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MESENCHYMAL STEM CELLS AND CRANIOFACIAL REGENERATION

Editors:
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Mesenchymal Stem Cells and Craniofacial Regeneration

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PREFACE

In humans, tooth loss cannot only lead to physical and mental suffering, but also affect the aesthetics, which compromise an individual's self-esteem and life quality. There are several dental diseases that can eventually result in tooth loss. With the development of tissue engineering, regenerating a whole tooth is now considered a promising strategy to treat tooth loss.

In addition, cranial and maxillofacial trauma is notoriously difficult to repair and presents a significant burden to the healthcare system. Current therapies usually utilize autologous tissue transplantation and artificial composite material reparation. However, limitations exist. Tissue engineering as an alternative to autologous transplantation provides new hope for restoration and reconstruction of missing tissue and avoids immunosuppression. It has been used to successfully regenerate many tissue types, such as bone and cartilage. The aim of regenerative medicine is to restore and replace tissue that has been damaged or lost through trauma.

Tissue engineering is an interdisciplinary technique that creates a biological substitute through application of the principles of engineering to biology and medicine to restore, maintain, and improve tissue function. The emerging discipline of regenerative medicine and tissue engineering is committed to a rational approach to maintain and preserve the microenvironment, which is based on growth factors for tissue induction, stem cells, and scaffolds.

Stem cell-based tissue engineering approaches have the capacity to regenerate damaged and diseased tissues. Induced pluripotent stem cell (iPS) cells are a type of pluripotent stem cell that is generated from somatic cells, contrary to natural course of cell differentiation. iPS cells are derived from differentiated cells or tissue, reprogrammed to an embryonic-like state. Reprogrammed human induced pluripotent stem cells (hiPS) are similar to human embryonic stem (hES) cells in many aspects, such as morphology, epigenetic status of pluripotent genes, surface antigen expression, telomerase activity and proliferation rate.

Mesenchymal Stromal Progenitor/Stem Cells (MSCs) are not a large rare population of non-hematopoietic stromal cells.They are present in the bone marrow and most connective tissues. MSCs can be isolated from a variety of tissues, such as endometrial polyps, umbilical cord, endometrial polyps, adipose tissue, menses blood, bone marrow, adipose tissue, *etc*. These sources are most practical for experimental and possible clinical applications due to the ease of harvest and large obtainable quantity. In recent decades, cell sheet technology (CST) has been exploited as a novel promising scaffold-free approach for cell culturing and delivering in tissue regeneration. Studies have demonstrated that MSCs facilitate cell viability and function

in the CST co-culture system to improve transplantation efficiency. There are extensive interests in developing strategies to enhance the formation of cell sheets with MSC for downstream applications.

Growth factors are naturally occurring proteins induced by cell surface receptor-mediated chemokines, mitosis, and angiogenesis. They participate in the growth, development, repair, reconstruction, and regeneration of tissues and organs.

The scaffold is a key factor in tissue regeneration. It provides a desired three-dimensional (3D) space that allows stem cells to adhere, proliferate, migrate, and differentiate. In the field of regenerative medicine and tissue engineering, biomaterials are critical for the encouragement and maintenance of biological activities. Scaffolds can be cell-instructive, structural and fabricated from smart materials. Design considerations include the creation of well-defined materials, cell-material interactions, and controlled-release bioactive agents. Since three-dimensional printing (3DP) technology was first invented in 1980s, it has been used in a range of fields, including architecture, manufacturing, engineering, education and medicine. In dentistry, 3DP provides a rapid and precise technology that helps diagnosis, treatment planning and treatment of various dental diseases. In craniofacial regeneration, 3DP helps to design and fabricate dental prosthesis and implants, and it also improves craniofacial tissue engineering. Not only scaffolds can be fabricated by 3DP, biologics such as stem cells and growth factors can also be delivered. Thus, 3DP technology has become a major tool in craniofacial regeneration.

Overall this book brings together research spanning from diverse backgrounds ranging from materials science and engineering, biology and clinical medicine, to discuss various aspects of craniofacial and dental regeneration. We hope this book will add further insight for basic and applied researchers as well as clinicians involved in tissue engineering and regenerative medicine, thus contributing to further advances in dental medicine.

This book provides an in-depth overview of current knowledge about mesenchymal stem cell and its applicant in craniofacial regeneration, including concepts and features of mesenchymal stem cells, induced pluripotent stem cells,craniofacial regeneration, new methods on scaffold fabrication, tooth regeneration and three-dimensional printing in dentistry. We sincerely hope that the publication of this book will provide an overview for researches and clinician who are concentrated on the field related to this promising subject .

