfluidic chip with PDMS coated bottom surface is shown in Fig. 3. Fabrication method is based on photolithography. First SU-8 was spin-coated on the silicon wafer with a speed of 2700 RPM for 30s; then after baking it for 40 mins, UV light exposure was conducted under specific shadow mask, the pattern used is the same as

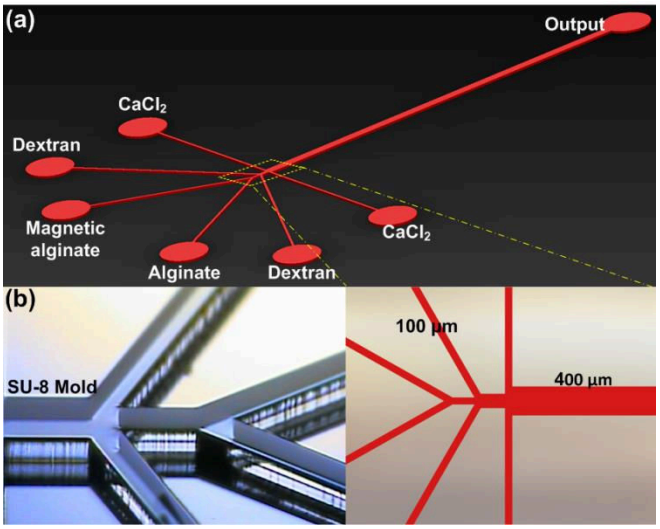

Fig.2. Illustration of the fluidic chips used in the fabrication of magnetic hydrogel fiber. (a) Pattern design of microfluidic chips with the exact size; (b) SU-8 mold used for fabricating microfluidic device.

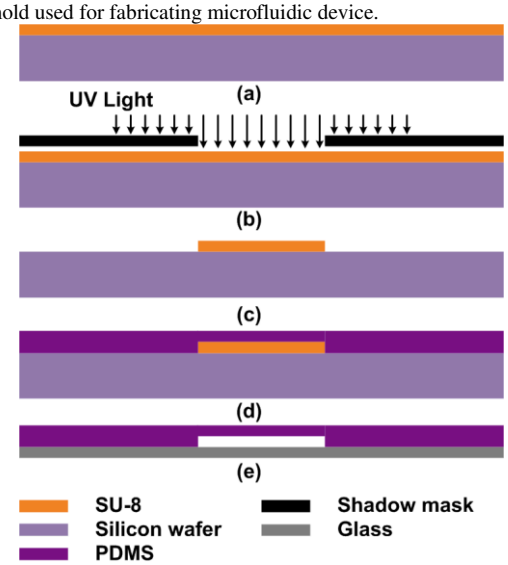

Fig.3. Fabrication process for the microfluidic device. (a) SU-8 was spin-coated on the silicon wafer; (b) UV light exposure under specific shadow mask; (c) SU-8 model was achieved after development; (d) PDMS was spin-coated on the SU-8 mold; (e) Peeling off the PDMS from the wafer and bonding it on the cover glass.

mentioned above. SU-8 mold was achieved after PM thinner development. Finally PDMS was spin-coated on the SU-8 and it solidified after 24 hours at room temperature. After peeling off the PDMS from the wafer, it was bonded on the cover glass after oxygen plasma treatment. The whole fabrication process was as shown in Fig. 3. Holes were made on the ends of the channel and the silicone tube was put in the holes. All solutions were injected by the digitally controlled syringe pumps (KDS 111, Muromachi Kikai Co., Japan).

B. *Fabrication of Magnetic Hydrogel Fiber*

MRI contrast media solution that is a hydrophilic colloidal solution of superparamagnetic iron oxide coated with carboxydextran was added into the alginic acid sodium solution. Before the experiment we mixed the 4ml 4% alginic acid sodium with equal amount of MRI contrast media. The mixture was sonicated for 1 hour inside ultrasound device

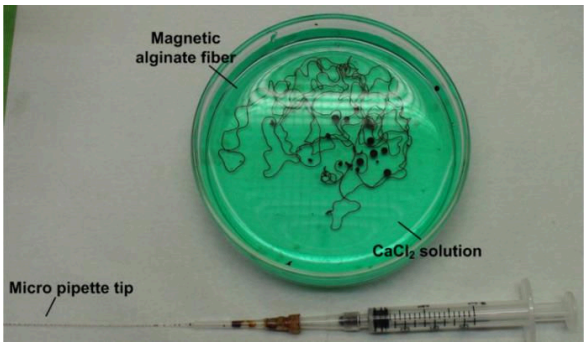

Fig. 4 Injection of magnetic alginate solution for solidification. A syringe is connected with a micro pipette for achieving magnetic fiber with small diameter of 0.8mm.

