DOI 10.5162/IMCS2012/3.2.2

Toward functional engineered tissues as biosensors using hydrogels and dielectrophoretic technique

Javier Ramón-Azcón [a,1], Samad Ahadian [a,1], Raquel Obregón [b], Gulden Camci-Unal [c], Serge Ostrovidov [a], Vahid Hosseini [a,d], Kosuke Ino [b], Hitoshi Shiku [b], Ali Khademhosseini [a,c,e,], Tomokazu Matsue [a,b]*

[a] WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan. [b] Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan. [c] Department of Medicine, Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139, USA and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [d] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA
Mail corresponding author: javier@bioinfo.che.tohoku.ac.jp

Abstract:

Microscale technologies have been emerged as powerful tools for tissue engineering. Such technologies render precise positioning for the cells in order to define the cell-cell and cell-extracellular matrix (ECM) interactions mimicking the structure of native tissue constructs. Dielectrophoresis (DEP) method is a suitable microscale technology to do that having notable characteristics in cell manipulation such as being high accurate, rapid, scalable, and capable of handling both adherent and non-adherent cells. DEP could be used in combination with new biomaterials to embed the cells within a given pattern, allowing precise positioning of cells in order to define interactions between neighboring cells. In this investigation, we propose the gelatin methacrylate (GelMA) as a promising hydrogel for the cell dielectropatterning due to low viscosity and ionic concentration. Combined application of the GelMA hydrogel and DEP technique could be useful for precisely creating complex and cell-responsive microtissues in a rapid, accurate, and scalable manner. In this study, we proposed also the interdigitated array of electrodes as a novel platform to electrically stimulate the 3D engineered muscle tissue. The attained muscle myofibers were analyzed and quantified in terms of myotube characteristics and gene expression. These engineered tissues have the potential to serve as biosensors to examine biologically active reagents and could be applied to pharmacological screening and environmental monitoring.

Key words: Engineered tissue, skeletal muscle, dielectrophoresis, hydrogel, electrical stimulation、C2C12 cells, HUVEC cells.

i

Dielectrophoresis as microscale technology

Microscale technologies have been emerged as powerful tools for tissue engineering and biological studies. Such technologies render precise positioning for the cells in order to define the cell-cell and cell-extracellular matrix (ECM) interactions mimicking the structure of native tissue constructs. DEP is a powerful microscale technology that could be used in combination with new biomaterials to embed the cells within a given pattern, allowing precise positioning of cells in order to define cell-cell and cell-ECM interactions [1].

Hydrogels are attractive soft materials for biological applications due to their high water content and biocompatibility. In this study, we suggest the application of a novel synthetic hydrogel, namely, gelatine methacrylate (GelMA) synthesized by our research group [2] to make 3D cell patterns with the aid of the DEP technique. GelMA is a photopolymerizable hydrogel comprised of modified natural ECM components [3], making it a potentially attractive material for cell-based studies and tissue engineering applications.

Fabrication of muscle and vascularized engineered tissues

As example, in this work we have evaluated human umbilical vein endothelial cells (HUVEC) as a model cell type for the potential applications in vascularized tissue engineering and rodent myoblasts (C2C12) for potential

skeletal muscle tissue engineering applications since all of these potential applications demand highly elongated, organized, and aligned ECM-cell constructs to mimic the complexly organized microarchitecture in vivo.

The protocol to produce the functional tissues consists in: (i) surface treatment with silanes agents and polyethylene glycol-diacrylate (PEG-DA) hydrogel polymer. (ii) Cell patterning using DEP forces in GelMA prepolymer (see figure 1). (iii) Polymerization of the GelMA and cultivation of the cells for 5 days.

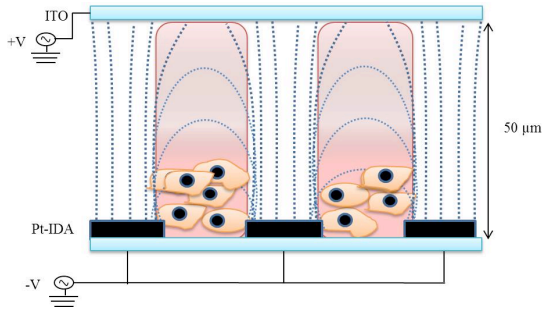

Fig. 1. Schematic representation of the DEP device for pattering cells.

