Chapter 2

Micro-Nanomechatronics for Biological Cell Analysis and Assembly

2.1. Introduction of micro-nanomechatronics in biomedical fields

On the one hand technological advancement on the top-down fabrication process, or micro-machining, provides nanometer structures. On the other hand, the bottom-up fabrication process, or chemical synthesis such as self-assembly or supermolecule techniques, also provides nanometer structures. In fact, both approaches reach the nanometric scale with the present limitations of physical/chemical aspects. "Nanotechnology" has an important role in the combination of the top-down and bottom-up approaches. It is considered that the multi-scale technique: from the atomic scale to the meter scale will be realized in the near future.

The multi-scale technique is widely applied to the fields of research in the biological and medical fields (Figure 2.1). Against the background of an aging society, social demands to develop resource saving technology and highly efficient energy systems required the integration of the academic disciplines of engineering, biology and the medical sector with multi-scale technology. This integration will be applied for various applications. In the engineering field, the bio-micro-nanomimetics system and biologically driven micro-nanosystem are investigated to realize the system in semi-set or

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wet environments. The biological system has quite a highly efficient energy consumption system, for example a flagellar motor of bacteria has almost 100% energy efficiency [MOR 09]. The current mechatronics system will be inactive enough to take into account such a biological system. In the biological field, the single cell analysis system and single cell nanosurgery system are investigated as an evolutional technology to analyze/manipulate a single cell. Current group analysis can be obtained for the averaged information as a cell group. The micro-nano robotics control technique will be achieved to obtain individual information or manipulation in single cells. In the medical field, the regeneration medicine, tissue medicine and alternative healthcare are readily investigated.

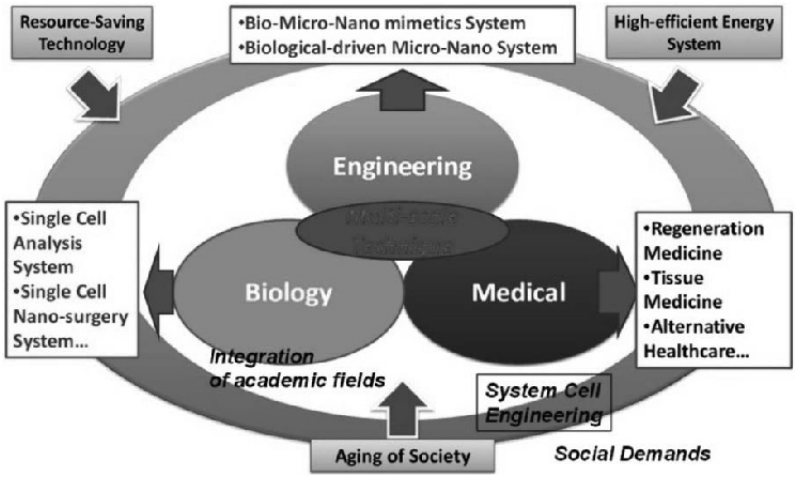

Figure 2.1. *Micro-nano robotic control techniques for biological/medical applications*

The current direction of research is moving to synthesis from analysis of the biological system as shown in Figure 2.2. The multi-scale technique is developed for cell analysis in single cell/group cell/tissue levels. The significant improvement of regeneration engineering/biology will be achieved by three-dimensional construction *in vitro* from "*in vivo*" biological functions. Stem cell engineering is a key technology to realize tissue/ organs developed by embryo stem cells (ES cells). By reducing the snit factors of the induced pluripotent stem cells (iPS cells), to be changed to cancer cells, this research field will be dramatically improved in the near future [YEE 06].