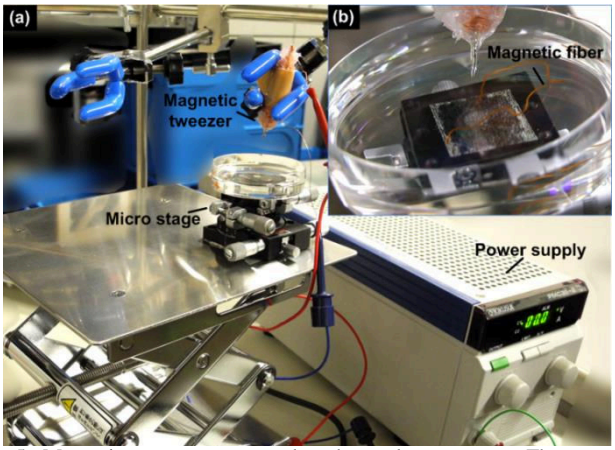

Fig.5. Magnetic tweezers system based on electromagnet. The magnet consists of a single coil enclosing a soft iron core. The core is needle shaped at the end, the magnetic flux is then bundled and the magnetic field reaches a maximum near the tip. (a) device structure; (b) enlarged view of magnetic tweezers.

under the temperature of 40 °C to help MRI contrast media uniformly distributed inside the alginate solution. During the mixing, aggregation of magnetic materials may happen; therefore the mixture was filtrated before use in the case that the aggregation blocks the microfluidic channels. Pressure control for each channel of the fluidic chips is very important to generate laminar flow and steady gelling of alginate. In addition, during the experiment, calcium chloride solution was avoided to meet the alginate solution directly inside the channel. The buffer solution was injected first with the rate of 100 μL/h, and then the other two solutions were injected with the rate of 50 μL/h. Tweezers were used at the output part of the channel to collect the hydrogel fiber and assist the fiber coiling.

For the proof-of-concept experiments, another kind of larger diameter of magnetic hydrogel fiber was fabricated by wet spinning. A syringe was connected with micro pipette tip. The magnetic alginate solution was injected with syringe pump at a speed of 5 mm/min, then the fiber solidified inside $CaCl_2$ solution. The diameter of magnetic fiber is about 0.8 mm. The experimental setup is as shown in Fig.4. Lower injection speed results in that the solidified hydrogel aggregates at the tip of the pipette and the diameter of the fiber is non-uniform. On the contrast, higher injection speed has little influence to the thickness of the fiber.

C. *Magnetic Tweezers System for Manipulation*

Magnetic tweezer is an electromagnetic device capable of exerting a force of controlled magnitude and direction on magnetic objects. Our magnetic tweezer is composed by a solenoid electromagnet for aligning and patterning magnetic hydrogel fiber as shown in Fig.5. Enameled wires were winded on a cylinder soft steel rod. The delivering current ranges from 0 to 0.5A. The soft iron was polished as a sharp tip with a diameter of 0.2 mm, which is comparable with the diameter of magnetic fiber. A three axial controllable micro stage is mounted on the Jack and the petri containing the magnetic hydrogel fiber was placed on the top of the micro stage. The micro stage is with a precision of 0.1 micrometer for precious alignment and control of the magnetic hydrogel fiber. A programed power supply was used to control the input current of the electromagnet for obtaining the desired magnetic strength on the tip of the magnetic tweezer. A clamper stage was used to hold the magnetic tweezer and adjust the position, angle and orientation. Since the magnetic field from a solenoid is inversely proportional to the coil diameter, and the field gradient from a ferromagnetic tip is inversely proportional to the tip dimensions, the minimization of both of these parameters in the design of the magnetic tweezer is needed. In our design, the diameter of soft iron is 0.8 mm and a pretightening force of coiling is 3N for providing a higher current density along the soft iron.

