3D In Vitro Living Systems for Biological Application

Hossein Hosseinkhani

Graduate Institute of Biomedical Engineering, National Taiwan University of Science and Technology (TAIWAN TECH), Taipei, Taiwan

Summary

Many three dimensional (3D) models currently in practice require expensive equipment, large sample volumes, long incubation times and/or extensive expertise. Their most serious disadvantage, however, is that they are too far from the nature of human organs. Because of the above problems, research and development in drug discovery, regenerative medicine, biotech, and pharmaceutical industries is very costly, and it takes several years to bring a single drug/product to the market. The goal of our research is to merge biomaterials science, nanotechnology, and biological principles to generate 3D in vitro living organs, to be called “Human on a Chip.” The goal is to mimic organs/tissues in order to partially reduce the amount of in vitro testing, in vivo animal testing, and clinical trials, as well as to solve the problems noted above. In short, our goal is to jump “from lab bench to the market.” We propose to do all the above costly and time-consuming tests in a rapid and cost-affordable way. At the nanoscale, we play with the chemistry and materials to fabricate novel types of hydrogels that are similar to human organs, infusing the cell-laden hydrogels with extracellular matrix (ECM) molecules and gradients of signaling molecules to influence cell development and aggregation. On the microscale level, we employ fabrication technologies such as photolithography, adopted from the semiconductor industry, to mass-produce identical building blocks in a variety of shapes and sizes. These products will have to mimic the physical, chemical, and biological properties of natural organs and tissues at different scales, from molecules to cells to building blocks to organized clusters, if we are to reach our target, i.e., “Human on a Chip.” It is envisioned that the proposed method can be used to generate vascularized organs and tissues with controlled cell-cell and cell-matrix interactions that will be useful as in vitro diagnostics tools, for drug screening applications, and for transplantation.

Keywords: HTS, biosensors, 3D in vitro systems, human on a chip, microscale technology

1 Introduction

In the past few years, many high-throughput screening (HTS) technologies together with significant efforts to develop diagnostics tools such as biosensors have revolutionized the process of drug discovery to screen hundreds of thousands of compounds against disease targets to find novel drugs. Typically HTS studies are performed through a combination of modern robotics, data processing and control software, liquid handling devices, and sensitive detectors. However, robots and other equipment required for HTS studies are often expensive. Large volumes of cost-intensive chemicals are required to perform such tests. Another limitation is the time it takes to assay individual samples. In the end, even as these sets of facilities are fixed, the experimental data are not reliable for clinical application and, therefore, animal testing and clinical trials are required in order to get approval for the product (drug) after a very long processing time. The above problems are among the many limitations of current technologies using materials in many aspects of biological applications. Despite considerable effort at developing methods to enable testing of many conditions, it still takes a highly specialized and expensive screening lab to run a HTS operation, and these studies often are limited to large or moderately sized research institutions. The purpose of our study is to use microengineering, biomaterial sciences, and biological principles to develop 3D in vitro living organs with controlled microvasculature and tissue architecture. These 3D models will be useful for in vitro diagnostics and drug screening applications as well as for transplantation. Furthermore, 3D in vitro living organs contribute significantly to the development of microscale biosensors that will be more portable and scalable for point-of-care sample analysis and real time diagnosis. Modern biosensors based on micro- and nanoscale techniques have the potential to greatly enhance methods of detecting foreign and potentially dangerous toxins and may result in cheaper, faster, and easier to use analytical tools.