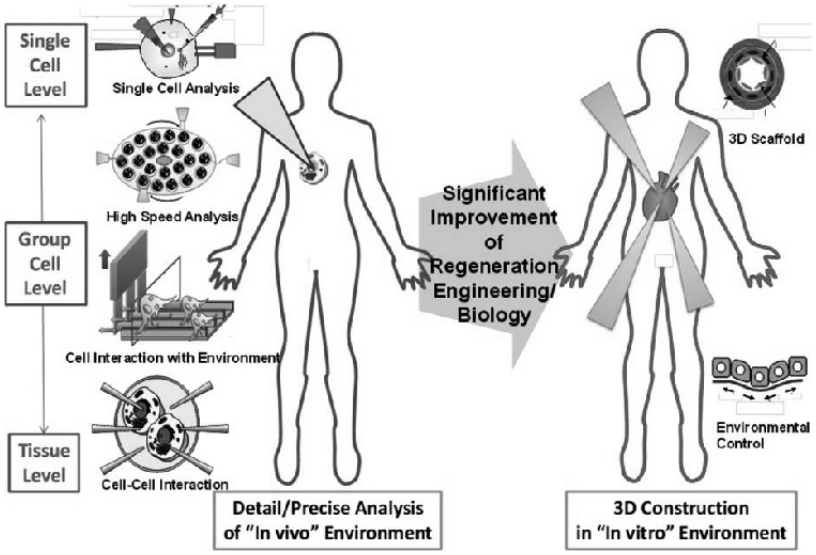

Figure 2.2. *The schematics of construction of in vitro system from analysis*

2.2. Configuration of micro-nanomechatronics

Mechatronics is a interdisciplinary field through synergetic integration of mechanical and electrical engineering with computer technology. For example, in the field of robotics, industrial robots are actuated by replacing the hydraulic actuator with electric motors. Miniaturizing technologies, such as micro-nanotechnology, grows dramatically in the engineering field around 2000. These technologies are commonly used in our daily lives, in applications for automobiles, computer peripherials, printers, cameras, amusements, robotics, automation, environmental monitoring, energy resource, biological/medical treatments and so on. Currently, the micro-nanomechatronics field is set out to improve device specification to have more greater efficiency, higher integration, higher functionality, lower energy consumption, lower cost, miniaturization and so on.

The relationship of required technologies for micro-nanomechatronics is shown in Figure 2.3. The material/sensing/actuation technologies are the fundamental level for micro-nanomechatronics. Their scientific discoveries

or technological developments are important to bring innovations in this field. Control technology is constructed from actuation and sensing technologies, but we need to consider the energy supply system. The fabrication technology is strongly related with material technology. Finally, the assembly technology is achieved on the basis of these technologies and makes a feedback loop from the micro-nano evaluation technology to check the requirements, safety issues, improvement of the system and so on. Through this flows of research configuration techniques are constructed for micro-nanomechatronics.

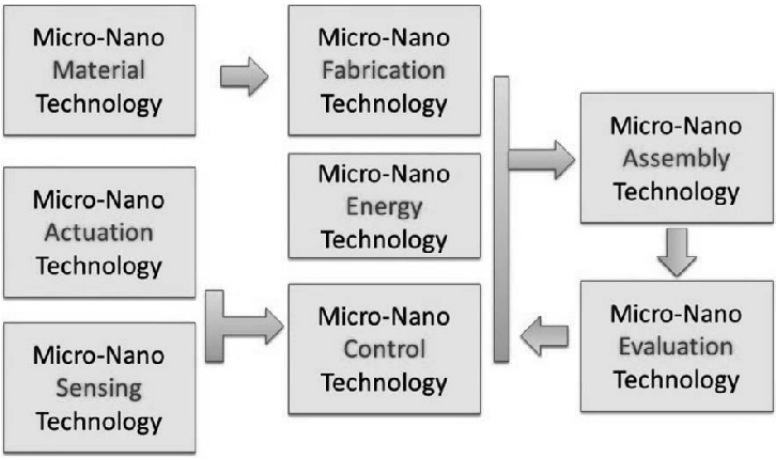

Figure 2.3. *Technological relationship for micro-nanomechatronics system*

The examples for each technique are summarized in Figure 2.4. Various methods are developed and investigated for each technique. To establish micro-nanomechatronics, these need to be selected and integrated on a case-by-case basis for the applications. From the application viewpoint, the environmental constraint is one of the issues when choosing the techniques. For example, the conditions of temperature, gas pressure, vibrations and wet/dry are important and need to be considered. For the biological application, the environmental condition should be tougher including the sterile/biocompatible/biodegradable conditions. Normally, biological cells are quite sensitive to the environment, and it is required to design the system with the environmental constraints factored in for biomedical applications.

