

MIT Open Access Articles

Cell bioprinting as a potential high-throughput method for fabricating cell-based biosensors (CBBs)

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

Citation: Xu, F. et al. "Cell Bioprinting as a Potential High-throughput Method for Fabricating Cell-based Biosensors (CBBs)." IEEE, 2009. 387–391. © Copyright 2009 IEEE

As Published: <http://dx.doi.org/10.1109/ICSENS.2009.5398245>

Publisher: Institute of Electrical and Electronics Engineers (IEEE)

Persistent URL: <http://hdl.handle.net/1721.1/71872>

Version: Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

Terms of Use: Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.



Cell Bioprinting as a Potential High-Throughput Method for Fabricating Cell-Based Biosensors (CBBs)

F. Xu^a, S. Moon^a, A.E. Emre^a, C. Lien^a, E.S. Turali^a, U. Demirci^{a,b}

^a *Bio-Acoustic-MEMS in Medicine (BAMM) Laboratory, Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

^b *Harvard-MIT Health Sciences and Technology, Cambridge, MA, USA.*

Abstract—Cell-based biosensors (CBBs) are becoming an important tool for biosecurity applications and rapid diagnostics. For current CBBs technology, cell immobilization and high throughput fabrication are the main challenges. To address these in this study, the feasibility of bioprinting cell-laden hydrogel to fabricate CBBs at high throughput was investigated and cell response was tracked by using lensless charge-coupled device (CCD) technology. This study indicated that (i) a cell-laden collagen printing platform was capable of immobilizing cells (smooth muscle cells) in collagen droplets with precise spatial control and pattern them onto surfaces, (ii) high post-printing cell viability was achieved (>94%) and the immobilized cells proliferated over five days, (iii) the immobilized cells maintain their biological and physiological sensitivity to environmental stimuli (e.g. environmental temperature change and lysis by adding of de-ionized water), as quantified by change in cell spread size (decreasing from $\sim 3000 \mu\text{m}^2$ at $t=0$ hour to $\sim 600 \mu\text{m}^2$ at $t=16$ hours), and (iv) our developed lensless CCD technology is capable detecting the cell morphology change under environmental stimuli, which is essential for the portability of the CBBs. These results show that printing cells encapsulated in biocompatible hydrogels could lead to fabrication of CBBs in a high throughput manner. Also, the lensless CCD systems can be used to monitor the morphological cell responses over a wide field of view (as large as $37.25 \text{ mm} \times 25.70 \text{ mm}$).

I. INTRODUCTION

Cell-based biosensors (CBBs) have several practical applications, such as environmental monitoring [1-4] and assessing the functional attributes of pathogens and toxins [5-7]. CBBs provide a better assessment of the overall affect of contaminants on a biological system compared to chemical-based sensors [8]. For example, CBBs have shown promise as a method to monitor toxicity in water, compared to other ecotoxicological assays [9].