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CHAPTER 1

Fundamental Concepts and Features of Mesenchymal Stem Cells: Proliferation, Differentiation, Migration and Immunomodulatory Characteristics

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Abstract: Mesenchymal Stromal Progenitor/Stem Cells (MSCs) are not a large population of non-hematopoietic stromal cells. This type of cells is usually present in the most connective tissues and bone marrow of the body. They can be isolated from a lot of tissues, such as endometrial polyps, adipose tissue, umbilical cord, menses blood, bone marrow, *etc*. MSCs have been defined following isolation and culture expansion, by their expression of different molecules including CD90, CD73 and CD105 and the negative markers like CD45, CD34 and CD14. MSCs have the proliferation ability in culture in uncommitted state, while retaining their pluripotency, which makes them attractive effector for biological cell-based tissue repair approaches. MSCs could be differentiated to several cell lineages. Furthermore, the exclusive biological properties of MSCs are mediated by paracrine mechanisms and by intensive immunomodulatory activity. The MSCs use for treatment has been fund and evaluated to be very useful in some pre-clinical animal models and in clinical trials. MSCs and MSC-like cells have low rates of homing to target tissues and organs, limiting therapeutic efficacy. Thus, researches of the homing mechanisms and homing factors of MSCs may lead to the

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development of therapies with the potential to promote clinical applications of MSCs.

Keywords: Cell migration, Differentiation, Homing, Immune, Mesenchymal stem cell (MSC), Stem cells.

1. INTRODUCTION

1.1. Overview

Mesenchymal stem cells are self-renewing, multipotent somatic cells that play vital roles in tissue homeostasis and repair and, as such, exhibit tremendous potential for tissue engineering and regenerative medicine. As one of the first few stem cell types isolated, mesenchymal stem cells are among the most-wel--characterized and have since been harvested from a variety of tissues. However, clinical translation of these cells is still hampered by issues including cell heterogeneity, maintenance of stem cell phenotype with extended *in vitro* culture and identification of the optimal strategies for controlling mesenchymal stem cell behavior to promote tissue healing and regeneration. To address this, we summarize the history as well as review the essential characteristics and properties of mesenchymal stem cells, including the isolation procedures of these cells, methods for maintaining their proliferation and stemness *ex vivo* as well as growth factors and signaling pathways essential to mesenchymal stem cell migration, chemotaxis and homing. The purpose of this chapter is to provide readers with essential concept of MSC isolation, maintenance and biology for optimal deployment of these cells in tissue engineering applications.

1.2. History

The history of mesenchymal stem cells began with their isolation from bone marrow tissue before additional populations were isolated from other tissues. In 1970, Friedenstein and colleagues showed that bone marrow comprise hematopoietic non-adherent cells along with an uncommon population of plastic-adherent cells, after entire bone marrow cells were placed in plastic culture dishes with medium supplemented with 10% fetal calf serum. These cells have the ability to form colonies derived from single cells. After two days, these adherent cells begin to proliferate and can differentiate into mature mesenchymal cell

lineages [1]. The term Colony Forming Unit–fibroblasts (CFU-f) refers to the round colonies of fibroblastic cells generated from the primary clones of adherent cells. Friedenstein also found part of the colony can differentiate into small region bone or cartilage. They studied the proliferative capacities and phenotypic qualities of CFU-f [2] and found that these cells present multipotential property which can differentiate into chondrocytes, adipocytes, osteoblasts and myoblasts. Dependent on the age and health situation of the bone marrow donor, very donor shows a particular frequency of CFU-f. According to Caplan's propose [3], since the cells differentiate into mesenchymal cell lineage, or mesenchymal stromal cells, they are currently categorized as mesenchymal stem cells (MSC) [4, 5]. Whether these cells ought to be viewed as pure stem cells or as multipotent progenitors of mesenchymal lineages has been very widely discussed. Thus, adoption of the expression "multipotent mesenchymal stromal cells" has been proposed in place of "mesenchymal stem cells" [6, 7]. In 1974, Friedenstein and his fellows firstly identified the MSCs [8], despite the fact that Caplan already initially applied the term "mesenchymal stem cell" in 1991 [3]. Subsequent to MSC isolation from bone marrow, MSCs were isolated from a lot of tissues including adipose tissue, cutaneous tissue, fetal hepatic and pulmonary tissue.

1.3. Definition

The definition of MSCs depends singularly on the examination of cell populations *in vitro*. This is because, despite years of investigation, the natural position and role of MSCs within the body origin tissue remains unclear. Confounding such analysis is the absence of specific MSC markers to permit their unambiguous identification [7, 9, 10]. Since MSCs are removed from their natural environment and grown on artificial plastic dishes in a combination of exogenous chemical and physical growth conditions, the MSC phenotypes and abilities in both *in vivo* and *in vitro* settings may not necessarily be the same. For example, MSCs are rearrangement *in vitro* phenotype operating experience, gain new markers while losing the expression of others [11]. Because of increasing interest in the use of MSCs as a part of cell-based therapy [12, 13], the need to recognize MSCs definitively is not only scientific but also clinical and administrative interest [11, 14]. As described above, no single marker can unequivocally identify the MSC and other cell types differentiate. In 2006, the International Society for Cell

CHAPTER 2

Three-dimensional Printing in Dentistry: An Advanced Technology for Craniofacial Regeneration

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Abstract: Since three-dimensional printing (3DP) technology was first invented in 1980s, it has been used in a range of fields, including architecture, manufacturing, engineering, education and medicine. In dentistry, 3DP provides a rapid and precise technology that helps diagnosis, treatment planning and treatment of various dental diseases. In craniofacial regeneration, 3DP helps to design and fabricate dental prosthesis and implants, and it also improves craniofacial tissue engineering. Not only scaffolds can be printed by 3DP, stem cells and growth factors can also be controlled and printed by 3DP. 3DP technology has become a major tool in craniofacial regeneration.