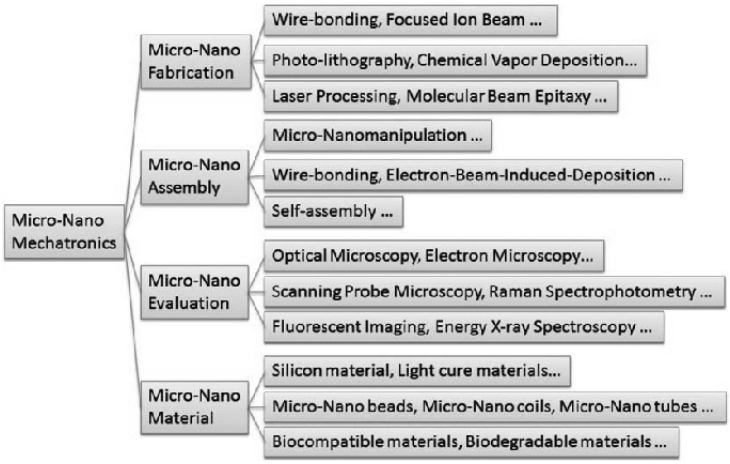

Figure 2.4. *Examples of techniques on micro-nanomechatronics*

Examples of actuation mechanisms are summarized in Table 2.1. From the viewpoint of physical interactions, the actuation technology is mainly divided into contact or non-contact ways. For a micro-nanomanipulation system, the piezo-driven mechanisms are widely used because of their good performance in the positioning accuracy. For biological cell manipulation, the manipulation system is required to approach the targeted cells under fluidics, which is needed to keep the cells in a viable condition. Through contact manipulation, the fluidics surrounding the targeted cell is affected during manipulation, making some turbulence. On the other hand, the non-contact manipulation mechanism is effectively used for biological cell manipulation in wet environments without direct physical contact to the fluidics. The electrical and magnetically driven mechanisms have larger actuation forces by comparison with optical manipulation. However, optical manipulation is superior on the local actuation of a targeted object by its narrower actuation range.

Examples of sensing mechanisms are summarized in Table 2.2. The local and highly accurate sensing mechanisms are important for the micro-nanomechatronics applications. To select the sensing mechanism, the various limitations need to be considered; such as (1) environments (wet/dry/vacuum, etc., sterile or non-sterile environments, etc), (2) space and (3) energy supply limitations. To construct the control system, the sensing mechanism needs to be combinable with the actuation mechanism as an micro-nanomechatronics system.

Micro-nano actuation mechanism	Physical interaction	Accuracy	Actuation range	Durability	Environmental condition
Electrical motor driven	Contact	++	+++	+++	Dry
Hydraulic pressure driven	Contact	++	+++	+	Dry
Piezo-driven	Contact	+++	+	++	Dry
Biological cell driven	Contact	+	+	+	Wet
Bacteria driven	Contact	+	+	+	Wet
Optically driven	Non-contact	+++	+	++	Dry, wet
Electric driven	Non-contact	++	++	++	Dry, wet
Magnetically driven	Non-contact	++	++	++	Dry, wet

Table 2.1. *Examples of actuation mechanisms for micro-nanomechatronics*

Micro-nano sensing categories	Examples
Positioning sensing	Laser meter, ultrasonic sound meter, piezo sensing, microscopic imaging, etc.
Force sensing	Strain gauge, electric capacitance, electrostatic gauge, piezo cantilever, etc.
Electromagnetic sensing	Flowmeter, particles charges, photodetectors, etc.
Chemical sensing	Gas sensing, ionic sensing, biosensing, bioindicator, etc.
pH Sensing	Indicator, potential difference mechanism, field effect transistor, etc.
Temperature sensing	Indicator, infrared intensity, resistive change, etc.
Cell activity sensing	Indicator, fluorescent dye, fluorescent protein, physical/chemical interaction, etc.