Microscale technologies are potentially powerful tools for addressing some of the challenges in 3D in vitro models. MicroElectroMechanical Systems (MEMS), which are an extension of techniques used in the semiconductor and microelectronics industries, can be used to control features at length scales <1 μm to > 1 cm³. In the past few years, microfabrication has been used increasingly in biomedical and biological applications. This is partially due to the emergence of such techniques as soft lithography to fabricate microscale
devices without the use of expensive “clean rooms” and photolithographic equipment. These techniques are compatible with cells and are now being integrated with biomaterials to facilitate the fabrication of cell-material composites that can be used for biomedical engineering (Lindstrom et al., 2010). In addition, microscale technologies provide an unprecedented ability to control the cellular microenvironment in culture and to miniaturize assays for high-throughput applications. Initial experiments used micromachining technologies on silicon surfaces to generate vascularized systems. Subsequent work on the replica molding of biocompatible polymers such as poly(dimethylsiloxane) (PDMS) from patterned silicon wafers has resulted in the fabrication of biocompatible scaffolds. More recently, microfabricated capillary networks have been fabricated out of biodegradable elastomers such as poly(DL-lactide-co-glycolide) (PLGA), polyurethane, and poly(glycerol sebacate) (PGS). However, there are potential disadvantages with the use of these polymers, including their rigid mechanical properties and bulk degradation kinetics. Alternative methods of fabricating scaffolds with micro- and nanoscale resolution include 3D printing, microsyringe deposition, tissue spin casting, and electrospinning of nanofibers. These approaches, however, are difficult to perform and to scale up for fabrication of large 3D organs. A potentially powerful approach to engineering the microvasculature is to use cell-laden hydrogels. Recently, it has been demonstrated that biomaterials made from hydrogels can be molded to fabricate microchannels. Although this technology has not yet been used for 3D living models, the ability of this approach to mimic organs and tissues is useful in engineering complex, vascularized organs.

One of the main features of the proposed approach is that it will use micro-engineered cell-laden hydrogels for 3D in vitro living organs. These hydrogels mimic the natural tissues in that they provide a 3D environment for cells. To use hydrogels in various biological applications, it is desirable to control their mechanical properties which affect cell attachment, differentiation, viability, and proliferation. Therefore, hydrogels that can mimic the mechanical, biological, and physical properties of native tissues need to be generated. Despite significant progress, however, many current approaches to fabricating hydrogels fail to result in the synthesis of constructs with the desired mechanical and chemical properties. Limitations in generating robust hydrogels that can withstand the in vivo environment include the need for low overall concentration of material, the required degradation, and the need for cytocompatibility. Our research aims to use interpenetrating networks (IPNs) as a powerful method of modifying hydrogel properties. Furthermore, we will be able to tailor the mechanical, physical, chemical, and biological properties of these hydrogels. Over the years, much has been attempted in generating tissue-engineered products. One strategy for engineering 3D engineered tissues is to cultivate cells within biodegradable scaffolds made from either natural or synthetic materials. A major challenge in 3D tissue engineering is that cells quickly lose their differentiated function. This is in contrast to the behavior of cells in the body, which have the capacity to regenerate. Thus, it is desirable to formulate alternative approaches to more precisely control the organization of cells and the vascularization of engineered tissues. Traditional 3D scaffolding approaches are not suitable for generating such complex structures due to lack of control of the tissue architecture and cell-cell interactions. In particular, cells in 2D culture, as well as within traditional 3D scaffolds, simply do not organize as they do in normal tissue; their metabolic properties are, therefore, unsuitable for tissue engineering applications. Our research plan aims to make an advance in 3D models by developing the basis for fabricating tissues made from cell-laden hydrogels with engineered microvasculature. Although engineering microscale features into tissue engineering scaffolds has been attempted before, in this proposal we will use a cell-laden hydrogel, which will eliminate the difficulties associated with other microfabricated tissue engineering scaffolds, such as uniform cell-seeding.

2 3D in vitro technology

Materials technology aims to develop a technique that draws from microscale engineering, novel biomaterials, and biological principles to overcome the limitations of the current approaches to generating 3D in vitro living organs. These include the inability to generate 3D constructs that mimic the complexity of native tissue structure, or to generate vascularized structures within a 3D tissue culture system. The success of hydrogels as tissue implants or biomedical devices is strongly dependent on their bulk properties. The ability to seed cells directly within macroporous hydrogels will be important for overcoming challenges associated with uniform cell seeding density and vascularization. However, most hydrogels lack the desired mechanical and biological properties associated with human tissues in the body. IPN composed of different types of natural hydrogels is a very attractive technology for overcoming the above problem. We used three different hydrogels in our previous studies (Hosseinkhani et al., 2006a,b,c, 2007a,b,c, 2010; Tian et al., 2008; Mohajeri et al., 2010). Mixtures of collagen/fibronectin, photocrosslinkable HA and self-assembled PA are used to synthesize IPNs (Fig. 1) comprised of the various combinations of the three different hydrogel precursors to generate a library of IPN hydrogels.