Keywords: Craniofacial Regeneration, Dentistry, Three-dimensional Printing.

2.1. INTRODUCTION

Three-dimensional printing (3DP) is a process in which three-dimensional objects are created by fusing or depositing materials layer by layer. The materials used for 3DP can be plastic, metal, ceramic, biomaterials or even living cells [1, 2]. 3DP is

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also known as additive manufacturing (AM), rapid prototyping (RP), or solid free-form technology (SFF) [1]. This new technology allows complex physical structures to be fabricated accurately and rapidly.

Since its first invention in 1984, 3DP has been developed into different modeling systems and its application has been expanded to various areas [1-8]. 3DP originally was only used to produce an object with designed shape, but now it can generate more functional products such as food, clothes, houses, auto parts, gun and aircraft. It is bringing radical changes to our daily life. In medical field, 3DP can be used in tissue and organ fabrication, in the creation of customized prostheses, implants, and anatomical models, and in pharmaceutical researches regarding drug dosage forms, drug delivery, and new drug discovery [1]. With the constantly evolving, 3DP has been brought to the dentistry field. It is making a difference in various dental specialty fields including endodontics [9-14], oral surgery [15-23], prosthodontics [24-27], periodontics [28, 29], orthodontics [30-34], and implant [35-45].

2.2. THE HISTORY OF 3DP

The concept of 3DP was first introduced by Chuck Hull in 1984. It was described as stereolithography (SLA), the printing of successive layers of materials to create a 3D object [2]. Later in 1988, Hull and the company 3D Systems developed the first 3D printer termed “Stereolithography Apparatus”, and introduced the first commercially available 3D printer called SLA-250 [1, 46]. Subsequently, a new technique named “Fused Deposition Modeling” (FDM) was engineered by Scott Crump at Stratasys in 1990. In 1991, the Solid Ground Curing (SGC) technology by Cubital, and the Laminated Object Manufacturing technology (LOM) by Helisys were both commercialized [47].

In 1992, Selective Laser Sintering (SLS) system from DTM corporation and the Soliform Stereolithography system from Teijin Seiki were introduced to 3DP market [47]. In 1993, the first apparatus termed “3D printer” was patented by Massachusetts Institute of Technology (MIT) to attain plastic, metal and ceramic parts [48]. In 1996, Stratasys introduced a new 3DP machine named “Genisys” that used an extrusion process similar to FDM. In the same year, the company Z Corporation launched Z402, a concept modeling 3D printer based on MIT’s inkjet

printing technology. Also in 1996, three new 3DP systems emerged to the market: Personal Modeler 2100 from Ballistic Particle Manufacturing (BPM) technology, DuPont's Somos Stereolithography technology and Zippy Paper lamination systems [47].

In 2005, Z Corporation launched the first high definition 3D color printer named Spectrum Z510 [49], and in 2006 the RepRap, a desktop 3D printer for plastic objects printing, was introduced to market. It was a self-replicating 3D printer and provided an open source option to print the majority of its own parts [46, 49]. In 2007, Z Corporation introduced the ZPrinter 450, which could automatically remove and recycle loose powder material from printer. In 2009, the FDM patent was expired, and in 2014, SLS patent was expired. Since then various types of 3D printing system have been released. According to Wohlers 2014 Report, the worldwide 3D printing industry is now expected to grow from $3.07 billion in revenue in 2013 to $12.8 billion by 2018, and exceed $21 billion by 2020 [50].

The medical application of 3DP was dated back to 1996 when the idea of 3D-printed organs was proposed by Dr. Gabor Forgacs. In 1999, Dr. Atala's group at the Wake Forest Institute for Regenerative Medicine for the first time created artificial scaffolds in the shape of the desired bladder and coated it with cells taken from their patients, and successfully grew an artificial bladder [51]. This set the stage for true "bioprinting". According to the First International Workshop on Bioprinting and Biopatterning, bioprinting was defined as "the use of material transfer processes for patterning and assembling biologically relevant materials, molecules, cells, tissues, and biodegradable biomaterials with a prescribed organization to accomplish one or more biological functions" [52]. In the early 2000s, 3DP technology was applied in medical and dental fields to print dental implants and custom prosthesis [1]. At these early stages of bioprinting, only biomaterial scaffold was printed but not living cells. In the past decade, the 3D bioprinting technology was greatly evolved. It has been used to print not only the scaffolds for organs regeneration, but also print protein and DNA patterning, as well as multi-cellular assemblies and tissue patterning [52]. Though 3DP is still very far from being able to print organs, it is possible to print artificial scaffolds in the shape of an organ with living cells [5].