D. *Computational Model of Static Magnetic Fields*

For the transient analysis of magnetic field generated by the solenoid, electromagnetic conversion problem is governed by Biot-Savart law:

$$d\boldsymbol{B} = \frac{\mu_0}{4\pi} \times \frac{\boldsymbol{I}\, d\boldsymbol{l} \times \boldsymbol{r}}{|\boldsymbol{r}|^3} . \tag{1}$$

where μ_0 is the permeability of the vacuum, magnetic constant, $\boldsymbol{r}$ is the displacement unit vector in the direction pointing from the wire element towards the point at which the field is being computed, $\boldsymbol{dl}$ is a vector, whose magnitude is the length of the differential element of the wire, and whose direction is the direction of conventional current, and $\boldsymbol{B}$ is the magnetic flux density.

When placing a magnetic hydrogel fiber in magnetic field, surface magnetization current and volume magnetization current inside the ferromagnetic material is generated after its magnetization in the magnetic field, the magnetic force is interpreted as the magnetic force on molecular current. For isotropic medium it can be calculated as:

$$\boldsymbol{F}_M = \iiint_V (\nabla \times \boldsymbol{M}) \times \boldsymbol{B}\, dV . \tag{2}$$

E. *Cell Seeding inside Hydrogel Fibers*

We used the C2C12 cells for evaluating the cell compatibility of alginate hydrogel. C2C12 is a mouse myoblast cell and can easily cultivate. For the real cell cultivation, alginate solution is needed to be treated by the autoclave firstly and then filtrated with syringe filter. In order to remove the cells from culture dish, the old culture media

was discarded and 4ml PBS was injected near the edge of culture dish so as to not flow directly to the cell surface, then PBS was removed. 1.5mL of Trypsin EDTA was injected and placed inside the incubator for 3 minutes to detach the cells from each other. 3.5 mL of culture media was injected to stop the reaction then 15mL tube was put with 4mL of any solution inside centrifugal machine. Finally the dish was put with new cultivation media and the dish was placed inside incubator at 37 °C. The culture media was renewed in every 2 days. We observed the hydrogel encapsulated with C2C12 cells for three times, the first day, the second day and the eighth day of cultivation. The results are explained below. As during the observation, we cannot make sure the observation area is same every time, we chose the observation site that has an average cell number of all the hydrogel fiber. Through all the process of cultivation and observation, the dishes were sprayed with ethanol after every operation to avoid bacteria or fungi proliferating inside or around the culture dishes.

III. Results and Discussion

A. *Fabrication of Segmented Hydrogel Fiber*

By using the fluidic chips with the fluidic pattern as shown in Fig.2, the input solutions to all the channels were as shown in Fig. 6. The magnetic section of hydrogel fiber can be obtained by controlling the inputs of magnetic alginate solution and nonmagnetic alginate solution at different time sequence. At the central section of the channel, calcium chloride solution, the dextran buffer and the alginate solution will confluent. With the flow of all the solutions, the calcium chloride solution diffuses inside the channel and solidifies the alginate solution to become hydrogel fiber. The laminar flow at the central part of the fluidic chip is as shown in Fig.6(b). This laminar flow is important that turbulent flow can cause the alginate solution solidify inside the channels and make fiber jam inside channel, which finally makes the fluidic chip blocked by the solidified fiber. Before the experiment, it is also necessary to remove the air bubbles inside all the solutions for 2 hours, as the small amount of air bubbles may aggregate inside the fluidic chips and change the physical properties of laminar flow, which results in non-uniform thickness of the hydrogel fiber.

B. *Magnetic Hydrogel Fibers*

The magnetic hydrogel fiber fabricated with fluidic chips is as shown in Fig.7 and the magnetic alginic acid sodium was controlled with pressure control at the crossing of three channels. By injecting the magnetic alginic solution, the magnetic sections can be obtained. As the fluidic channel is with a height of 100 µm, the size of hydrogel fiber fabricated is about 100 µm in width and 40 µm in height. And from the experiments, we prove the mechanical strength of hydrogel fiber is enough for magnetic manipulation. The minimum pitches of the magnetic hydrogel fiber are related to the switching frequency of the magnetic alginate flow and nonmagnetic alginate flow. It has been reported that fibroblasts grow on scaffolds of pore sizes of between 5 and

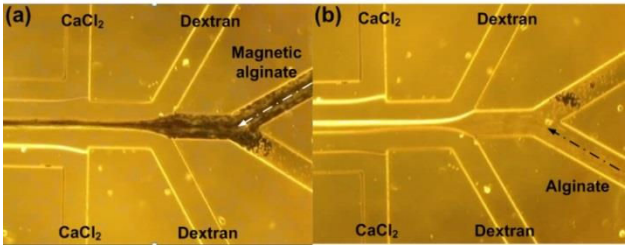

Fig. 6. Illustration of laminar flow at the central area of the fluidic chip during the fabrication of magnetic hydrogel fiber. (a) Input of magnetic alginate.(b) Input of nonmagnetic alginate.