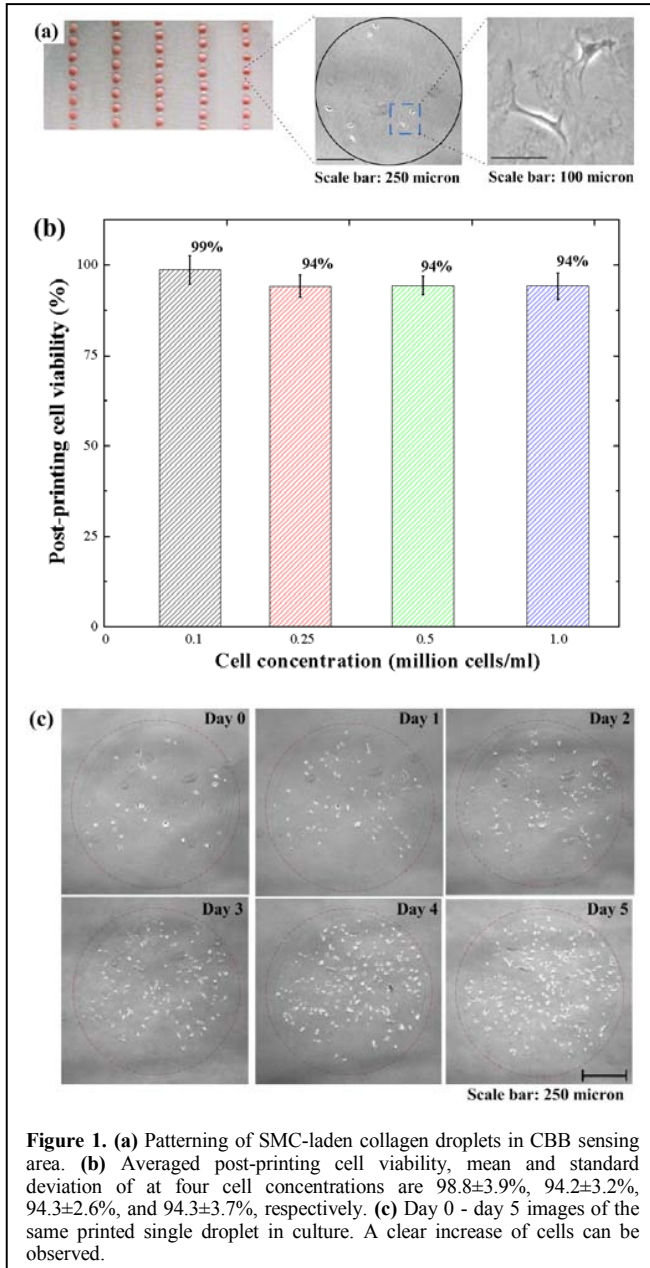
CBBs are generally composed of sensing element (cells) and reporting elements, such as metabolism [10], fluorescent probes and reporter genes [11], motility and adhesion [12]. However, the current CBBs suffer from several limitations. (i) Cell-substrate attachment is weak [13], especially for some

mammalian cell lines (e.g., neuroblastoma cells [14, 15]). (ii) It is difficult to make hundreds of CBBs with similar patterned sensing elements (cells) at high throughput to enable data collection for statistical analysis, e.g. for insecticide toxin evaluation and detection [16]. (iii) Most of current CBBs detect cellular responses in the form of cumulative fluorescent change from a large cell population [17]. Although this supplied the advantage of portability, it cannot measure important characteristics, such as spatial and temporal change of cell morphology (e.g. area [18]) and motility [17] for these needs.

To address the above limitations of current CBBs, we studied the feasibility of bioprinting cell-laden hydrogels (i.e., collagen) to fabricate CBBs at high throughput. Cell printing is a novel technology initially used in the tissue engineering field [18-21]. It can be used to pattern multiple cell types mixed with scaffolding materials to build complex structures *in vitro* at high throughput. This avoids the need to chemically pre-treat surfaces to immobilize the cells using binding as used with traditional patterning methods such as lithographic patterning. Cell bioprinting has been used to create a layered microorgan for *in vitro* pharmacokinetic model [22]. Besides cell-laden hydrogels (e.g., collagen) have potential to immobilize cells in 3D for CBBs, and hydrogels have high water content, biocompatibility and mechanical properties resemble natural cell microenvironment [23]. Various cell types may be entrapped or immobilized within these hydrogel scaffolds [17, 24], such as smooth muscle cells (SMCs) and neurons. Within the different hydrogels for immobilizing mammalian cells in CBBs, collagen generates special interest and offers significant promise [5, 24-26]. Collagen supplies several advantages, including different pore sizes that trap different cells by varying the collagen concentration [24, 27]. For example, a collagen-encapsulated B-lymphocyte cell line has been used as a biosensor for rapid detection of pathogens and toxins [5, 28].

We have created and characterized a cell printing system capable of providing precise spatial control to encapsulate cells and pattern them in 3D to create these model constructs at high throughput [29-31]. Such a high cell viability and

This work was supported by The Randolph Hearst Foundation and Brigham and Women's Hospital Department of Medicine Young Investigator in Medicine Award.



control on cell location and cell density are very important to fabricate CBBs at high throughput. Also, we have earlier developed CCD (Charge Coupled Device)-based fluorescence-free (label free) technology to monitor cells in a wide field of view [32]. This large area tracking capability is very helpful since we will pattern multiple cell-laden droplets in the sensing area of CBB (**Figure 1a**). In this study, we (i) immobilized SMCs in collagen droplets at high throughput using cell printing technology since our lab is specially interested in testing toxicity and possible future drugs for smooth muscle cell applications, (ii) tested the viability of the immobilized SMCs and their biological properties (proliferation), (iii) investigated biological and physiological sensitivity of immobilized cells to environmental stimuli (temperature change and adding of de-ionized water, and (iv)

tested the feasibility of developed CCD technology for wide-area cell morphology detection, which is essential for the portability of the CBBs. We quantified the response by using the cell morphology variation (i.e., spread size), which has been used as an indicator of cell viability and response to environmental stimuli, such as pH [33].