CHAPTER 3

Induced Pluripotent Stem Cells: Proliferation, Migration, MicroRNA, Signaling Molecules

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Abstract: Since it was first demonstrated that induced pluripotent stem cells (iPS cells) could be derived from mature cells, significant progress has been made in the field of acquisition, characteristics, identification and application of iPS cells. Until now, diverse means have been proven to generate iPS cells successfully in many biological species and more cell types. Meanwhile, researchers continue to target the efficiency of induction. To identify the characteristics of induced pluripotent stem cells and attest to their pluripotency, one must verify the expression of new derived stem cell genes and proteins, doubling times, methylation patterns, teratoma formation, embryoid body formation, viable chimera formation and capacity to differentiate into all cell types. In other words, induced pluripotent stem cells are theoretically similar, or even same, to natural pluripotent stem cells, for instance embryonic stem (ES) cells. Furthermore, iPS cells have the potential to take the place of ES cells eventually, from which numerous ethical difficulties arise for the treatment of a mass of diseases, for use in therapeutics for drug discovery, disease modeling and regenerative medicine, *etc*. However, many problems exist as barriers to clinical transformation, including risk for induced oncogenesis and the stability of reprogramming. Here we summarize the current

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acquaintance of iPS generation and evaluate the advantages and disadvantages of their applications in clinical medicine.

Keywords: Biological safety, Biometric identification, Cellular biological properties, Cell differentiation, Cellular reprogramming, Induced pluripotent stem cell, Investigation of drugs, Pluripotency, Regenerative medicine.

INTRODUCTION

During normal development, cells are generally endowed to increasingly differentiated fates. But according to research achievements for cellular reprogramming, the process of differentiation is reversible. iPS cells are a type of pluripotent stem cell that is generated from somatic cells, contrary to natural course of cell fate. The iPS cell technology was first introduced by the Japanese investigator Shinya Yamanaka [1] who transfected four genes (containing Oct3/4, Sox2, c-Myc and Klf4, OSKM) into mouse embryonic fibroblasts (MEFs) *via* retroviral transduction and converted somatic cells into pluripotent stem cells. The Nobel Prize of 2012 was granted to him for his contribution in discovering that mature cells can be reversibly reprogrammed to become pluripotent status.

Among the pluripotent stem cells, the embryonic stem cell is the best-known, because, as the beginning and origin of life, they can differentiate into all kinds of cell lines. However, since the usage of embryonic stem cells relate to impairing the embryo [2], significant ethical opposition has been raised. Furthermore, the source is limited, and patient-matched embryonic stem cell lines cannot currently be obtained. There are too many obstacles to the clinical production of embryonic stem cells.

In contrast, iPS cells are derived from differentiated cells or mature tissue, reprogrammed to an embryonic-like state. Reprogrammed human induced pluripotent stem cells (hiPS) are similar to human embryonic stem (hES) cells in many fields, such as morphology, epigenetic status of pluripotent genes, surface antigen expression, telomerase activity and proliferation rate. Beyond that, it has been demonstrated that hiPS cells have the capacity to differentiate into cell types of all three germ layers *in vitro* and *in vivo*. The cell source is extensive, and they can be used to give each individual his or her own pluripotent stem cell line.

Autologous donors also reduce the likelihood of immune rejection of transplantation. Thus, hiPS cell is a promising cell classification as a replacement of embryonic stem cells in the field of regenerative medicine, thus mitigating ethical risks. However, more and more evidence indicated that hiPS cells and hES cells are not identical at many aspects. There are also verified differences in inheritance among different iPS cell lines that may show that differences in the somatic cell source or even genetic variability among similar cells lead to discrepant pluripotent stem cell.

In recent studies, it has been demonstrated that more than one outcome of iPS cell states exists from the usage of a secondary reprogramming means, in which the initial differentiated cells are mouse fibroblasts continued derived by OSKM containing doxycycline. A novel category of steady-state pluripotent cells was emerged, as given F-class status after the fuzzy presentation of cell colonies in culture. It is a new condition that deserves to be studied.

Although various methods have been established to generate iPS cells, with some success in terms of degree of pluripotency, each means has advantages and disadvantages with respect to future clinical applications.

While iPS cell technology has developed more rapidly than ever, it has not advanced to a stage where the related techniques of cell therapy or organ synthesis are safe and reliable. Carcinogenicity is one of the significant risks blocking the usage of iPS cells in human while there are challenges like low efficiency, incomplete, genomic insertion posing serious risks that limit the use in clinic. Thus, the task of iPS cell application continues to be both challenging and full of opportunities.

2. IPS CELLS: METHODS OF ACQUISITION, PROMOTING GROWTH AND INCREASING INDUCED EFFICIENCY

2.1. Selection of Proper Recipient Cells

Since the successful derivation, in 2006, of iPS cells from adult mouse fibroblasts through the ectopic co-expression of four related genes, fibroblasts have been the most common cell type to reprogram, likely because these cells are readily

CHAPTER 4

Craniofacial Defects and their Regeneration: Destruction and Regeneration of the Periodontium, Craniofacial Tumors, Trauma, and Congenital Defects

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Abstract: Injuries in the cranial and maxillofacial region are clinically important because this region contains the most exposed parts of the body and is important for expression of social etiquette. Currently, the most common methods used to repair cranial and maxillofacial defects are autologous tissue transplantation and artificial composite material reparation. However, these methods have limitations. In recent years, tissue engineering has been developed as an alternative method of craniofacial reparation and reconstruction. Stem cell therapy is an emerging strategy that may potentially be used for the reconstruction of craniofacial defects.