Table 2.2. *Examples of sensing mechanisms for micro-nanomechatronics*

2.3. Micro-nanomechatronics for single cell analysis

The micromanipulation of biomicroparticles such as microorganisms and cells has become essential in various industries, such as in the food industry, the medical industry, for environmental purification and so on. Many microorganisms such as oil-degrading bacterium, basophilic bacterium and thermopile bacterium have already been discovered. By using the useful characteristics of these microorganisms, the development of new medicine, the production of useful materials and environmental purification has been achieved. For example, the recombination of microorganisms are used for the production of biological plastic (polyhydroxyalkanpoate (PHA)) [ABE 10] or the degradation of biodegradable plastics (poly-butylene succinate-co-adipate (PBSA)) [KIT 11]. These are examples of microorganism application to the post-petrochemical industry amid energy problems such as a decrease in oil resources.

However, microorganisms which have so far been discovered are estimated as less than 10% of the total microorganisms. For the remarkable advancement of microbiology and bioengineering, it is important to develop a new technology of manipulation and separation and discover unknown microorganisms and their characteristics. Much research on cell analysis has been done for the investigation of the unknown properties of them. There are several different methods to understand the biological properties of cells. For example, the group cell analysis and single cell analysis are based on the direct observation of each specimen. Both methods are important, but each method has its own characteristics. With the former method, the time-course of cell properties such as an increase in cell volume and DNA synthesis during the division cycle has been studied. Group cell analysis is very important for application in industry. However, it is only possible to obtain general information on each cell.

In recent years, single cell analysis has received much attention with the development of biotechnology [WAK 05, ROM 07]. For the actual microbial community, it is a heterogeneous population as shown in Figure 2.5. After stable phase through the exponential growth phase, this population divides into three phases such as viable but not culturable, apoptosis and adaptation to a new environment. Essentially, the cell population is not homogeneous, and the mechanism of differentiation to the heterogeneous is not known. Microbial activity is not uniform, and the mechanism of gene expression is not known. In addition, gene expression is dynamically changing, and its

property is unknown. For single cell analysis, an extraction of a single target cell from a large number of samples and continuous monitoring of the cell are necessary. For these reasons, "biomanipulation" is quite an important technique, which is defined as the technique for manipulation of biological cells from the micro-nano scale to the macro scale, based on single cell manipulation and the observation of single cell reactions.

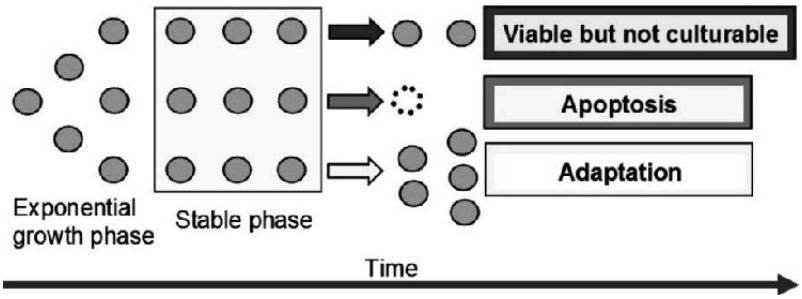

Figure 2.5. *Mechanism of microbial community*

The major examples of biomanipulation techniques are categorized in Table 2.3. The purposes of biomanipulation are classified into major three directions. The first direction is the "analysis of biological cells" such as "(1) mechanical interaction, (2) cell–cell interaction and (3) measurement/ imaging". The second direction is "manipulation of biological cells" such as in "(4) gene manipulation/injection, (5) separations/immobilization, (6) environment control and (7) molecular manipulation". The third direction is "assembly of biological cells" such as "(8) shape control of cell/tissue and (9) 2D/3D cell assembly". Until now, the contribution of "biomanipulation" has mostly been the investigation of analysis and manipulation applications. Recently, based on this knowledge and techniques, the assembly applications are given greater attention as a current research topic.

In the following sections in this chapter, we introduce our recent results on the semi-closed microchip for the single cell analysis and biological cell assembly using photo-linkable resin based on single cell analysis techniques.

