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Progress in Bio-manufacturing

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In this paper we made a thorough and systematic expatiation of the definition, connotation and composition of bio-manufacture. Based on the different development stages and process of bioengineering, its main research direction and corresponding researches were reviewed. Furthermore, from the viewpoint of disciplinary crossing, integration and development, the main technological development direction of bio-engineering in the coming years was pointed out.

Key words bio-manufacturing, organ, cell assembling, tissue engineering

1 Bio-manufacturing engineering

In 1995, the concept of bio-forming was put forward[1]. The processes of growth forming, removal forming, casting forming and discrete stacking forming were listed as the four main forming processes, which highly generalized the contemporary and future forming methods. The chairman of "the Committee of Challenges and Prospects of Manufacturing in the 21th Century", Dr. J. Bolinger, also brought out the concept of "bio-manufacturing" in 1998[2]. Later, some researchers in China also mentioned the concept of bio-manufacturing[3]. It's obvious that the concept of bio-manufacturing has already been noticed. However, as the definition and connotation of the concept was not clear back then, it didn't play a guiding role in manufacturing. With the application of manufacturing, especially rapid prototyping technology, in bio-medicine the concept of bio-manufaturing has become more and more explicit.

Biomanufacturing can be defined in a wide as well as narrow sense.

In a wide sense: bio-manufacture, or BM for short, includes biomimic manufacturing, biomass and organism manufacturing and other manufacturing related to biology and medicine.

In a narrow sense: BM mainly means organism manufacturing, which directly or indirectly assembles single cell or multicellular aggregates with the principles and methods of modern manufacturing science and life science, and accomplishes the forming and manufacturing of the organisms with metabolic characteristics. After culturing and training, they can be used for repair or replacement of human tissues and organs. From some other aspects, organism manufacturing is the extension of the Tissue Engineering appearing at the end of 1980s.

Bio-manufacturing, starting from the simple and explicit concept that "all life phenomena can be explained with glossaries in physics and chemistry" and "life is completely an outcome of physics and chemistry", gets its own philosophical idea that any complicated life phenomena can be simulated under artificial conditions with physical and chemical theories and methods and tissues and organs can be manufactured. Organism manufacturing is not equal to life manufacturing because it does not deal with the origin of life, but only with tissues, organs and other biomimic products through assembly of zoetic units.

2 Progress in bio-manufacturing

Manufacturing of human tissues and organs in vitro to repair or reconstruct disabled tissues and organs, has been a dream beyond the memory of men, and is also a long-term goal of bio-manufacturing engineering. The earliest artificial organs are mechanical, such as ventricular assist device (VAD) and total artificial heart (TAH) (For example, the Abio Cor devices developed by the Aboimed company in Massachusettes, USA), and many kinds of artificial skins and vasa constructed by inanimate polymers. Then it becomes partially mechanical and partially biological, such as the mixed bio-artificial livers. Up to now, it has become totally bio-artificial analogues of natural tissues and organs. At this stage, researchers haven't mastered the exquisite skills as those in nature. Manufacturing of human organs needs several steps, starting with single tissues, then complicated tissues, functional tissues, then some kinds of organs and finally all kinds of organs. At present, some breakthroughs have been made in the first few steps.

Recently, non-degradable and non-biocompatible biomedical models have been applied in clinic. For example, the invisible ortho-aligner produced by the Time Angles Company of Beijing, China, on a large scale for teeth correction. Biomedical physical models for repairing craniums, pelvis and occlusalf surface have become popular now and physical model has gradually become indispensable reference models for bone surgeon (such as traffic accident, war etc.).

It is now quite mature to manufacture non-degradable implants with good biocompatibility and customized prostheses. Metal (or nonmetal) artificial limbs and the activation of the interface of these implants with the host tissues are gradually popularized. Repair of individual ear and occlusalf surface, plastically reconstructing occlusalf surface, and implanted cranium scaffold, designing and constructing reticulum lamina and other reparative medical devices have all benefited from bio-manufacturing, and suggest a new direction of technology and product development focused on bio-manufacturing.