Fabrication of a highly organized cell structure is crucial in making many functional tissues, which recapitulates the function and structure of the corresponding tissue in vivo. To asset the functionality of the tissue, it was evaluated the cell alignment, circularity and elongation within GelMA and compared with standard PEG-DA hydrogel of C2C12 and HUVEC cells. Viability of the tissue was also evaluated with calcein AM/ethidium homodimer for both types of cells within the hydrogels.

Electrical stimulation

In order to prepare a viable tissue, we proposed the interdigitated array of electrodes as a novel platform to electrically stimulate (ES) the 3D engineered muscle tissue, which had the following advantages: (a) Since the electrodes were permanently positioned on the substrate, they could provide facile, highly producible, and well-quantified electric field; (b) It was feasible to fabricate high resolution and complex electrode designs relevant to physiological feature sizes and architectures; (c) The ES was synchronized in the whole tissue; (d) Lower energy was needed to create a specified electric field as compared to the conventional setups for the ES. The attained muscle myofibers were analyzed and quantified in terms of myotube characteristics and gene

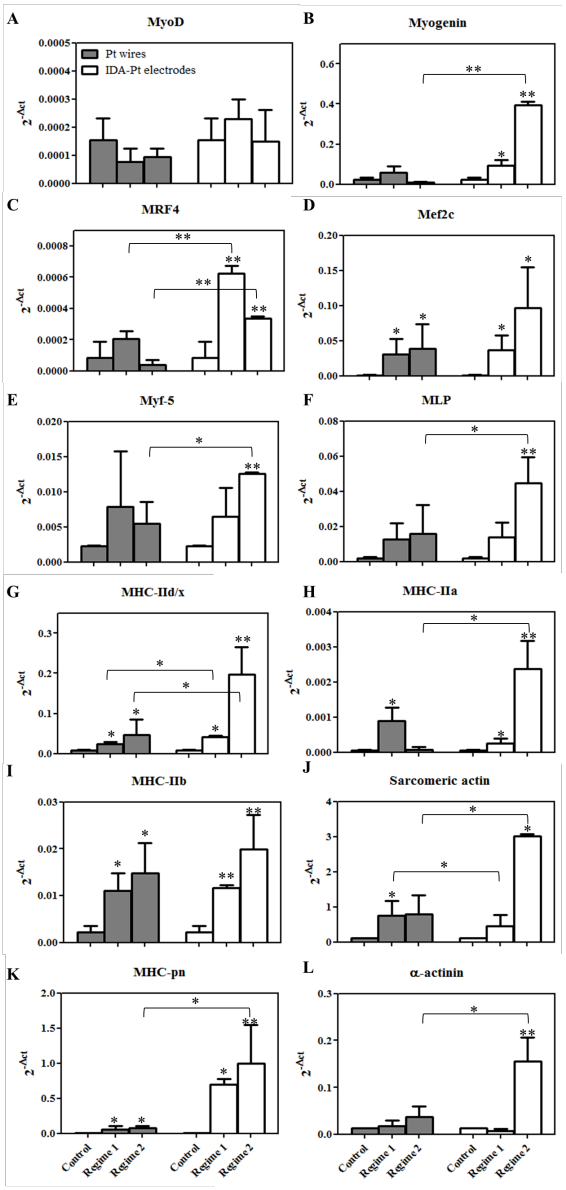

*Fig. 2. Changes in the expression levels of MyoD (A), Myogenin (B), MRF4 (C), Mef2c (D), Myf-5 (E), and MLP (F) MHC-IId/x (G), MHC-IIa (H), MHC-IIb (I), Sarcomeric actin (J), MHC-pn (K), and α-actinin (L) as a result of different ES paradigms of regime 1 (voltage 0.5 V, frequency 1 Hz, and duration 10 ms), regime 2 (voltage 6 V, frequency 1 Hz, and duration 10 ms), and control (Without ES). Expression levels were normalized with respect to the internal reference gene GAPDH (*p <0.05 and **p <0.001).*

expression. It was shown that the engineered muscle tissues stimulated through the interdigitated array of electrodes demonstrated superior performance and maturation compared to the corresponding tissues stimulated through the conventional setup (i.e., through Pt wires in close proximity to the muscle tissue).