Purpose	Techniques	Examples	Issues
1) Mechanical interaction	Biomechanics, differentiation, growth condition, etc.	Mico-nano handling, μTAS, BioMEMS/NEMS, etc.	Local control, 3D positioning, 3D force sensing, individual/local sensing, etc.
2) Cell–cell interaction	Intercellular materials, protein–protein interaction, etc.	Micro-nano probe, regeneration engineering, etc.	Local sensing, Minimally invasive technique, *in vitro* realization of *in vivo* system, etc.
3) Measurement/ imaging	Real-time measurement, gene expression, etc.	AFM, nano-probe optical tweezers, etc.	Real-time imaging, local force/torque/ positioning measurement, etc.
4) Gene manipulation/ injection	Stability in the cell transportation, expression, etc.	Micro-nano-probe, injection, μTAS, BioMEMS/NEMS, etc.	Viability, high speed, mass production, high efficiency, gene expression, etc.
5) Separations/ Immobilization	On-chip cell analysis, gene expression, etc.	Optical tweezers, DEP, electrophoresis, etc.	High speed, *in situ*, low damage, etc.
6) Environmental control	Chemical, mechanical, electrical stimulation, mechanical stimulation, etc.	Micro-nano-probe, μTAS, BioMEMS/NEMS, etc.	Local control, density gradient real time, probes, etc.
7) Molecular manipulation	Cell modeling, functional protein synthesis, etc.	Optical tweezers, nano-probe, electrophoresis, magnetically driven, etc.	Protein synthesis, mutation, functional control, etc.
8) Shape control of cell/tissue	Chemical control of cell surface, shape and pattern control, etc.	Micro-nano fabrication, SPM, μTAS, BioMEMS/NEMS, etc.	Local chemical control, cell selection/shape/ pattern, function control, etc.
9) 2D/3D Cell assembly	Control of extracellular matrix/cytokine, etc.	Scaffold, bioprinting, spheroid, cell origami, cell sheet engineering, etc.	High speed, high efficiency, complex tissue organization, etc.

Table 2.3. *Examples of biomanipulation techniques*

2.4. Semi-closed microchip for single cell analysis

For single cell analysis, two types of systems have mainly been investigated. One is the open chambers such as a petri dish [TAK 08, INO 08] and the other is the closed chambers such as a microchip [HUN 05, WAN 10]. The open chambers allows probe manipulation. Probe manipulation is one of the most important techniques for single cell analysis, because probe-type devices realize manipulating arbitrary single cell and controlling the environment of the arbitrary position. The probe-type devices also perform the measurement of cellular status [CLA 07], for example temperature distribution. However, it is difficult to prevent the effects of the external environment, such as evaporation of a solution and contamination when the open chambers are used.

On the other hand, the closed chambers can prevent the effects caused by the external environment. However, it is difficult to use the probe-type devices on conventional microchips. Generally, non-contact manipulation techniques like optical tweezers [MAR 03, ARA 04] or fluid force [UVE 09, SUN 06] are needed for cell manipulation on the microchip. These non-contact manipulation techniques have some disadvantages. For example, the optical tweezers can only apply a pN order force to cells, and it is difficult to manipulate large cells like an egg cell. It is also difficult to manipulate the target single cell when the fluid force is used to manipulate cells. The closed chambers have to fabricate complex microchannels [CAR 06, TAN 07] before experiments to use them for single cell analysis.

Hence, the desire to develop new devices that have the advantages of both open and closed chambers. We proposed a semi-closed microchip, which realizes the probe manipulation and keeping the seal [TAK 10]. Figure 2.6 shows the schematic of a microchip for cell manipulation. Cell culture, cell analysis and target cell harvesting can be realized continuously in the semi-closed microchip under various environments. The proposed semi-closed microchip is shown in Figure 2.7. The microchannel is fabricated in the microchip to realize the exchange of solution in the bath. To realize the probe manipulation, the semi-closed microchip has the bath in the middle of microchannel. The probe-type devices can be inserted into the microchip through this bath. The bath is sealed off by thin oil film, which can prevent the evaporation of the solution in the bath. The probe-type devices can be inserted and taken out, still keeping the seal of the bath.