Researching and manufacturing scaffolds for tissue engineering with biodegradable and biocompatible materials has attracted more and more attention these years. At present, significant achievements have been obtained in structural tissue engineering. Structural tissues are tissues with one sort of cells and simple structures, such as skin, bone, cartilage, muscle and so forth. Several kinds of tissue engineered cartilage and skin have been proved by FDA, USA. Since the 1990s, research in this area has been strongly supported through "973" and "863" programs, national natural science fund and other national key plans, which has established its own status in the international community. As early as in 1994, the Second Medical University of Shanghai has succeeded in culturing human auricular cartilage on the back of nude mouse, which evoked worldwide repercussions. Furthermore, the Research and Development Center of Shanghai Tissue Engineering has successfully carried out several skin and skeletal reconstruction operations.

Tsinghua University together with the Fourth Military Medical University and Institute of Chemistry, Chinese Academy of Sciences, have launched rapid forming tissue engineering research, and obtained some good results in bone, cartilage and other tissues. Artificial bone scaffolds of PLGA/TCP with through-pores (Figure 1), bBMP, bFGF and other growth factors made through low temperature deposition technology has successfully repaired 15 mm segmental bone defects in the radii of rabbits and 20 mm segmental bone defects in the radii of dogs and articular cartilage defects of rabbits. The artificial bone may be used clinically as an excellent material for bone repairing[4].

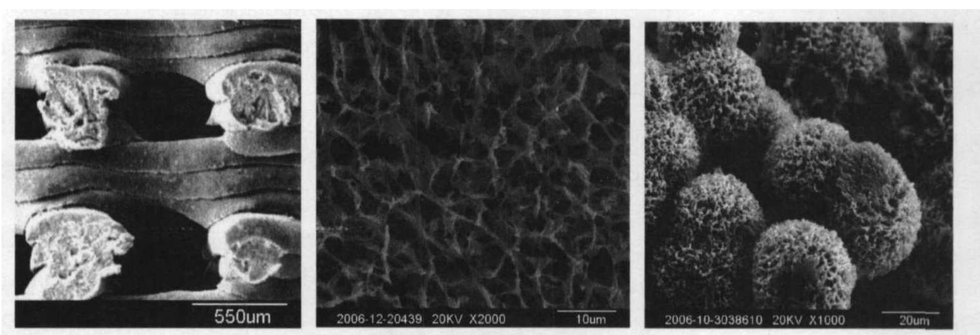

Figure 1   A nano-artificial bone with three level hierarchical porous structure

It still needs to expatiate the mechanism for manufacturing of full-functional internal organs due to complicated structures of organs and the large quantity and varities of cells involved, unclear control over cells and tissues and regeneration, and unresolved construction of vascular network in vitro. Constructing vascular network plays a significant role in constructing complicated organs. There are various trials all over the world and some kinds of products have entered the market. But it is still far from being applied to organ reconstruction. An example is the fabrication of branched and multilayered blood vessel scaffolds based on Melted Extrusion Manufacturing (MEM) technology in Tsinghua University.

The research on repairing and constructing various internal organs has been going on for nearly 20 years and gained considerable results. In recent years, engineered cardiac tissue patches or strips have been successfully constructed. Such work is mainly carried out by Telaviv University (Israel), Harvard University and Massachusettes Institute of Technology (MIT, USA), Neuremberg University (Germany), and Tokyo Woman's Medical University (Japan)[5], Academy of Military Medical Science and Bio-manufacturing Center of Tsinghua University (China)[6]. The results make it possible to repair large scale structural cardiac tissues, and easier for drug screening and cardiac electrophysiological study.

Recently, researchers in Newcastle University have achieved exciting results in liver tissue engineering[7]. They have constructed a liver tissue less than 1 inch (about 2.5 cm). It may be used for replacing small disabled liver defect or for drug tests for human and animals. This result has laid a foundation for eventually constructing complete liver with its own blood supply and fibrous skeleton systems.