An example is the fabrication of five different IPN of collagen and HA at the following mass ratios: i) 20% collagen, 80% HA, ii) 40% collagen, 60% HA, iii) 50% collagen, 50% HA, iv) 60% collagen, 40% HA, and v) 80% collagen, 20% HA. These concentrations allow us to measure the mechanical properties of the entire spectrum of each binary IPN. Finally, IPN consisting of all three of the precursor solutions at 20 different concentrations again allow us to study the properties of the entire spectrum of IPN. Based on testing at least five different concentrations for each of the three binary IPN conditions (i.e., IPN of HA-PA, HA-collagen and collagen-PA), as well as the 20 different concentrations of triple IPN (i.e., HA-PA-collagen (Fig. 2), the total number of experiments can easily be performed using standard laboratory techniques.
techniques. With these approaches it is possible to synthesize and test biological and mechanical properties of the library of hundreds of different polymers by using liquid-handling robots, thus allowing us to easily screen for thousands of polymer combinations and concentrations. The polymers are synthesized on the arrays in triplicates to ensure statistical validation. We will synthesize a library of IPN hydrogels and analyze their mechanical properties by a high-throughput nanoindentor technique. The nanoindentor approach can be used to accurately and rapidly assess the load-displacement responses of the various polymers within the library. Briefly, the arrays are printed in triplicates using a microarrayer (MicroGrid TAS) on a standard PDMS substrate that is fitted with small microwells to ensure proper positioning of the individual hydrogels. These wells act as a placeholder and will not affect the unconfined compression testing since they will not be deep relative to the height of the hydrogels. With this approach we are able to design a hydrogel that can mimic mechanical properties of human organs and tissues. In general, brain tissues exhibit elasticity between 0.1kPa and 1kPa, muscle tissue ~10kPa, and collagenous bone approximately 100kPa. The human heart has an elasticity of ~31 kPa.
will be duplicated and continued to culture the 3rd and the 4th and more layers of the cells, by which we are able to create in vitro living organs to be used as intelligent diagnostic tools.

To generate micropatterned arrays of libraries on IPN hydrogel and to enable their testing in a high-throughput manner without the use of expensive equipment or large reagent volumes, these hydrogel arrays can be manufactured at a central facility, dried and stored until testing. The ability to fabricate the microarrays at a central location eliminates the need for the end users to employ robotics or other types of equipment. To test each sample, the microgel array is simply sealed against an array of microwells containing cells in culture media. Upon exposure to the aqueous environment the chemical is released. By this approach, we are able to develop the individual components for this system, including an array of chemical-containing hydrogels immobilized on a hydrogel slide and an array of microwells, as shown in Figure 3.

![Fig. 3: Schematic representation of the in vitro based biochip](image)

A detection system for released proteins from cells in the microwells. Antibodies are printed on the array and binding of the proteins to the antibodies can then be detected by ELISA and a microarray scanner.

### 3 Fabrication technologies for 3D models

Fabrication technologies such as photolithography are borrowed from the semiconductor industry to mass-produce identical building blocks in a variety of shapes and sizes. For fabrication of 3D in vitro living systems we use an advanced lab of microfabrication facilities based on lithography technology through collaboration with the semiconductor industry toward the future development of biochip technology. The use of microfluidic channels or micromolding techniques to deposit cells and materials on specific regions of a substrate that may also be used for microfabrication is important as well. To engineer 3D living organ models, soft and photo-lithography techniques are followed. In this approach, the first layer of cells is cultured in IPN hydrogels; following photolithography, a cell-laden microwell will be formed. The next step is culturing the 2nd layer of the cells inside the empty microwell. This process
By incorporating the engineered organs inside arrayed microfluidic channels, we can fabricate the tissue-based diagnostic tools for a drug screening system, for instance. A technique required by these proposed experiments is to pattern an array of hydrogels containing different drugs on a PDMS substrate. A commercially available spotter (MicroGrid II TAS) is utilized to selectively immobilize desired combinations of matrix molecules within each microwell, as well as a second microarrayer (PerkinElmer) that utilizes piezoactuation to print droplets of liquids on substrates. The fabrication process of hydrogel arrays requires access to a standard robotic spotter, off-the-shelf materials, and 1,000 times less reagents than conventional methods of testing molecules on chemicals (Fig. 3). Approximately 3,000 unique spots are patterned in less than 30 minutes, making the process amenable to high-throughput screening. Furthermore, microarray printers can be used to print high-molecular-weight PEG molecules that can be used to fabricate our proposed arrays.