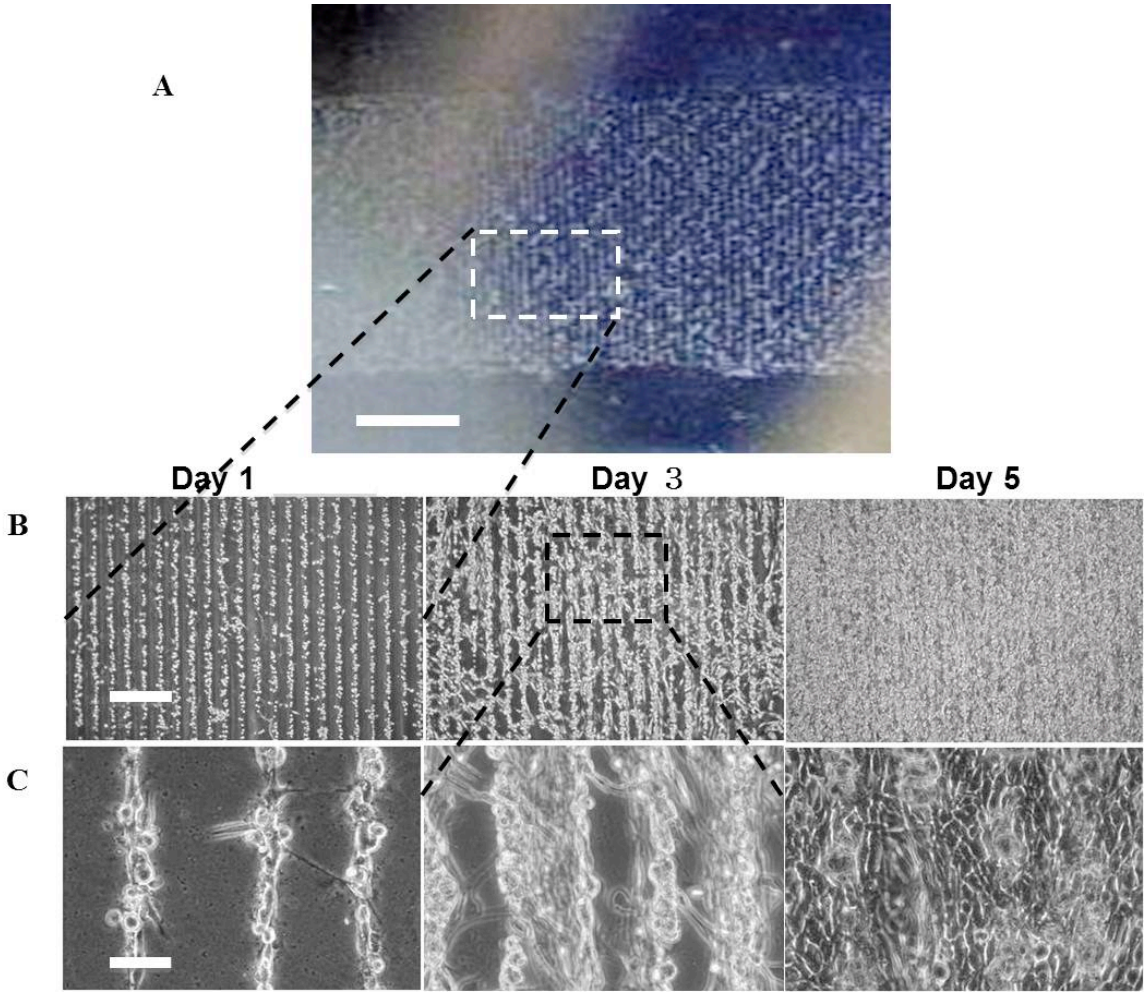

Fig. 3. Picture of aligned C2C12 muscle cells within 5% gelatin methacrylate (GelMA) hydrogel as obtained by the dielectrophoresis (DEP) technique using 50 μm electrode-50 μm gap device (A). Phase contrast images of the aligned C2C12 muscle cells within 5% GelMA hydrogel at different culture times (B and C). Scale bar shows 0.25 cm, 400 μm, and 50 μm in A, B, and C, respectively.