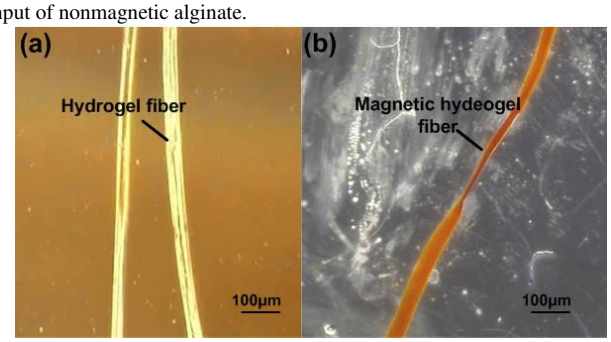

Fig.7. Optical image of hydrogel fiber. (a) Nonmagnetic hydrogel fiber; (b)magnetic hydrogel fiber.

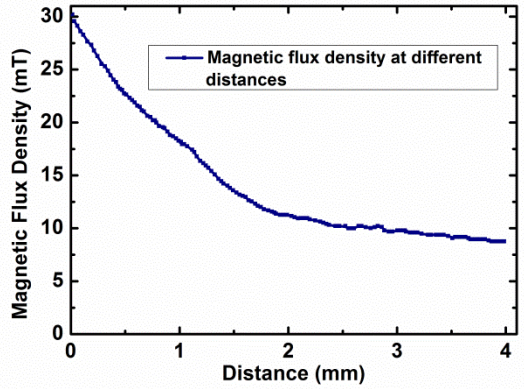

Fig.8. Magnetic flux density as a function of distance from the tip of magnetic tweezer.

15 µm, hepatocytes on scaffolds with pores of approximately 20 µm, skin cells regenerate on scaffolds with pores of between 20 and 150 µm and neovascularization occurs on scaffolds containing pores of 5 µm [19]. This hydrogel microfiber can be used to encapsulate many kinds of living cells and patterned as scaffolds in tissue regeneration.

C. *Magnetic Tweezers System*

Compared to other micro manipulation methods, such as optical laser tweezers, acoustical standing waves, micro pipette and dielectrophoresis techniques, magnetic approach can satisfy the requirements of high forces, reduced charging and heating of samples, and remote controllability[20, 21]. In our study, a magnetic tweezers system based on electromagnet is as shown in Fig.5 to control fiber in 3D space. The magnetic tweezers system is mounted on a micro stage for positioning the tip above the magnetic fiber and manipulating the fibers. Polymer glue was covered on the surface to make the device water-proof. When giving a current of 0.2 A, the magnetic flux density at the tip of the electromagnet is 30 mT. The magnetic field is used to control

the magnetic hydrogel fibers and to align them with specific shapes. Fig.8 shows the magnetic gradient changes as a function of distance from the tip of magnetic tweezers. With the given condition of magnetic alginate fiber fabricated in this study, the minimum magnetic flux density at the tip of magnetic tweezer is about 23.5 mT. This valve can be obtained by adjusting the distance of tip and magnetic fiber from Fig.8, or by regulating the input current. However it is better to use the levitation force to manipulate the fiber magnetically in order to avoid rigid contact between the fiber and tip, as this rigid contact increases the risk of fiber rupture.

D. Manipulation of Magnetic Hydrogel Fiber

Magnetic alginate fiber fabricated by wet spinning was used to prove the feasibility of the proposed method. The thickness of the fiber is 0.8mm and can be attracted to the tip of magnetic tweezer. As indicated in Fig.9, the fibers can be manipulated and the speed and trajectory can be controlled by the micro stage. The positioning resolution is under sub-micro level. It is in principle possible to achieve stronger magnetic forces on the alginate fiber by using higher input current to the coil, winding additional coil turns, or improving the percentage of MRI contrast media during the preparation of magnetic alginate solution.