Keywords: Adipose tissue regeneration, Bone defects, Bone regeneration, Cartilage regeneration, Congenital defects, Craniofacial defects, Cartilage defects, Periodontal tissue regeneration, Periodontium defects, Regenerative medicine, Reparation and reconstruction, Scaffolds, Soft tissue defects, Soft tissue regeneration, Stem cells, Tissue engineering, Tooth loss, Tooth regeneration, Trauma, Tumors.

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4.1. INTRODUCTION

The cranial and maxillofacial region contains the most significantly exposed parts of the human body and important organs for expression of social etiquette. Cranial and maxillofacial defects commonly occur secondary to congenital malformation, infection, cancer, and trauma, but the clinical management of these defects remains challenging [1]. Other than a small number of congenital malformations, most such craniofacial defects are caused by acquired factors and are thus often called acquired defects. The most frequent cause of these defects in the first half of the 20th century was infectious diseases, especially sequelae of gangrenous stomatitis. However, oral and maxillofacial defects caused by traffic accidents and tumor resection have steadily increased with developments of the social economy and surgical treatment of craniofacial tumors [2 - 5].

Rapid development of craniofacial reconstruction and reconstruction surgery has been promoted with increasing emphasis on quality of life and formal establishment of the unity of form and function recovery. Surgical repair and reconstruction involves the correction of deformities and reconstruction of defects of various tissues and organs of the whole body, and its basic technology can be used in various clinical disciplines. Cranial and maxillofacial reparative and reconstructive surgery involves correction of deformities and reconstruction of defects of the oral and maxillofacial region. It not only involves oral and maxillofacial surgery but is also related to the clinical disciplines of craniofacial surgery, head and neck surgery, plastic surgery, otolaryngology, and other fields.

A patient's quality of life will substantially decline by severely disfiguring facial injuries. The craniofacial region has various specific functions, such as protection of the brain and optic tracts, mastication, speech, breathing, and hearing. The craniofacial region is also important for self-esteem and social recognition [6, 7]. Therefore, cranial and maxillofacial defects cause not only great physical damage but also psychological damage.

The three basic principles of reparation and reconstruction in any body region are repair of the defect, improvement in shape, and restoration of function. This is also true for reconstruction of oral and maxillofacial defects. To achieve success,

a surgeon must be mindful of the functional and aesthetic requirements in planning for reconstruction [8]. In clinical practice, the repair method differs among different degrees of craniofacial defects. The development of surgical materials has led to the establishment of autologous tissue transplantation and artificial composite material reparation as routine methods for reparation of craniofacial defects. Autograft surgery for craniofacial defects has been widely used; most such procedures involve the use of a free flap and autologous bone graft. Hydroxyapatite (HA) and implants comprising artificial composite materials are also widely used in the clinical setting.

The cranial and maxillofacial region comprises an intricate bony/cartilaginous structural framework, secretory organs, muscle, and a sophisticated skin/subcutaneous structure, all of which are attached through a vast neurovascular network and numerous ligaments. Because of the limited source of autologous tissue, even the most sophisticated autologous reconstructive technology is insufficient to restore a wide range of maxillofacial defects. Therefore, massive craniomaxillofacial defects caused by trauma, tumor resection, or congenital malformations are still a unique challenge for reconstructive surgeons [9].

In the past 10 years, experimental studies involving facial vascularized allografts have increased the possibility of clinical use of these allografts in patients with disease, trauma, and congenital deformities. This technique may now be considered a viable option for restoration of complex craniofacial defects, the treatment outcomes of which are still not ideal. Vascularized allotransplantation allows for optimal anatomical reconstruction and provides desired functional, aesthetic, and psychological results superior to those obtained by traditional methods. Patients' functional status often recovers, allowing them to make facial expressions such as smiling and to perform various functions such as smelling, eating, drinking, and speaking [6]. With recent progress in oral maxillofacial surgery and immunotherapy, composite tissue allotransplantation now allows for the performance of tissue reconstruction with composites made of all necessary components. However, craniomaxillofacial allotransplantation has some shortcomings, such as the need for lifelong immunosuppression and mismatch between the patient's and donor's skeletal framework and defects, providing suboptimal results [9].

CHAPTER 5

Cell Sheets Engineering And Transplantation in MSCs Regeneration

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Abstract: Transplantation of mesenchymal stem/stromal cells (MSCs) is an emerging treatment for various hard-to-treat diseases. In recent decades, cell sheet technology (CST) has been exploited as a novel promising scaffold-free approach for cell culturing and delivering in tissue regeneration. Results have demonstrated that MSCs facilitate cell viability and function in the CST co-culture system to improve transplantation efficiency. There are extensive interests in developing strategies to enhance the formation of cell sheets with MSC for downstream applications. Novel fabrication techniques have been emerging in recent years, including temperature-responsive system, electroactive responsive system, oxide surface assistant electrochemical system, light responsive system, PH responsive system, magnetite nanoparticles and magnetic force system. This chapter summarizes recent studies in cell sheet engineering and its combination with MSC for tissue regeneration.