It's not easy to construct internal organs with complicated structures and functions such as heart, liver and kidney. The research group of Regeneration Medical Institute, Wake Forest University (America), has successfully cultured human bladders in vitro using cells of the patients themselves. The constructed bladders have been applied in seven patients with good results[8]. In recent years, research on constructing internal organs from cells through directly controlled assembly technique has become a hot issue[9,10].

On the whole, China has made considerable progress in manufacturing bio-artificial organs and has an important impact on the international artificial viscera organ research, although the research is not yet so systematic and in-depth. Currently the Center of Organism Manufacturing of Tsinghua University, the Structure Project Research Center of Academy of Military Medical Sciences, Institute of General Surgery of Chinese PLA General Hospital have achieved initial results and some have reached the world's advanced level in the study of organ reengineering of heart, pancreas and liver[11].

Based on principles of discreteness and stacking, three dimensional controlled cell/matrix assembling technology is the forefront research area of manufacturing of tissues and organs which rose at the beginning of the 21st century. In manufacturing science, the proposal of discrete-stacking principle has connected the manufacture science and life science and promoted them to integrate. The core principle of discrete-stacking is actually a controlled assembling concept. It includes different level controlled assembly from macroscopic units, mesoscopic units to microscopic units. In the macroscopic dimension, it is biomaterial microdroplet assembly which is now the main parts of bio-manufacturing. In the mesoscopic dimension, it is cell assembly that is now the hot issue of bio-manufacturing and the research on it has just started both in China and the world at large. Currently only Clemson University, South Carolina University, Bath University, Tsinghua University and Germany's Freiburg Research Center have started research in this field. In a microscopic dimension, molecular assembly is still in the stage of being discussed out of interest, and no substantive progress has been reported. Manufacturing visceral organs, such as the heart, liver, kidney, lung, is the dream of people, which differs from manufacturing structural tissues, such as bone and skin. Manufacturing visceral organs involves a higher level life and manufacturing science, and has a close relationship with manufacturing science in the molecular and cellular level of manipulation and assembly. Three dimensional controlled cells assembling technology has become one of the very hopeful visceral organs manufacturing technology. It includes the accurate three-dimensional arrangement (transport, control, connectivity and development) of a wide variety of cell types and the inducement expression of different genome in different time and location, etc. These problems to a considerable extent involve the formation of life and evolution. With the interdisciplinary development of life science and manufacturing science, it is expected that mechanism of manufacturing artificial human organs with whole functions will come true before 2020.

3 The important technology development direction of Bio-manufacturing engineering

Artificial human organ manufacturing is still in the exploratory stage. The exploration of the mechanism still needs to continue for 10 to 20 years, which shows the long-term characteristic of artificial human organ manufacturing. On the other hand, manufacturing artificial human organs involves many important fields, and it needs long-term and deep integration of different disciplines. The study of mechanism of artificial human organ manufacturing should be combined with the key technology and application. Mutual promotion can lead to healthy and rapid development, which will benefit humankind.

To sum up, at this stage, there are key research areas about mechanism and application of artificial human organ manufacturing.

3.1 Organ manufacturing technology and hardware and software systems

Organ manufacturing is the method that scaffolds and growth factors are assembled to create a similar natural human organ under the control of digital organ model drives cells. The available low-temperature deposition manufacturing (LDM), cell printing, biological drawing and three-dimensional directly controlled cell assembling technology are unable to meet various requirements of bio-manufacturing. Development and research of multi-cellular and multi-materials space controlled assembly technology is inevitable. Different cells or extracellular matrices were stored in different materials barrels. According to the actual components proportion of certain location of a digital model, multi-nozzle technology or single nozzle with multiple material carrier-composition was used to accurately locate cells and other biological materials into complex gradient physical structure which can imitate natural human organs.

3.2 Physical simulation system

Through construction of three-dimensional tissue precursors, analysis of biological information and modeling of physiological function, control of different cells number and space composition, regulation of signal transduction among cells it is possible to establish small biologic modules which can truly simulate the specific physiological system function. These modules can be used to study the mechanism of interaction of various cells and the functional characteristics of biological systems, which offers new method in the fields of drug screening, disease pathogenesis, diagnosis and prevention.