To extend the semi-closed microchip as a useful single cell analysis system, it is necessary to develop and integrate other devices such as a pump,

a probe-type device and filter. We developed the probe-type devices. The sizes of cells are generally 10–100 μm, and the volumes are less than 1 nl. So it is necessary to develop the probe-type device that can control the spout and suction volume easily in nl in order to realize single cell manipulation under a microscope. We proposed a novel probe-type device "thermal gel-actuated probe", which uses thermal gel as an actuator to realize the spout and suction in an nl order. The spout and suction can only be controlled to switch the heater on and off. The thermal gel-actuated probe can be fabricated at a low cost; so disposable use can be realized by the thermal gel-actuated probe.

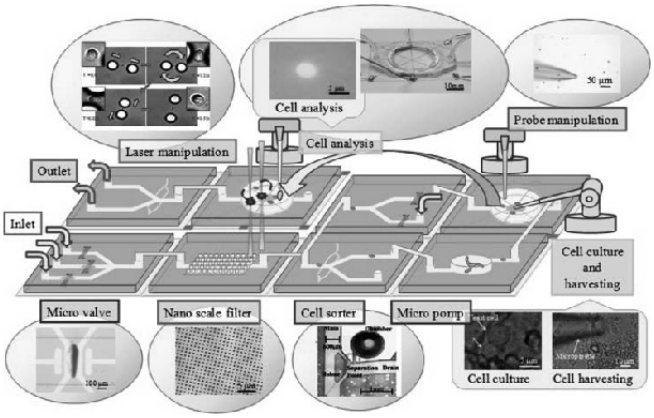

Figure 2.6. *Configuration of microchip for cell manipulations. For a color version of this figure please see www.iste.co.uk/mechatroneng.zip*

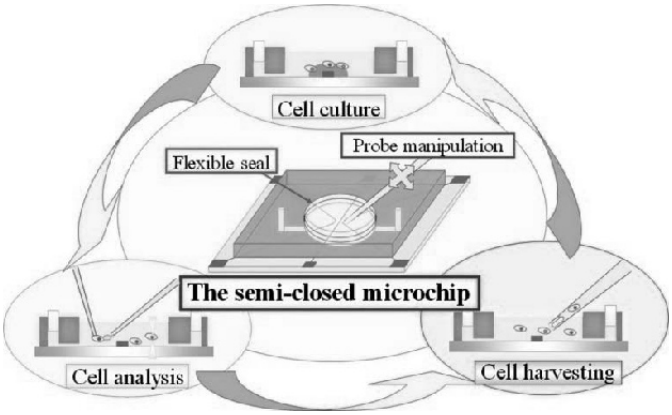

Figure 2.7. *Conceptual figure of semi-closed microchip*

Cell harvesting by the thermal gel-actuated probe was demonstrated under the microscope using a semi-closed microchip. Indium tin oxide (ITO) electrodes are fabricated as heaters at the center of the bath. A poly-(N-isopropylacrylamide) (PNIPAAm) solution, which shows a gelation mnore than 32°C, can be gelled by applying voltages to these electrodes. Cells in the semi-closed microchip can be fixed by this generated thermal gel. This cell fixation realizes the stable observation under a microscope during cell culture and cell analysis in the semi-closed microchip. The thermal gel-actuated probe was set on the micromanipulator. The suction and the spout of the solution were controlled only to switch the heater on and off. Figure 2.8 shows the experimental results. The thermal gel-actuated probe was attached to the target yeast cells at first. After positioning of the thermal gel-actuated probe, the target yeast cells were harvested. This result indicates that the thermal gel-actuated probe can harvest the target cells that are fixed by the thermal gel.