II. MATERIALS AND METHOD

A. High throughput printing of SMC-laden collagen droplets

The cells used in this study were primary SMCs isolated from rat bladder tissue. SMC suspensions were mixed with reconstituted collagen (0.2 mg/ml) for printing. SMC-laden collagen droplets were patterned, see **Figure 1a**. We generated 20-160cell-laden collagen droplets per second with our system. The patterned droplets are the sensing units in the CBB and each droplet acts as a separate monitoring area.

B. Test of viability and proliferation of immobilized SMCs in printed collagen droplets

We assessed the post-printing cell viability in collagen droplets using a fluorescent live/dead viability staining (Invitrogen, Carlsbad, CA). Briefly, we first washed the cell-laden collagen droplets with PBS and stained the immobilized cells for 10 minutes at 37 °C with live/dead staining solution. We then washed these droplets with PBS prior to imaging to reduce background. Cell proliferation was characterized as a function of days (0-5 days), using a bright-field microscope (Nikon TE2000).

C. Measurement of cytotoxicity

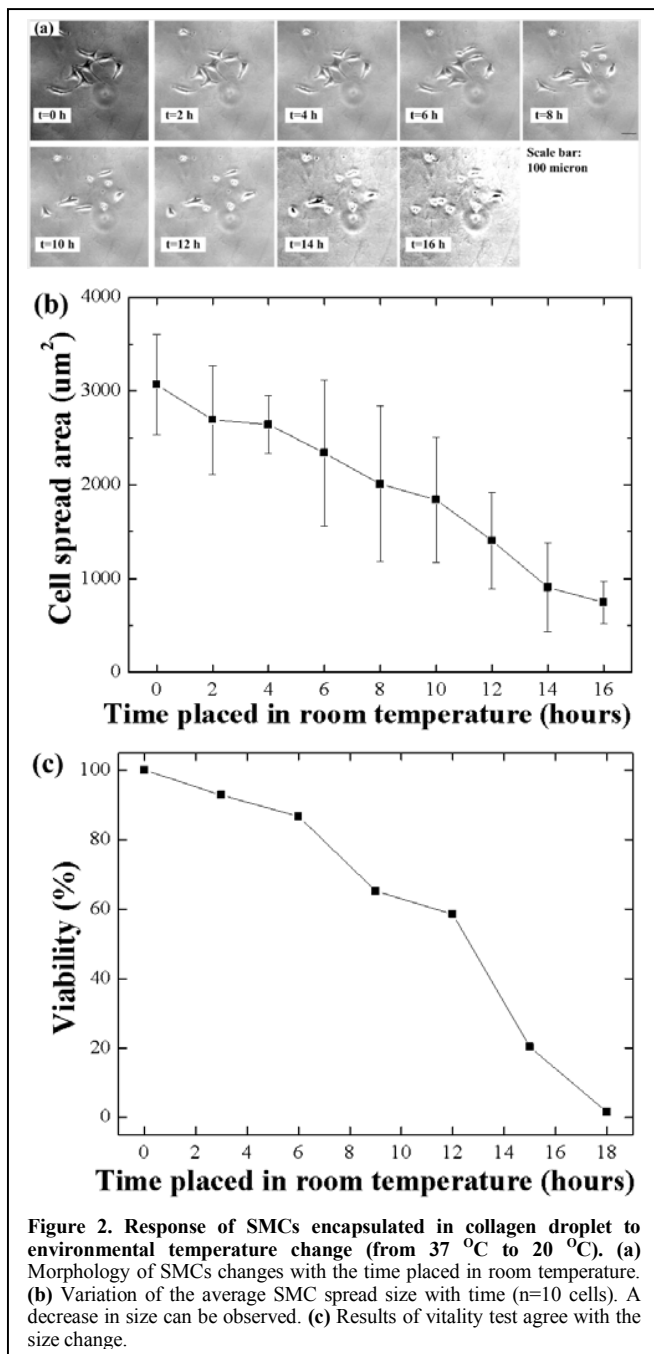
Healthy SMCs adhere to the surface which is imperative for subsequent cell functions like proliferation and synthesis of an extracellular matrix, and they lose this adherent morphology when they are damaged or dead [34]. The SMC morphology change response to environmental stimuli was observed with time for two cases: environmental temperature change from 37 °C to room temperature (20 °C), and adding of de-ionized water to lyse the cells. The printed cell-laden droplets were cultured in an incubator (37°C, 5% CO₂, sterile, Forma Scientific, CO₂ water jacketed incubator) for 24 hours to give the immobilized cells enough time to spread. The SMC-laden droplets were then placed under a bright-field microscope (Nikon TE2000) to record the morphology variation under stimuli (e.g. environmental temperature change).