Fig. 10(a) shows when three steel pillars are placed on a larger magnet, the electromagnet could be used to coil the alginate fibers along the three pillars. The illustration of the hydrogel constructs is as shown in the dash box. For fabricating complex hydrogel constructs, more magnetic pillars are needed to achieve arbitrary shape hydrogel constructs. As shown in Fig. 10(b), a conventional patterning device, comprising poly(methyl methacrylate) (PMMA) with a thickness of 5 mm and steel wires with a diameter of 0.7 mm, and an interval of 3 mm, is utilized to generate dot pattern of magnetic field on the surface. A neodymium magnet with the surface magnetic flux density of 0.035 T is mounted under the patterning device. Around the cylindrical steel area, the magnetic gradient is higher and with maximum value of magnetic gradient at Y direction of 48.6 mT when a neodymium magnet with the surface magnetic flux density of 0.38 T is placed under the patterning device. As the tip of the electromagnet is 0.8 mm in diameter, it is convenient to use the magnetic tweezer to pattern the magnetic hydrogel fiber on the surface of the patterning device. In the experiment, we cut the magnetic hydrogel fiber into pieces beforehand, then put all the small pieces of magnetic hydrogel fiber inside the calcium chloride solution. By adjusting the position of micro stage, the relative position of magnetic tweezer and the patterning device will change. The magnetic hydrogel fiber is attracted on the tip of the magnetic tweezer and moved inside calcium chloride solution.

E. Cell Cultivation inside Hydrogel Fiber

C2C12 cells are a mouse myoblast cell. Under appropriate conditions, these cells differentiate into contractile myotubes and produce characteristic muscle proteins. The cell cultivation results in 8 days are as shown in Fig. 11. The three

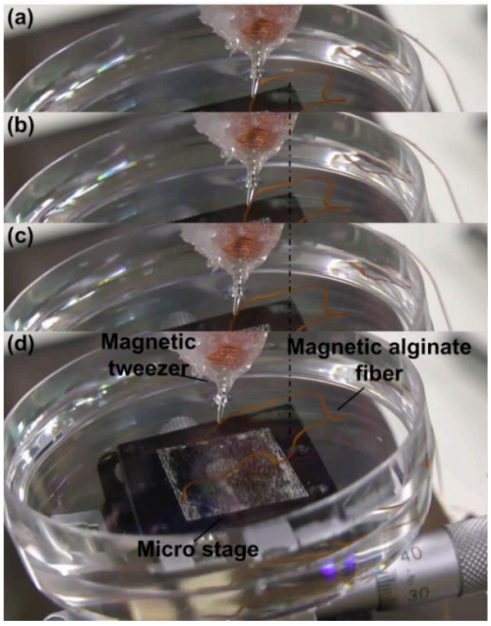

Fig.9. Experiments for manipulating magnetic alginate fiber at different time.(a) at time 0; (b) at time 1s; (c) at time 2s;(d) at time 4s.

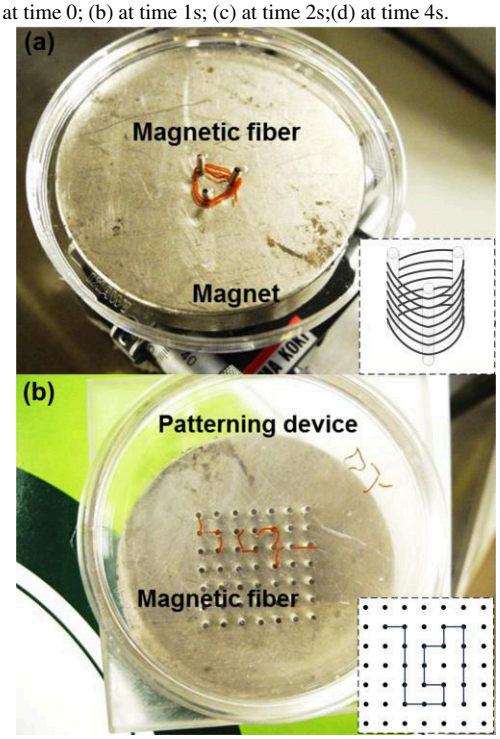

Fig.10 Manipulation of magnetic hydrogel fiber with magnetic tweezers system. (a) coiling magnetic hydrogel fiber on three steel pillar; (b) aligning magnetic hydrogel fiber on a patterning device.