3.3 Cells source of organ manufacture

Human organs are composed of cells. The cells coming from the patients themselves are essential for organ manufacturing. Since the number of cells taken from corresponding organs is small, the differentiation and expansion capacity of the cells are limited and cell sources have become a huge bottleneck. Stem cells are cells that come from body and have more primitive traits, tremendous potential proliferation and differentiation ability. Through the proliferation and differentiation of stem cells from human body, enormously different cells can be obtained which will become the major cell source of bio-manufacturing and provide important basis for the construction of human tissues and organs in vitro.

3.4 Extracellular matrix mimiking

Matrix is the extracellular environment of cell survival. To provide cells with environment similar to the human body, matrices should be mimicked. The main contents include: to select the matrices from the existing biological materials and to modify the surface, to further study the biocompatibility, blood compatibility, mechanical properties, formability and the aging properties, as well as the influence on the secretion, adsorption and control-release of growth factors and other factors of these materials. The ultimate goal is the "seamless" connection of biomimiking extracellular and cells.

3.5 Human organ biological modeling

Digital Anatomy Model of human organ is one of the prerequisites of bio-manufacturering. Based on organ information obtained by CT/MRI tomography technology, expert system software and reconstruction of digital model can be developed which can effectively describe complex composition of organs and functional gradients. This involves a lot of theoretical and technical details such as geometric modeling, materials modeling, functional modeling and system optimization from local to global based on fractal theory. Based on the process of organ manufacturing and biomimick principles, it is of great significance to address the vascular function, organization of matrix structure and to create hierarchical structural models for precursors with natural three-dimensional environment for cell survival.

3.6 Artificial organs revascularization

In the construction of human organs in vitro, larger branched vascular systems, such as arteries and vein, can be induced by using the biomimic vascular scaffolds. For the capillary network, it can be constructed through reserving angiogenesis channel, pre-implanted endothelial cell and growth factor in the manner of vascular occurrence or regeneration. So it is essential to study the mechanism and rules of vascularization in developmental biology, to find micro-environment conditions of vascular and microvascular networks forming, and to guide the construction of artificial blood vessels and the vascularization of the artificial organs.

3.7 Design and optimization of bioreactors

It is necessary to culture and train the precursors of artificial organs in vitro using bioreactors that can simulate human body temperature, liquid, gas, pH, stress and strain, electrical signal transduction and many other factors. This makes the precursors accept stimulation of various exogenous signals, continuously self-assemble and reconstruct which eventually leads to the formation of functional human organs. The study includes not only equipments, but also the basic scientific knowledge about developmental biology and regenerative biology.

Natural cells survive in 3D environment, and interconnect with surrounding cells. Such linkages directly present in vitro as the formation of cell clusters. The traditional cell culture methods are two-dimensional. Cells settle with gravity and grow on plane, losing three-dimensional balanced state in natural tissue, and thus leading to the failure of in vitro organ formation through cell culture and reconstruction.

The precursors manufactured by three-dimensional cell controlled assembling technology simulate the three-dimensional growth state of cells in natural environment to some degree. But in the traditional static culture, cell density in the scaffold is usually high. Material exchange with culture medium is relatively inadequate. Sometimes gravity affects cell growth and movement in three-dimensional scaffolds. If cells are cultured in a rotary cell culture system (RCCS) with microgravity and swirl liquid movement, the exchange between cells and culture medium will be greatly strengthened, which helps free growth, contact, communication and reproduction of cells in the three-dimensional fiber scaffolds. Precursors manufactured in this way have solved the problem of spatial location of different cells. In a microgravity environment, the homogeneous and heterogeneous cells can interact sufficiently so as to achieve the integration of the structure, the development of function and eventually the construct of tissues.