III. RESULTS AND DISCUSSIONS

A. Viability and proliferation of immobilized cells

One major challenge with CBBs is to maintain cell viability, e.g. for CBBs using cardiomyocytes and neurons [35, 36], and the cell viability after immobilization determines the actual working life of the sensor [5]. For example, the B-lymphocytes were cultured in collagen for up to four days before they were used for cytotoxicity testing [5]. We tested the post-printing viability of the encapsulated SMCs and their proliferation over five days.

Cell viability in cell-laden collagen droplets after printing was assessed using a live/dead assay. The cell viability was analyzed for four different initial cell concentrations and the

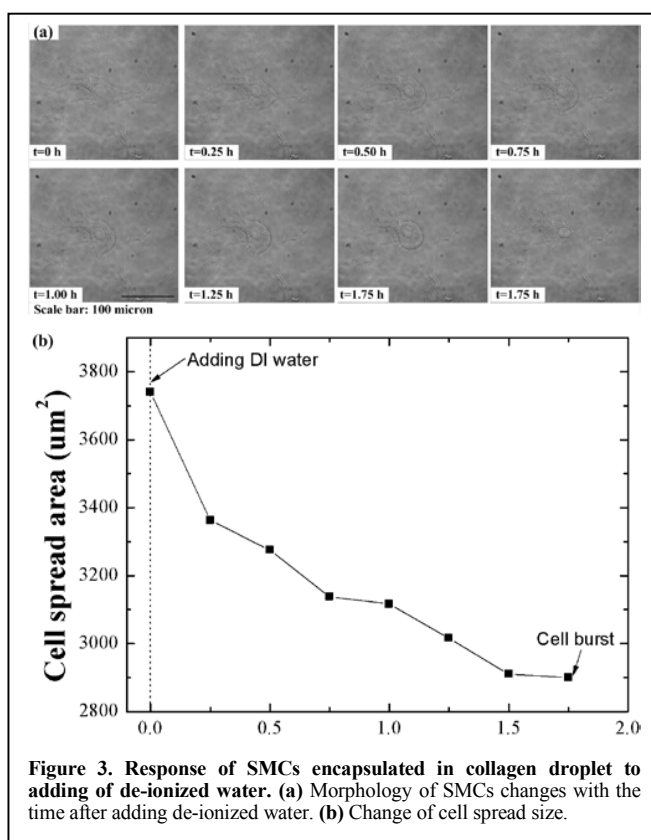


average cell viability of SMCs was assessed (n=10 per concentration), **Figure 1b**. Average cell viability (mean \pm standard deviation) for each initial number of cells per droplet case were 98.8 \pm 3.9%, 94.2 \pm 3.2%, 94.3 \pm 2.6%, and 94.3 \pm 3.7% for cell concentration of 0.1, 0.25, 0.5 and 1.0 million cells/ml, respectively. This indicates that overall cell viability was >94.2 \pm 3.2% (absolute values) repeatably and reliably for 40 droplets tested. On average, the cell viability before printing was 97.1% in medium. We also studied the SMC proliferation in single printed collagen droplets. The images of a typical printed cell-laden droplet from day 0 to day 5 are

given in **Figure 1c**. The total cell count within the droplets increased as cells proliferated in culture over 5 days.