figures present the cell cultivation condition in the first day, the second day and the eight days respectively. Compared the cell number in the image, cells grow well inside the hydrogel fibers. Fibroblast structures are shown in the figures. Due to the sterilization during the operation, there is no other cells pollute the C2C12 cells. In the experiments, we also confirm the hydrogel degrade and its mechanical properties become weaker after 8 days cultivation inside incubator. The C2C12 cells grow healthily inside the hydrogel fiber and take up most of the hydrogel. Apart from these, it is good to show that the alginate hydrogel fiber is biocompatible, biodegradable

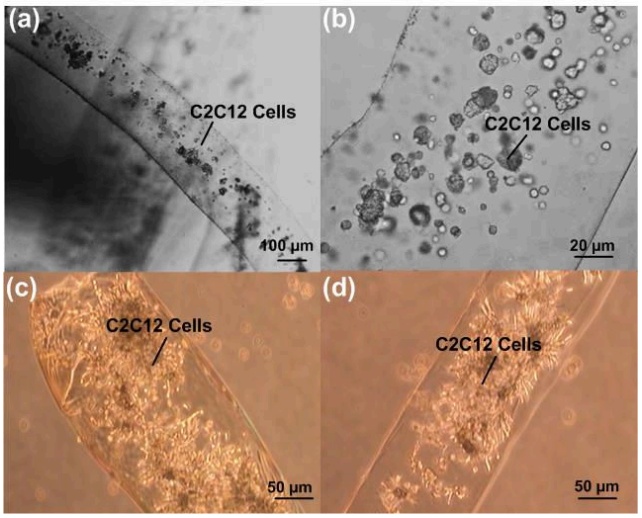

Fig.11. Cultivation results of C2C12 cells after a week inside the hydrogel fibers. (a) on the first day; (b) on the second day; (c)-(d) on the eighth day.

and with good cytocompatibility. These properties are crucial for the hydrogels as base material in the engineered scaffold fabrication.

Therefore, the proposed method is useful for fabricating segmented magnetic hydrogel fiber used for tissue regeneration by in-vitro cultivation. However there still exist some limitations in this study, such as 3D structure construction is inaccessible due to magnetic support is needed for aligning the patterning the magnetic hydrogel fiber in 3D space. Future challenges of this research will include the thickness control of the segmented hydrogel fiber, the manipulation of magnetic hydrogel fiber for constructing 3D complex assembly and cell cultivation in 3D complex hydrogel construct.

IV. Conclusions

An improved method for fabricating segmented magnetic hydrogel fiber based on microfluidic device was proposed for the scaffold application in tissue regeneration. The fabricated hydrogel fiber was made of alginic acid sodium and with a diameter of 34 μm. MRI contrast media solution was added into the alginic acid sodium solution to append magnetic material inside fiber. The magnetic material inside the hydrogel fiber was successfully regulated by the microfluidic chip and controlled at desired length with syringe pump. Magnetic tweezer system based on solenoid electromagnet was utilized to evaluate the magnetic response of the magnetic hydrogel fiber. Evaluation results showed the magnetic hydrogel fiber could be patterned and aligned under the constructed magnetic control system. The cultivation results of hydrogel fiber with C2C12 cells shows the proposed methods hold great potential in tissue engineering.

Acknowledgment

The authors appreciate the help rendered by Natsuki Takei in Department of Micro-nano Systems Engineering, Nagoya University for C2C12 separation and cultivation.

References

[1] S. Martel, O. Felfoul, J. B. Mathieu, A. Chanu, S. Tamaz, M. Mohammadi, M. Mankiewicz, and N. Tabatabaei, "MRI-based Medical Nanorobotic Platform for the Control of Magnetic Nanoparticles and Flagellated Bacteria for Target Interventions in Human Capillaries," *International Journal of Robotics Research,* vol. 28, pp. 1169-1182, Sep 2009.

[2] C. Hu, T. Uchida, C. Tercero, S. Ikeda, K. Ooe, T. Fukuda, F. Arai, M. Negoro, and G. Kwon, "Development of biodegradable scaffolds based on magnetically guided assembly of magnetic sugar particles," *Journal of Biotechnology,* vol. 159, pp. 90-98, 2012.

[3] P. Pouponneau, J.-C. Leroux, and S. Martel, "Magnetic nanoparticles encapsulated into biodegradable microparticles steered with an upgraded magnetic resonance imaging system for tumor chemoembolization," *Biomaterials,* vol. 30, pp. 6327-6332, 2009.