Keywords: Cell sheet technology, Co-culture, Mesenchymal stem cell, Micro-patterning, Tissue engineering, Vascularization.

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1. INTRODUCTION

Recent years have witnessed the boosting development of regenerative medicine, which is targeted for functional restoration of damaged tissues with the transplantation of cells, tissues or even organs. In recent decades, substantial progress has been achieved in the realms of cell-based therapies and tissue engineering in regenerative medicine, though challenges still remain. For example, direct injection of single-cell suspensions, the most common method for cell transplantation therapy, cannot avoid huge cell loss in the host tissue site, and the island-like cell aggregation also discounts the healing [1, 2]. In addition, biodegradable scaffolds, one of the main components in tissue engineering, may lead to infiltration of pathological fibrous tissue or extracellular matrix (ECM) during scaffolds degradation [3, 4]. To overcome these issues, novel alternative approaches are needed.

Among all the constructive efforts, Okano and Yamada brought up the methodology named “cell sheet engineering” in the 1990s [5, 6], also referred as cell sheet technology (CST) in this book. CST is relied on the utilization of a temperature-responsive polymer, poly-N-isopropylacrylamide (PIPAAm), as the culture surfaces covalently grafted on conventional culture polystyrene dishes or glass substrates at nanometer level thickness. The hydrophilic/hydrophobic feature of the PIPAAm surface can be reversibly alternated in response to external temperature changes, which leads to thermally modulated cell adhesion and detachment. The spontaneous cell detachment facilitated by PIPAAm-grafted surfaces, enables a non-invasive harvest of cultured cells, in avoid of using trypsin, dispase and collagenase, which could cause cell damage and cell death during conventional cell culture for harvesting. All the cell surface proteins, external cell matrix and cell-cell/cell-ECM interactions are well preserved in the scaffold-free intact monolayer sheet [5, 6]. Furthermore, the collected monolayer sheet can either be directly transplanted to targeting sites straightway or accumulated to produce three-dimensional tissue-like structures with little cell migration in the transplanted site [7]. According to their sizes and shapes cell sheets can be classified into the following types (Table **1**).

There are other cell sheet materials, in addition to temperature-responsive

polymers, in response to external stimuli to trigger cell detachment and promote transfer efficiency. So far the well investigated external stimuli that could influence the features of cell sheet materials include delicate mechanical force [8], pH [9], ionic concentration [10], magnetic force [11, 12], electrochemical polarization [13], and light [14], just to name a few. Temperature-responsive PIPAAm and its derived polymer membrane is the most frequently used in CST due to the safety and simplicity consideration [15 - 18].

Table 1. The advantages and disadvantages of the methods of cell sheet technologies.

Forms	Structure/ cell types	Characteristics	Application
Monolayered Cell Sheet	2D homotypic heterotypic	The basis of CST. Easy to fabricate. Either be directly transplanted to host sites or be further constructed. It is fragile, tends to shrink, wrinkle, clump and break when manipulating. Suitable for thin tissue transplantation without vascularization supply.	skin corneal epithelium bone periodontium urothelium islet
Multilayered Cell Sheet	3D homotypic hoterotypic	Most frequently applied and promising form of CST When MCSs are mounted, ECM deposited trans-sheets quickly. More stable structure, mimic to the real microenvironment with better cell interactions. Suitable for regenerating tissues or organs with high-density. Need vascularization supply.	bone, cartilage, periodontium kidney liver heart, blood vessels skin
Cell Sheet Fragment	up to1mm in diameter	Injectable Easier to transport to some narrow and deep locations with minimized damaging to the structure and the efficacy of cell transplantation is raised.	bone heart periodontium
Cell Sheet Pellet	3D microtissue	Formed by wrapping a cell sheet to a small global structure, which adds mechanical support and reduces instability. While the cellular bioactivity and nutrition supply in CSP for generating large constructs remain an issue.	dental-pulp

CST has been widely used along with various cell types for the regeneration of different types of tissues, including cardiac tissue, bone, tendon, cartilage, liver, corneal, bladder, pancreas, renal, skin, esophageal, blood vessel, and periodontium [4, 19 - 31]. Up to now, several clinical studies have shown curing

CHAPTER 6

New Methods of Scaffold Fabrication: Cell-instructive Scaffolds, Structural Scaffolds, Scaffolds Fabricated from Smart Materials Able to Respond Sensitively to Environmental Cues

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Abstract: In the field of regenerative medicine and tissue engineering, biomaterials are critical for the encouragement and maintenance of biological activities. Scaffolds can be cell-instructive, structural and fabricated from smart materials. Design considerations include the creation of well-defined materials, cell-material interactions, and controlled-release bioactive agents.