B. Response of immobilized cells to environmental temperature change

Cell response has been used as an indicator of cell viability and response to environmental stimuli, e.g. pathogens or toxic agents [37-40]. For example, in the widely used electrical cell-substrate impedance sensing (ECIS) system [37], the sensing is achieved by measuring changes in the impedance resulting from changes in cellular morphology ('motion' or spreading on a surface) caused by external perturbations. Here, we also used the cell spread size to quantify the immobilized cells to environmental temperature change. **Figure 2a** shows the morphology of SMCs immobilized in printed collagen droplet changes with the time placed in room temperature (t=0, 2, ..., 16 hours). A decrease in size can be observed (**Figure 2a**), as shown by the average spread cell size in **Figure 2b**. This can be further verified by the viability tests performed every three hours after placing the cell-laden droplets in room temperature, **Figure 2c**. The morphology of the cells were also observed at each stage and compared to the viability values to find an indirect label-free link between the cell live/dead state and its morphology. Although cell morphology is not a true measure of the cellular life, we were able to see a relationship that follows the same trend. The cells lose their adherent state as they become more and more uncomfortable with their environment. Eventually, they acquire a spherical shape when they die.

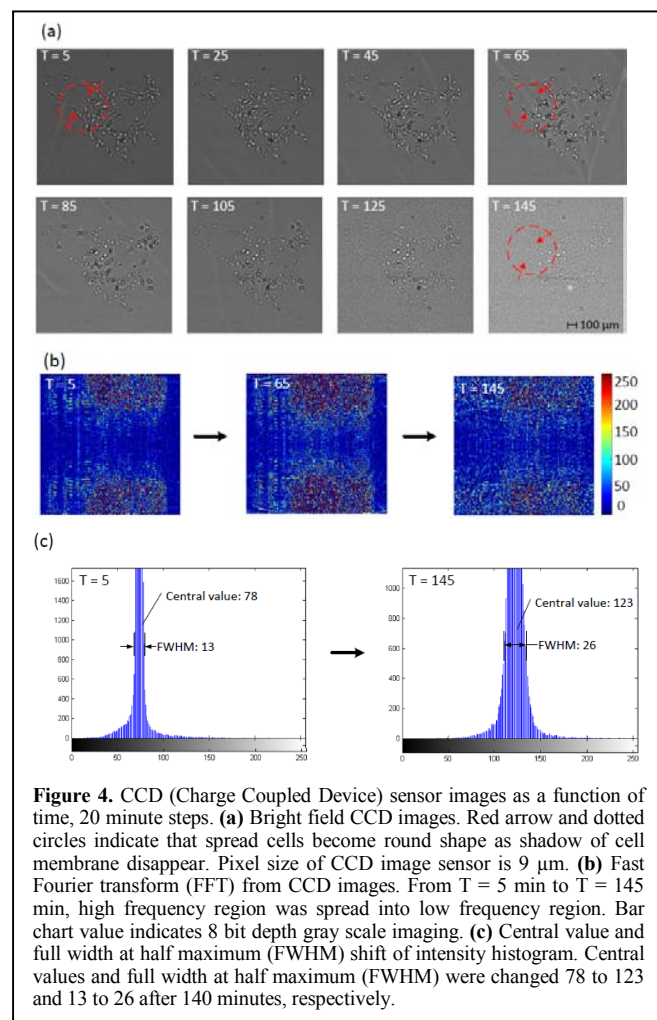


C. Response of immobilized cells to adding of de-ionized water

To mimic the toxic agents, de-ionized water was added to the printed cell-laden collagen droplets. The response of the immobilized SMCs was quantified by recording the cell spread size change. **Figure 3a** shows the cell morphology at eight time points ($t=0, 0.25, 0.5, 0.75, 1.0, 1.25, 150, 1.75$ hours) after adding 1 ml de-ionized water onto the glass slide containing the cell-laden droplets within 100 μl medium. The size was quantified as given in **Figure 3b**. Similar with the response to environmental temperature change, cell spread size decreased after adding de-ionized water. But this process was much faster (< 2 hours).