4 Conclusions

Generally, bio-manufacturing science and technology has solid basis of material science, biology science and manufacturing technology. It breaks the gaps between traditional manufacturing science and life science, and leads manufacturing science to a new stage. With the development of artificial internal organs based on bio-manufacturing science and technology, stem cell technology, and the related science and technology, it must lead to changes of medicine and biotechnology. Human organ bank will store the second set of important organs of people, which are ready to save the lives of thousands and improve the quality of life.

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References

[1] Yongnian Yan, Jianguo Wang, Yaohua Wu. Modern shaping science and rapid prototyping processes. Symposium on information control problems in manufacturing, 1995.

[2] 颜永年,熊卓,张人佶等. 生物制造工程的原理与方法, 清华大学学报,2005,45(2),145—150.
Yan Yongnian, Xiong Zhuo, Zhang Renji, et al. Principles of organism manufacturing engineering. Journal of Tsinghu University (science and technology), 2005, 45(2), 145—150.

[3] 吴永辉,李涤尘,卢秉恒等. 基于快速成形的生物制造工程研究,机械工程学报,2000,36(9).
Wu Yonghui, Li Dichen, Lu Bingheng, et al. Research for biological manufacturing engineering based on rapid prototyping. Chinese Journal of Mechanical Engineering, 2000, 36(9).

[4] 熊卓. 骨组织工程支架的低温沉积制造及应用基础研究:[博士学位论文]. 北京:清华大学机械工程系, 2003.
Xiong Zhuo. Low-temperature deposition manufacturing and fundamental research of bone tissue engineering scaffolds. Beijing: Mechanical engineering department, Tsinghua University, 2003.

[5] Vara D. S., Salacinski H. J., Kannan R. Y., et al. Cardiovascular tissue engineering: state of the art. Pathologie Biologie, 2005, 53 (10): 599—612.
[6] 颜永年,刘海霞,王小红等. 细胞直接受控组装技术的实现及研究进展,科学通报,2005,5(11),1—2.
Yan Yongnian, Liu Haixia, Wang Xiaohong, et al. Realization and progress of direct controlled cell assembling techniques. Chinese Science Bulletin, 2005, 5(11), 1—2.
[7] FIONA MacRAE. British scientists grow human liver in a laboratory. http://www.dailymail.co.uk/pages/live/articles/news/news.html? in_article_id = 413551&in_page_id = 1770&ico = Homepage&icl = TabModule&icc = NEWS&ct = 5, 31st October 2006.
[8] Atala A, Bauer SB, Soker S, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet, 2006, 367 (9518): 1241—1246.
[9] Xu T, Jin J, Gregory C, et al. Inkjet printing of viable mammalian cells. Biomaterials, 2005, 26(1): 93—99.
[10] Yan YN, Wang XH, Pan YQ. Fabrication of viable tissue-engineered constructs with 3D cell-assembly technique. *Biomaterials*, 2005, 26(29): 5864—5871.
[11] 刘海霞. 细胞直接三维受控组装技术研究: [博士学位论文]. 北京:清华大学机械工程系,2006.
Liu Haixia. Studies on three dimensional direct controlled cell assembling technology. Beijing: Mechanical engineering department, Tsinghua University, 2006.

Zhu Zuoyan Met with Rockefeller University Life Professor Cai Nanhai

On Feb. 1, 2007, Prof. Zhu Zuoyan met with Prof. Cai Nanhai, life professor of Rockefeller University and Foreign Member of CAS.

Prof. Cai, as a life professor at Rockefeller University, is renowned in the field of plant molecular biology and has published a series of papers in world top scientific journals. Over the years, he has been keeping close attention to the development of China's basic research. Many Chinese biologists have visited and worked in his laboratory.

During the meeting, issues of mutual interests were discussed, such as the development trend of major research plan in life sciences, the training of young talents, the internationalization of Chinese academic journals, etc. Prof. Cai pointed out that international cooperation in the field of science policy making is of strategic significance in identifying scientific frontiers and suggested that NSFC join hands with NSF of the United States to support bilateral workshops in this field.

(Liu Xiuping; Chen Huai)