Keywords: Cell-instructive, Controlled release, Extracellular matrix, Scaffold.

1. INTRODUCTION

Biomaterials are critical components that encourage and sustain biological activities in regenerative medicine and tissue engineering to provide an appro-

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priate environment for cell behavior. Mimicking the extracellular environment should be considered in cellular scaffold design to render them capable of facilitating cell behavior, such as proliferation, adhesion and differentiation. Scaffolds can be cell-instructive, structural and fabricated from smart materials which could respond sensitively to environmental cues. The fabrication of materials and interactions between cells and materials should be taken into consideration when the materials are designed. Obviously, the interaction of materials with cells or local tissue has important effects in the regeneration process. Knowledge of materials characteristics and cell-material interactions is necessary for guiding cell phenotypes and tissue formation.

2. CELL-INSTRUCTIVE SCAFFOLDS

Scaffolds for tissue engineering should match some physical and biochemical characteristics. Cell-instructive scaffolds that may mimic the extracellular environment offer a favorable environment for the natural behavior of cells. In this review, we discuss fabrication strategies such as spinning and self-assembly techniques to provide well-designed scaffolds, related factors of cell-instructive scaffold for cell behavior, and methods for loading of bioactive molecules.

2.1. Synthesis and Fabrication of Cell-instructive Scaffolds

Both natural and synthetic materials are available for the construction of cell-instructive scaffolds [1]. Natural materials frequently employed include polysaccharides (*e.g.*, alginate, chitosan) and proteins (*e.g.*, collagen, gelatin) with biological activity and biocompatibility [2]. However, natural materials have the disadvantages of rapid degradation rates and high variability, as well as obstacles in sterilization and purification [3, 4]. In contrast, synthetic materials provide control for their physico-chemical properties and can be modified [5]. Moreover, composite materials overcome the limitations of single-component materials that lack the desired mechanical properties or cell–matrix interactions. Kuo *et al.* [6] promoted cell adhesion and viability on poly(lactide-co-glycolide) (PLGA) /chitosan scaffolds with type I collagen. Polyesters such as Poly(ε-caprolactone)(PCL),poly(glycolic acid) (PGA), poly(lacticacid) (PLA)and their copolymers are usually applied in synthetic polymers [7]. However, their acidic

degradation products may result in a strong inflammatory response [8], rendering them unfavorable for cell adherence to the surfaces of polyesters due to their hydrophobicity [9].

Various approaches, such as spinning and self-assembly, have been used in the creation of polymeric fibers for the synthesis and fabrication of scaffolds with a three-dimensional network [10 - 12]. Other techniques have also been introduced for the fabrication of nanofibrous or microfibrous assemblies, such as thermally-induced phase separation, printing, and UV light and photomask application.

2.1.1. Spinning

Nanofiber or microfiber assembly scaffolds have been made by the physical bonding of fibers through spinning, such as wet-, electro- and melt-spinning. As a phase inversion technique, wet-spinning produces microscale polymeric fibers by an immersion precipitation process [13]. Electro-spinning that is able to mimic the physical functions of the extracellular matrix (ECM) has been used to produce polymeric nanofibrous assemblies for tissue regeneration (Fig. **1**). This technique formed fibers with a diameter from nanometers to micrometers by a polymeric solution through a capillary tube to a highly electric field [14,15]. For instance, periodontal ligament cells (PDLSCs) seeded on an electro-spun gelatin membrane showed good attachment and proliferation after 7 days [16]. Recently, live cell electro-spinning was used for the direct preparation of scaffolds with living cells. Jayasinghe and his colleagues [17] developed multiple core-vessel-like structures by the cell electro-spinning technique. This structure was comprised of various cell types around the core. Contrary to the electro-spinning method, melt-spinning is free of solvents and thus avoids the volatility and toxicity issues caused by solvents. Melt-electro-spinning writing (MEW) generates accurate fiber deposition and scaffolds with specific designs and shapes by layer-by-layer fabrication. Scaffolds designed by MEW have been demonstrated to support cell attachment, proliferation and infiltration [18].

CHAPTER 7

Tooth Regeneration: Dentin Regeneration, Periodontal Ligament Regeneration, Dental Pulp Regeneration

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Abstract: A tooth is a compound organ, which is composed of calcified tissues of enamel, dentin and cementum, and a soft connective tissue of dental pulp in which blood vessels and nerves are protected. Periodontal ligaments anchor teeth into the alveolar bone in the jaw to ensure the proper function of teeth. In humans, tooth loss can not only lead to physical and mental suffering, but also affect the aesthetics, which compromise an individual's quality of life and self-esteem. With the development of tissue engineering, regenerating a whole tooth for clinical tooth replacement is now considered to be an acceptable scientific objective. This subject has a number of challenges for investigators over complicated disciplines including biology, dental medicine and biomaterial science. This chapter will summarize the current knowledge related to tooth regeneration, especially focus on dentin regeneration, periodontal ligament regeneration, and dental pulp regeneration.

Keywords: Growth factors, Regulation mechanism, Scaffolds, Stem cells, Tooth regeneration.