D. Feasibility of using lensless CCD technology to capture the cell morphology change

We investigated the feasibility of using CCD sensor (9 μm pixel size) to track the morphology change of the immobilized cells under environmental stimulus (from 37 $^{\circ}\text{C}$ to 20 $^{\circ}\text{C}$). Images were taken automatically as a function of time with 20 minute steps. During the process, some cell shadows disappeared and some decreased in size. **Figure 4a** gives the bright field CCD images, where red arrow and dotted circles indicate that adhered spread cells became round shaped as the



cell membrane shadows disappeared. The obtained lensless CCD images were processed using Fast Fourier Transfer transform (FFT), which showed a different frequency distribution between round shape and elongated rod shape of cell images, **Figure 4b**. From 5 min to 145 min, high frequency region (red index: over 200 intensity value) was spread into low frequency region (blue index: less 100 intensity value). Central value and full width at half maximum (FWHM) shift of intensity histogram were calculated (**Figure 4c**). These normalized intensity values, 0 to 255 for 8 bit gray scale image, changed from 78 to 123 and 13 to 26 after 140 minutes, respectively. This means FWHM became wider as the cell outline become unclear.

IV. CONCLUSIONS

Cell-based biosensors (CBBs) are promising tools for environmental monitoring, biosecurity applications and rapid diagnostics. With CBBs, information about the physiological effects of analytes can be obtained [2, 41], such as toxins, pathogens and drug candidates on living systems. However, current CBB technology has challenges with cell immobilization, low throughput fabrication and limited portability. To address these, in this study, we investigated the feasibility of bioprinting cell-laden hydrogel to fabricate CBBs at high throughput. This study indicated that cell printing can be used to immobilize cells (e.g. smooth muscle cells) in hydrogels (e.g. collagen) with high post-printing cell viability and to pattern them on surfaces. The immobilized cells maintained their biological properties (proliferation over five days) and their biological and physiological sensitivity to environmental stimuli (e.g. environmental temperature change and lysis with de-ionized water). The series shape changes of SMCs in collagen gel matrix can be detected using FFT analysis and intensity histogram by a wide area CCD sensor. These results show that printing cells with biocompatible hydrogels could lead to fabrication of CBBs in a high throughput manner.

REFERENCES

- [1] C. Ziegler, "Cell-based biosensors," *Fresenius J Anal Chem*, vol. 366, pp. 552-9, Mar-Apr 2000.
- [2] D. A. Stenger, G. W. Gross, E. W. Keefer, K. M. Shaffer, J. D. Andreadis, W. Ma, and J. J. Pancrazio, "Detection of physiologically active compounds using cell-based biosensors," *Trends Biotechnol*, vol. 19, pp. 304-9, Aug 2001.
- [3] S. Belkin, "Microbial whole-cell sensing systems of environmental pollutants," *Curr Opin Microbiol*, vol. 6, pp. 206-12, Jun 2003.
- [4] K. Viravaidya and M. L. Shuler, "Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies," *Biotechnol Prog*, vol. 20, pp. 590-7, Mar-Apr 2004.
- [5] P. Banerjee, D. Lenz, J. P. Robinson, J. L. Rickus, and A. K. Bhunia, "A novel and simple cell-based detection system with a collagen-encapsulated B-lymphocyte cell line as a biosensor for rapid detection of pathogens and toxins," *Lab Invest*, vol. 88, pp. 196-206, Feb 2008.
- [6] T. H. Rider, M. S. Petrovick, F. E. Nargi, J. D. Harper, E. D. Schwoebel, R. H. Mathews, D. J. Blanchard, L. T. Bortolin, A. M. Young, J. Chen, and M. A. Hollis, "A B cell-based sensor for rapid identification of pathogens," *Science*, vol. 301, pp. 213-5, Jul 11 2003.