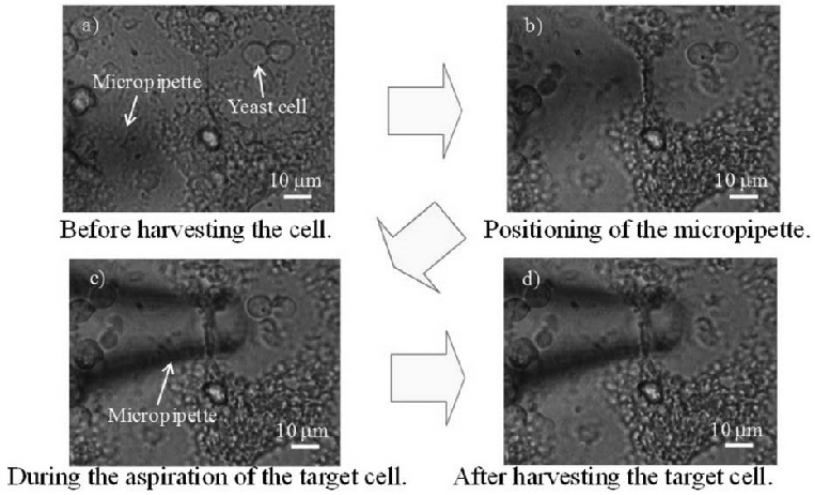

Figure 2.8. *Single cell harvesting using semi-closed microchip*

2.5. Biological cell assembly using photo-linkable resin based on the single cell analysis techniques

The cells inside real tissues and organs are usually arranged with a large amount according to certain patterns and shapes, such as neural cells, skin and blood vessels [TAK 10]. In tissue engineering, important issues are cell

encapsulation inside certain structures and high-throughput assembly of these structures [USH 02].

There are many kinds of cell manipulation methods, such as surface adhesion, optical tweezers and dielectrophoresis (DEP). By using surface adhesion, it is possible to manipulate cells without damaging the cells. The position of cells is not flexible because surface adhesion depends on the surface property, which is not easy to change [SUU 05]. Optical tweezers is a low-damage approach and easily controlled. An automatic cell manipulation system is constructed. But the manipulation force is weak as compared with the flow resistance [LAN 93]. Compared with other methods of cell manipulation, DEP is easier to control, and it is non-contact, which means less damage to the cells. Because of the low physiological stress caused by DEP, the cells remain viable after treatment and can be cultured for further purposes [BER 04]. By DEP force, it is possible to manipulate particles with great selectivity. It is possible to allow the separation of cells or the orientation and manipulation of nanoparticles and nanowires. Consequently, DEP force is widely used in cell manipulation, such as sorting, separation and patterning [HU 11].

The convenient methods for cell immobilization are based on such an aspiration, pressure of solution and fluidic structure [JAE 08]. The advantage of aspiration and pressure is that the fixing force is large, whereas the disadvantage is in damaging the cells [VAL 10]. By special fluidic structures, cells are immobilized inside a microfluidic chip. The immobilized cells are difficult to be analyzed further [TSU 10]. On-chip fabrication based on photo-crosslinkable resin via UV illumination is a creative way for immobilizing cells. Cells are directly immobilized inside the microfluidic chip. There are several advantages, such as high speed, low cost and their arbitrary shape [TIX 04].

To culture the cells in a tissue, taking account of the deficiency of oxygen and nutrients and the accumulation of wastes, thus the survival rates of cells are quite important issue. Basically, these problems are not easy to solve *in vitro*. The conservation limit of the thickness of stacks of a cell sheet by diffusion of oxygen, nutrients and wastes is less than 200 μm [DI 06]. Hence, a blood vessel structure is important to construct with three-dimensional tissue.

For tissue engineering, it is required to construct cell patterns and immobilize patterned cells inside certain structures. We proposed a novel method of forming a cell pattern through the DEP forces and immobilizing

cells by photo-crosslinkable resin inside microfluidic chips as shown in Figure 2.9 [YUE 11]. High-speed cell manipulation, including patterning and concentration control by DEP was demonstrated. Movable microstructure embedding cells is on-chip fabricated.