1. INTRODUCTION

"A tooth is a compound organ, which is composed of calcified tissues of enamel, dentin and cementum, and a soft connective tissue of dental pulp in which blood

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vessels and nerves are protected". Periodontal ligaments anchor teeth into the alveolar bone in the jaw to ensure the proper function of teeth [1, 2]. Teeth are formed by epithelial–mesenchymal interactions [3]. The mesenchyme originates from neural crest cells and the epithelium originates from pharynx or stomodeum. During early development, the ectomesenchyme of the first brachial arch and the frontonasal process is formed by the lateral and anterior migration of cranial neural crest cells [4]. "Because the interactions between cells and tissues constitute a central mechanism regulating the development of all multicellular organisms, the development of a tooth is a very similar process to that for other skin derivatives such as feathers, scales and hairs" [2]. A thickening of the oral epithelium is the first morphological sign of tooth development. And it yields the vestibular lamina on the vestibular side and the dental lamina on the lingual side. The dental lamina induces the teeth and the vestibular lamina forms a groove between the cheek and the teeth. The dental lamina proliferates greatly in a specific location corresponding to the future teeth. And then oval or round structures known as placodes invade the mesenchyme, and will form the tooth buds. Mesenchyme will start to condense around the buds after the penetration of the buds into the under lying mesenchyme. The mechanism of cell growth and morphogenesis in the following cap stage and bell stage is very self-controlled and complicated, and gives rise to the establishment of the form of the tooth crown in the exact location (Fig. **1**). The dental papilla will be formed by the mesenchymal cells, and eventually will differentiate and form the pulp tissue and the odontoblasts. The enamel-forming ameloblasts and the dentine-forming odontoblasts differentiate terminally during the bell stage of tooth development and both of them are unique to teeth. "This takes place at the interface of the epithelium and mesenchyme and is regulated by interactions between the two tissues" [5 - 7]. Dentin, a bone-like hard tissue, is mineralized by collagenous extracellular matrix which is secreted by the odontoblasts. The mineralization of the enamel, which is the hardest tissue in the body, is directed by the enamel matrix deposited by the ameloblasts. The periodontal ligament including cementoblasts will be generated by the dental follicle that is formed by enamel organ, the outer cells of the condensed mesenchyme around the epithelium. The roots of the teeth develop after crown morphogenesis, and subsequently the teeth erupt into the oral cavity [8].

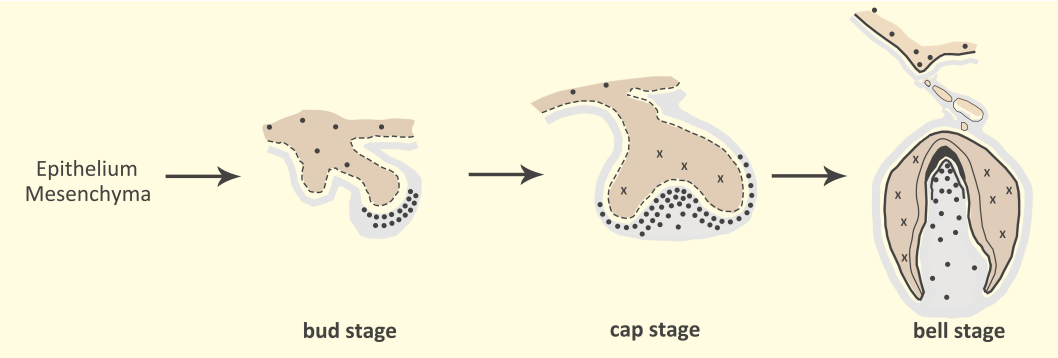

Fig. (1). The stage of tooth.

In humans, tooth loss cannot only lead to physical and mental suffering, but also affect the aesthetics, which compromise an individual's quality of life and self-esteem [4].There are several dental diseases that can eventually give rise to tooth loss. "Dental caries, known as tooth decay or cavity, is one of the most common disorders in humans, second only to common cold. It is an infectious disease primarily caused by bacterial colonies, which breakdown hard tissues of the tooth such as enamel and dentin, as well as soft tissue of the tooth known as dental pulp". Periodontal disease is another major cause for tooth loss. Bacterial plaque damages the periodontal tissues around the teeth and makes it unstable. Resection of oro-maxillo-facial tumors may include the extraction of teeth. In myriads craniofacial anomalies such as cleft palate, teeth can be congenitally missing [5 - 8]. Besides diseases, facial trauma may also lead to tooth loss in both children and adults. In clinical work, it is a great challenge for contemporary dentistry or stomatology to restore dentition defect or deficiency. Nowadays, dentures or dental implants are the most usual ways to restore missing teeth in contemporary dentistry or stomatology. Comparing with dentures, dental implants do not need to cause any damages of the consecutive tooth, and become favorite choices in many countries. However, it is too expensive for a large segment of the population to afford dental implants. And it can fail and will not adapt with surrounding bone that necessarily remodels throughout life, despite being the currently preferred treatment modality [9].

With the development of tissue engineering, regenerating a whole tooth for clinical tooth replacement is now considered to be an acceptable scientific

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