- [7] T. Curtis, R. M. Naal, C. Batt, J. Tabb, and D. Holowka, "Development of a mast cell-based biosensor," *Biosens Bioelectron*, vol. 23, pp. 1024-31, Feb 28 2008.
- [8] S. Kohler, S. Belkin, and R. D. Schmid, "Reporter gene bioassays in environmental analysis," *Fresenius J Anal Chem*, vol. 366, pp. 769-79, Mar-Apr 2000.
- [9] B. Ekwall, F. A. Barile, A. Castano, and e. al, "MEIC evaluation of acute systemic toxicity: Part VI. The prediction of human toxicity by rodent LD50 values and results from 61 in vitro methods," *ATLA*, vol. 26, pp. 617-658, 1998.
- [10] H. M. McConnell, J. C. Owicki, J. W. Parce, D. L. Miller, G. T. Baxter, H. G. Wada, and S. Pitchford, "The cytosensor microphysiometer: biological applications of silicon technology," *Science*, vol. 257, pp. 1906-12, Sep 25 1992.
- [11] J. R. Zysk and W. R. Baumbach, "Homogeneous pharmacologic and cell-based screens provide diverse strategies in drug discovery: somatostatin antagonists as a case study," *Combinatorial Chemistry High Throughput Screening*, vol. 1, pp. 171-183, 1998.
- [12] C. R. Keese and I. Giaever, "A biosensor that monitors cell morphology with electric fields," *IEEE Engineering in Medicine and Biology*, vol. 13, pp. 402-408, 1994.
- [13] S. Pitchford, "Whole-cell functional assays," *Gen. Eng. News*, vol. 18, pp. 38-39, 1999.
- [14] W. S. Kisaalita and J. M. Bowen, "Effect of culture age on the susceptibility of differentiating neuroblastoma cells to retinoid cytotoxicity," *Biotechnol Bioeng*, vol. 50, pp. 580-6, Jun 5 1996.
- [15] F. M. Watt, P. W. Jordan, and C. H. O'Neill, "Cell shape controls terminal differentiation of human epidermal keratinocytes," *Proc Natl Acad Sci U S A*, vol. 85, pp. 5576-80, Aug 1988.
- [16] A. Natarajan, P. Molnar, K. Sieverdes, A. Jamshidi, and J. J. Hickman, "Microelectrode array recordings of cardiac action potentials as a high throughput method to evaluate pesticide toxicity," *Toxicology in Vitro*, vol. 20, pp. 375-381, 2006.
- [17] C. Mao and W. S. Kisaalita, "Characterization of 3-D collagen hydrogels for functional cell-based biosensing," *Biosens Bioelectron*, vol. 19, pp. 1075-88, Apr 15 2004.
- [18] T. Boland, T. Xu, B. Damon, and X. Cui, "Application of inkjet printing to tissue engineering," *Biotechnol J*, vol. 1, pp. 910-7, Sep 2006.
- [19] W. C. Wilson, Jr. and T. Boland, "Cell and organ printing 1: protein and cell printers," *Anat Rec A Discov Mol Cell Evol Biol*, vol. 272, pp. 491-6, Jun 2003.
- [20] T. Boland, V. Mironov, A. Gutowska, E. A. Roth, and R. R. Markwald, "Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels," *Anat Rec A Discov Mol Cell Evol Biol*, vol. 272, pp. 497-502, Jun 2003.
- [21] V. Mironov, T. Boland, C. Wilson, E. Roth, A. Gutowska, V. Kasyanov, C. Eisenberg, A. Neagu, G. Forgacs, and R. R. Markwald, "Organ printing: Computer-aided jet printer-based three-dimensional tissue engineering," Atlanta, GA, United States, 2002, p. 9.
- [22] R. Chang, J. Nam, and W. Sun, "Direct Cell Writing of 3D Microorgan for In Vitro Pharmacokinetic Model," *Tissue Eng Part C Methods*, vol. 14, pp. 157-66, Jun 2008.
- [23] J. A. Rowley, G. Madlambayan, and D. J. Mooney, "Alginate hydrogels as synthetic extracellular matrix materials," *Biomaterials*, vol. 20, pp. 45-53, 1999.
- [24] S. M. O'Connor, J. D. Andreadis, and K. M. Shaffer, "Immobilization of neural cells in three-dimensional matrices for biosensor applications," *Biosens Bioelectron*, vol. 14, pp. 871-881, 2000.