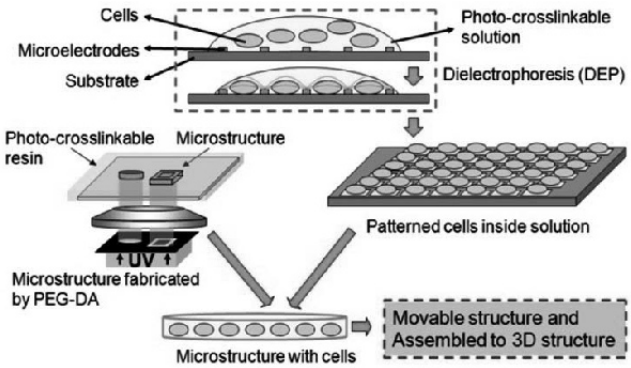

Figure 2.9. *A schematic drawing of the cell assembly method*

The experimental results show that the cell line patterns that contain hundreds of yeast cells can be formed by DEP within 1 s as shown in Figure 2.10. By applying DEP force, several microelectrodes are fabricated by ITO and Cr/Au, which are coated on the glass. The two kinds of DEP responses for yeast cell (W303) and other particles were confirmed experimentally. Based on the negative DEP phenomenon, cell traps generated by microelectrode are demonstrated. Position control and transportation of yeast cells are performed by using cell traps.

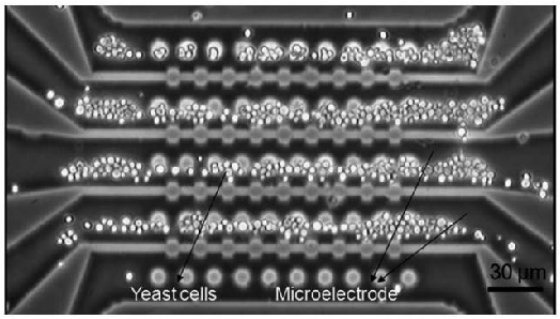

Figure 2.10. *The line pattern of yeast cells formed by n-DEP based on ITO microelectrode*

Figure 2.11 shows the assembly of a microstructure embedded with line-patterned cells. Figures 2.11(a) and 2.11(b) show the micro-channel before cleaning away the cells outside the microstructure. Figures 2.11(c) and 2.11(d) show the microstructures that contain three lines of yeast cell inside micro-channel after cleaning. The on-chip fabrication for arbitrary shapes of microstructures was performed using polyethylene glycol diacrylate (PEG-DA), which is the photo-linkable resin. With the cell patterning by DEP and immobilizing by on-chip fabrication, a microstructure that contains three lines of yeast cells is fabricated in the microfluidic channel, inside a PEG-DA and NaCl solution. It is designed for movable microstructures embedding microbeads of which, the concentration is controllable.

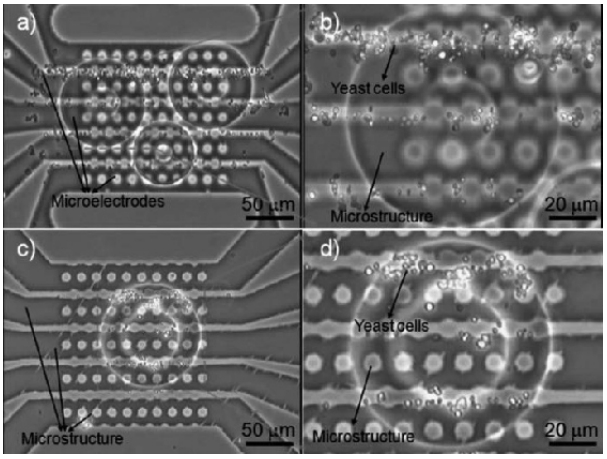

Figure 2.11. *Micro-structure embedded with line-patterned cells. For a color version of this figure please see www.iste.co.uk/mechatroneng.zip*

2.6. Conclusion

In this chapter, we introduced micro-nanomechatronics for biological cell analysis and assembly. The micro-nanomanipulation technique is a key technique to analyze and synthesize a biological system *in vitro*. We introduced the semi-closed microchip for the single cell analysis with a thermal gel-actuated probe. The biological cell assembly was also introduced by the DEP forces and immobilizing cells by photo-crosslinkable resin inside microfluidic chips. These research fields will be continuously investigated, becoming more sophisticated for realizing the various biomedical applications *in vitro* from the "*in vivo*" biological functions.

2.7. Acknowledgments

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