Two kinds of concepts will be applied here. First, the microfluidic channel in which the 3D-vascularized model is placed later carries out the chemical assay by flowing different chemicals through each channel. Second, the 3D vascularized models inside the gradient-generating chip are fabricated. By flowing the drug (or chemical) having a gradient through the channel, the 3D model is exposed to the chemicals at different concentrations and, therefore, we are able to investigate the chemical concentration effect on organs or tissues. The microfluidic platform containing multiple channels is fabricated using PDMS, and the flow networks having different reservoirs are connected to each chamber. The width of each channel is less than 1 mm. The microfluidic platforms are combined to fabricate the arrayed assay chip. Then the cell-laden hydrogel is introduced through the channel and is polymerized. The system including 3 kinds of cells is incubated, and we can fabricate the arrayed channel with the 3D engineered model inside. Finally, we can analyze the drug (chemical) effect on specific tissues by flowing different chemicals through the microfluidic channels.

The 3D engineered vascularized model is placed inside the gradient-generating chip. The proposed gradient-generating chip is simple and requires only a small amount of chemical because the flow is driven by osmotic pump. This chip can easily be used inside the cell incubator for a long time (>48 h) without changing media. The media or chemicals can be easily supplied, and the cells can be incubated for much longer times (i.e., more than 1 week). We put the media without chemicals in one reservoir and the media with chemicals in the other reservoir. Then the chemical gradient is generated inside the channel, and the 3D vascularized organs or tissues are influenced by the gradient of chemicals. By monitoring the tissues exposed to different drug or chemical gradients, we can investigate chemical gradient effect on cells, and this concept can be applied to the high-throughput screening of tissues according to different chemicals and concentrations.

This technique offers great flexibility in testing drug or chemical libraries. For example, various doses of the same chemical can easily be tested from the hydrogel microarrays to assess the minimum toxic dosage. This can be used in arriving at toxic dose levels without expensive and ethically challenging animal experimentation. In addition, the system can be modified to conduct other high-throughput experimentation. For example, various differentiation factors at different concentrations can be printed in the hydrogel slide, and their effects on the cell’s behavior can be studied in a multiplexed manner.

4 Milestones, deliverables, and economic potential

We anticipate that elucidation of the above goals will open many doors and lead to significant improvements in biological tools and the drug discovery process, as well as to identification and therapeutic approaches. The miniaturization of this approach allows one to perform many more experiments and to do so more simply than previously possible.

The 3D in vitro technology aims to develop a set of tools that are simple, inexpensive, portable, and robust and which could be commercialized and used in various fields of biomedical sciences, such as drug discovery, diagnostic tools, and therapeutic approaches in regenerative medicine. Our research program will be interdisciplinary, building the critical and experimental research media with the aim of overcoming the fundamental limits to information processing. It will enhance graduate education in the local universities, encouraging high quality researchers among local students to pursue research careers and create new knowledge in selected areas of focus. Its top academic and high-tech objective is to fabricate, investigate, and implement novel advanced micro- and nano-structures and superstructures based on ordered, highly functionalized materials to meet the demands of maximum efficient, active hydrogel materials of high and sustained reactivity as well as long term stability. It will enable new fundamental research and development for the next generation of biomedical materials, as well as the exploitation of such novel structures in the development of novel biomedical devices, transferring the knowledge to academia and industry for future implementation of novel knowledge and technology to the world.

In turn, it should increase the international competitiveness of the world in knowledge-intensive micro- and bioengineering of organ-based biosensors by focusing on high-impact research to generate new breakthroughs aimed at solving significant practical problems of biomedical sciences while seeking to extend the boundaries of understanding. It aims to raise the research profile of bioengineering as a vibrant center for medical and technological applications through a bottom-up approach that embraces elements of both basic and applied research to enhance the competencies in existing technologies and to seek out promising new areas and to develop an integrated, cutting edge research program by growing a pool of top research talent and developing the platforms on which local universities could create research breakthroughs of importance to the world.

Since advancement in device development technologies is a significant indicator of developed societies, the rapidly growing market for biomedical devices provides a competitive
advantage in R&D and commercialization for this field. Such advancement can be reached by helping local universities to set up and establish their own facilities using our technology created in local universities.

References

Correspondence to
Hossein Hosseinkhani, PhD
Graduate Institute of Biomedical Engineering,
National Taiwan University of Science and Technology (TAIWAN TECH),
Taipei 1067
Taiwan
Fax: +886 2 27303733
e-mail: hosseinkhani@mail.ntust.edu.tw