- [25] A. Desai, W. S. Kisaalita, and C. Keith, "Human neuroblastoma (SH-SY5Y) cell culture and differentiation in 3-D collagen hydrogels for cell-based biosensing," *Biosens Bioelectron*, vol. 21, pp. 1483-1492, 2006.
- [26] C. Mao and W. S. Kisaalita, "Characterization of 3-D collagen hydrogels for functional cell-based biosensing," *Biosens Bioelectron*, vol. 19, pp. 1075-1088, 2004.
- [27] C. E. Krewson, S. W. Chung, and W. G. Dai, "Cell-aggregation and Neurite growth in gels of extracellular-matrix molecules," *Biotechnol Bioeng*, vol. 43, pp. 555-562, 1994.
- [28] R. Chang, J. Nam, and W. Sun, "Direct cell writing of 3D micro-organ for in vitro pharmacokinetic model," *Tissue Engineering Part C*, vol. 14, pp. 157-166, 2008.
- [29] U. Demirci and G. Montesano, "Single cell epitaxy by acoustic picolitre droplets," *Lab Chip*, vol. 7, pp. 1139-45, Sep 2007.
- [30] U. Demirci and G. Montesano, "Cell encapsulating droplet vitrification," *Lab Chip*, vol. 7, pp. 1428-33, Nov 2007.
- [31] S. Moon, S. K. Hasan, Y. S. Song, F. Xu, H. O. Keles, F. Manzur, S. Mikkilineni, J. W. Hong, J. Nagatomi, E. Haeggstrom, A. Khademhosseini, and U. Demirci, "Layer by layer 3D tissue epitaxy by cell laden hydrogel droplets," *Tissue Eng*, p. in press, 2009.
- [32] S. Moon, H. O. Keles, A. Ozcan, A. Khademhosseini, E. Haeggstrom, D. Kuritzkes, and U. Demirci, "Integrating microfluidics and lensless imaging for point-of-care testing," *Biosens Bioelectron*, vol. 24, pp. 3208-3214, Apr 2 2009.
- [33] C. M. Lo, C. R. Keese, and I. Giaever, "pH changes in pulsed CO2 incubators cause periodic changes in cell morphology," *Exp Cell Res*, vol. 213, pp. 391-7, Aug 1994.
- [34] S. Choudhary, M. Berhe, K. M. Haberstroh, and T. J. Webster, "Increased endothelial and vascular smooth muscle cell adhesion on nanostructured titanium and CoCrMo," *Int J Nanomedicine*, vol. 1, pp. 41-9, 2006.
- [35] J. J. Pancrazio, S. A. Gray, Y. S. Shubin, N. Kulagina, D. S. Cuttino, K. M. Shaffer, K. Eisemann, A. Curran, B. Zim, G. W. Gross, and T. J. O'Shaughnessy, "A portable microelectrode array recording system incorporating cultured neuronal networks for neurotoxin detection," *Biosens Bioelectron*, vol. 18, pp. 1339-47, Oct 1 2003.
- [36] J. J. Pancrazio, N. V. Kulagina, K. M. Shaffer, S. A. Gray, and T. J. O'Shaughnessy, "Sensitivity of the neuronal network biosensor to environmental threats," *J Toxicol Environ Health A*, vol. 67, pp. 809-18, Apr 23-May 28 2004.
- [37] I. Giaever and C. R. Keese, "A morphological biosensor for mammalian cells," *Nature*, vol. 366, pp. 591-2, Dec 9 1993.
- [38] C. E. Campbell, M. M. Laane, E. Haugarvoll, and G. I., "Monitoring viral-induced cell death using electric cell-substrate impedance sensing," *Biosens. Bioelectron.*, vol. 23, pp. 536-542, 2007.
- [39] W. H. van der Schalie, R. R. James, and T. P. Gargan, 2nd, "Selection of a battery of rapid toxicity sensors for drinking water evaluation," *Biosens Bioelectron*, vol. 22, pp. 18-27, Jul 15 2006.
- [40] J. M. Farber and J. I. Speirs, "Potential use of continuous cell lines to distinguish between pathogenic and nonpathogenic *Listeria* spp.," *J Clin Microbiol*, vol. 25, pp. 1463-1466, 1987.
- [41] T. H. Park and M. L. Shuler, "Integration of cell culture and microfabrication technology," *Biotechnol Prog*, vol. 19, pp. 243-253, 2003.