Tissue characterization by gene expression

The myogenic regulatory factors (MRFs) are major transcriptional regulators of the myogenesis. The MRFs are a family of muscle specific transcription factors including MyoD, Myf-5, myogenin, and MRF4. MyoD and Myf-5 are expressed in proliferating, undifferentiated myoblasts, and regulate myogenic determination [4, 5]. The expression of sarcomere contractile proteins (i.e., sarcomeric actin, α -actinin, perinatal myosin heavy chain (MHC-pn), MHC-IId/x, MHC-IIa, and MHC-IIb) as markers of differentiation of skeletal muscle tissue [6] was also investigated to evaluate the fabricated muscle maturation.

Expression levels of target genes for muscle development were evaluated after applying the ES under paradigms 1 and 2 (figure 2). The electrically stimulated cultures through the IDA-Pt electrodes were compared to the corresponding samples stimulated by the Pt wires and those without ES (control samples).

Conclusions

A novel biomaterial (i.e., GelMA hydrogel) was employed to establish 3D microscale organization of the cells with the aid of DEP method. In this investigation, we suggested the application of the GelMA as a promising hydrogel for the cell electropatterning due to low viscosity and ionic concentration. It is found that the GelMA hydrogel has a superior performance to make myoblast and endothelial cell patterns with high fidelity as well as to maintain the cell viability and growth as compared to the PEG-DA. Combined application of the GelMA hydrogel and DEP technique could be useful for precisely creating complex and cell-responsive microtissues in a

rapid and scalable manner for both fundamental biological studies and tissue engineering applications. Stimulation is a crucial point in the maturation of the muscle tissue. In this study, we proposed also the interdigitated array of electrodes as a novel platform to electrically stimulate the 3D engineered muscle tissue. It was shown that the engineered muscle tissues stimulated through the interdigitated array of electrodes demonstrated superior performance and maturation compared to the corresponding tissues stimulated through the conventional setup (i.e., through Pt wires in close proximity to the muscle tissue). Combining different electrical techniques we have obtained an integrate device to fabricate and maturate skeletal muscle tissues. These engineered tissues are akin to their corresponding in vivo tissues in structure in order to ensure that the desired function is obtained and they are ideal candidates for drug screening, in the evaluation of several toxic drugs, like alpha-cypermethrin and 6-hydroxydopamine.

Acknowledgements

This work was supported in part by the WPI-Advanced Institute for Materials Research, Tohoku University, Japan.

References

[1] Matsue T, Matsumoto N, Uchida I. Rapid micropatterning of living cells by repulsive dielectrophoretic force. Electrochimica Acta. 1997;42(20-22):3251-3256. DOI: 10.1016/S0013-4686(97)00175-8.

[2] Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S, Khademhosseini A. Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials. 2010 Jul;31(21):5536-5544. DOI: 10.1016/ j.biomaterials.2010.03.064.

[3] Van Den Bulcke AI, Bogdanov B, De Rooze N, Schacht EH, Cornelissen M, Berghmans H. Structural and Rheological Properties of Methacrylamide Modified Gelatin Hydrogels. Biomacromolecules. 2000 Mar;1(1):31-38. DOI: 10.1021/bm990017d.

[4] Arnold H, Braun T. Targeted inactivation of myogenic factor genes reveals their role during mouse myogenesis: a review. International Journal of Developmental Biology. 1996;40:345–53.

[5] 29. Watt KI, Judson R, Medlow P, Reid K, Kurth TB, Burniston JG, et al. Yap is a novel regulator of C2C12 myogenesis. Biochemical and Biophysical Research Communications. 2010;393(4):619–24

[6] Taubman MB, Smith CW, Izumo S, Grant JW, Endo T, Andreadis A, et al. The expression of sarcomeric muscle-specific contractile protein genes in BC3H1 cells: BC3H1 cells resemble skeletal myoblasts that are defective for commitment to terminal differentiation. . The Journal of Cell Biology. 1989;108 (5):1799–806