[4] S. K. Suh, S. C. Chapin, T. A. Hatton, and P. S. Doyle, "Synthesis of magnetic hydrogel microparticles for bioassays and tweezer manipulation in microwells," *Microfluidics and Nanofluidics,* vol. 13, pp. 665-674, Oct 2012.

[5] A. Khademhosseini and R. Langer, "Microengineered hydrogels for tissue engineering," *Biomaterials,* vol. 28, pp. 5087-5092, Dec 2007.

[6] K. R. King, C. C. J. Wang, M. R. Kaazempur-Mofrad, J. P. Vacanti, and J. T. Borenstein, "Biodegradable microfluidics," *Advanced Materials,* vol. 16, pp. 2007-2012, Nov 2004.

[7] V. Liu Tsang and S. N. Bhatia, "Three-dimensional tissue fabrication," *Advanced Drug Delivery Reviews,* vol. 56, pp. 1635-1647, 2004.

[8] V. Mironov, T. Boland, T. Trusk, G. Forgacs, and R. R. Markwald, "Organ printing: computer-aided jet-based 3D tissue engineering," *Trends in Biotechnology,* vol. 21, pp. 157-161, 2003.

[9] N. E. Fedorovich, J. Alblas, J. R. de Wijn, W. E. Hennink, A. J. Verbout, and W. J. A. Dhert, "Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing," *Tissue Engineering,* vol. 13, pp. 1905-1925, Aug 2007.

[10] M. C. Cushing and K. S. Anseth, "Hydrogel Cell Cultures," *Science,* vol. 316, pp. 1133-1134, May 25, 2007.

[11] L. L. Y. Chiu, M. Radisic, and G. Vunjak-Novakovic, "Bioactive Scaffolds for Engineering Vascularized Cardiac Tissues," *Macromolecular Bioscience,* vol. 10, pp. 1286-1301, 2010.

[12] T. Takei, S. Sakai, H. Ijima, and K. Kawakami, "Development of mammalian cell-enclosing calcium-alginate hydrogel fibers in a co-flowing stream," *Biotechnology Journal,* vol. 1, pp. 1014-1017, 2006.

[13] R. T. Tranquillo, "The Tissue-Engineered Small-Diameter Artery," *Annals of the New York Academy of Sciences,* vol. 961, pp. 251-254, 2002.

[14] B. V. Slaughter, S. S. Khurshid, O. Z. Fisher, A. Khademhosseini, and N. A. Peppas, "Hydrogels in Regenerative Medicine," *Advanced Materials,* vol. 21, pp. 3307-3329, 2009.

[15] L. G. Griffith and G. Naughton, "Tissue Engineering--Current Challenges and Expanding Opportunities," *Science,* vol. 295, pp. 1009-1014, February 8, 2002.

[16] C. E. Schmidt and J. B. Leach, "Neural Tissue Engineering: Strategies for Repair and Regeneration," *Annual Review of Biomedical Engineering,* vol. 5, pp. 293-347, 2003.

[17] A. King, B. Strand, A. M. Rokstad, B. Kulseng, A. Andersson, G. Skjak-Braek, and S. Sandler, "Improvement of the biocompatibility of alginate/poly-L-lysine/alginate microcapsules by the use of epimerized alginate as a coating," *Journal of Biomedical Materials Research Part A,* vol. 64A, pp. 533-539, Mar 2003.

[18] S. Fu, A. Thacker, D. M. Sperger, R. L. Boni, I. S. Buckner, S. Velankar, E. J. Munson, and L. H. Block, "Relevance of Rheological Properties of Sodium Alginate in Solution to Calcium Alginate Gel Properties," *Aaps Pharmscitech,* vol. 12, pp. 453-460, Jun 2011.

[19] S. F. Yang, K. F. Leong, Z. H. Du, and C. K. Chua, "The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques," *Tissue Engineering,* vol. 8, pp. 1-11, Feb 2002.

[20] E. Mirowski, J. Moreland, S. Russek, M. Donahue, and K. W. Hsieh, "Manipulation of magnetic particles by patterned arrays of magnetic spin-valve traps," *Journal of Magnetism and Magnetic Materials,* vol. 311, pp. 401-404, Apr 2007.

[21] M. Barbic, "Magnetic wires in MEMS and bio-medical applications," *Journal of Magnetism and Magnetic Materials,* vol. 249, pp. 357-367, Aug 2002.