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☐ Review Article ☐

# Tissue Restoration, Tissue Engineering and Regenerative Medicine

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## Abstract

Recently, thanks to the rapid progress of new technologies in cell modulation, extracellular matrix fabrication and synthetic polymers mimicking bodily structures, the self-regeneration of bodily defects by host tissue has been considered by many researchers. The conventional science of art in biomaterials has been concerned with restoring damaged tissue using non-biological materials such as metals, ceramics and synthetic polymers. To overcome the limitations of using such non-viable materials, several attempts to construct artificial organs mimicking natural tissue by combining modulated cells with extracellular matrix-hybridized synthetic polymers have produced many worthy results with biologically functioning artificial tissues. The process involved in manufacturing biomaterials mimicking living tissue is generally called tissue engineering. However recently, the extension of knowledge about cell biology and embryology has naturally moved the focus from tissue restoration to tissue regeneration. Especially, embryonic and mesenchymal stem cells are attractive resources due to their potential for the differentiation of various tissue cells in response to signal transduction mediated by cytokines. Although no one knows yet what is the exact factor responsible for a stem cell's ability to differentiate between specific cells to generate specific tissue, what has been agreed is that delivering stem cells into the body provides a strong potential for the regeneration of tissue. In this review, the historical issues and future possibilities involved in medical tissue restoration and tissue regeneration are discussed.

**Key Words:** Tissue restoration, tissue engineering, tissue regeneration

## INTRODUCTION

Restoration of damaged tissue has long been attempted with medical treatment consisting of the use of drugs and various devices. Such medical devices have been fabricated with materials developed in cooperation with surgical procedures intending to preserve the remaining normal tissue and to replace the diseased tissue with artificially produced prostheses. Ranging from dressing materials that primarily protect against contamination to the body through open wounds during the healing process, to the highly advanced blood compatible materials that are used in producing artificial vessels, these materials have been regarded as playing a secondary role in treatment, after the primary role of surgery, in restoring lost tissue.[1]

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The restoration of defects has usually been limited to the support of mechanical functions for skeletal and circulatory systems or to the replacement of morphological defects for esthetic discomfort. Therefore, the bioinert materials been most important in this field. Metal has been the representative biomedical material, fabricated into various restorative materials for hard tissues, in spite of unavoidable concerns about corrosion and the release of metal ions that produce immune reactions during contact with tissue. Ceramics were introduced to medicine, especially as a restoring material for the skeletal system, in the 1960s and exhibited excellent biologically stable properties. Calcium phosphate is a natural component of hard tissues, and material scientists chemically synthesized various ceramics as defect restoratives or as coating materials for metals to improve their interactions with tissue. But due to the brittle characteristics of ceramics, the physiological load bearing area was contraindicative of their use.[2]

As the field of polymer science and its associated technology has grown, soft tissue restoration increased in scope. Since the first use of polyvinylchloride (PVC) to restore dissected vessels in 1952, synthetic poly-

mers have been regarded as one of the most important biomaterials. The malleable chemical and physical characteristics of synthetic polymers introduced the era of biochemically active materials in medical use. To protect against the formation of thrombosis oriented from blood plasma protein adhering to the polymer surface, many trials have explored the chemical modification of the lumen of artificial blood vessels by hydrophilic-hydrophobic phase segregation and/or grafting protein repellant on polymer surface, and these have made an outstanding contribution to the progress in biomaterials science.[3,4]

Although polymers have proven to be very useful materials, scientists had to consider carefully their interactions with tissues as their use entailed greater life risks than the use of metals and ceramics. And the need for control of polymer deterioration, which gradually occurs following contact with body fluid and which triggers unpredictable host immune responses that can not be avoided since the polymer is synthesized by chemicals, has promoted rapid progress in biocompatibility research. Research in material biocompatibility has proceeded in cooperation with progress in the understanding of cell biology related to the host cellular response to biomaterials.[5] And the better understanding of molecular biology has allowed researchers to modify conventional restorative biomaterials to maintain biologically stable or functional activities by hybridizing tissue components with polymers.[6]

## TISSUE ENGINEERING FOR TISSUE REPLACEMENT

Autograft is the best method for replacing defects, but the problem of deficiency of host donor site has never been overcome. Although the development of immune suppressing agents has expanded the allograft era, the number of donors with perfectly matching recipient HLA is still limited, and the horror of the possible transmission to recipient of a life-threatening virus, such as hepatitis and HIV, is another clinical problem.[7]

As tissue consists of cells, extracellular matrix and ionic body fluid, the concept of tissue restoration with materials mimicking natural tissue has been considered. By modifying conventionally available biomaterials with tissue components including cells, biomaterials can become biological material. Furthermore not only physical restoration, but also the possibility of replacing tissue defects with biochemically functioning materials instead of autograft and/or allograft has been investigated.[8]

Extracellular matrix plays the fundamental role of providing a suitable living environment for cells and maintaining tissue structures. By hybridizing extracellular matrix with polymers, cultured cells can be combined with the materials, and the biological properties of the resultant product are preferred for use as biomimicking material for tissue replacement.[9] A scaffold is the construction of an artificial tissue structure using polymers. Collagen, the main structural protein with cell adhesive properties in mammalians, is the representative extracellular matrix mostly hybridized to polymers. Specific cell adhesive components in extracellular matrix such as glycoproteins like fibronectin, vitronectin and laminin, and peptide sequence like arginine-glysine-aspartic acid (RGD) are also selectively hybridized to the polymer surface to provide increased cell bindings.[10] The cultured endothelial cells adhering onto the lumen of the polymer-made artificial vessel can almost completely prevent thrombosis, because the cell themselves are the cells' lining vascular lumens.[11] Otherwise, the collagen hybridized polymer membrane demonstrates cell proliferative conduction to the collagen layer, and firstly is used as a dermal dressing material promoting natural skin wound healing by proliferating host dermal fibroblasts.[12]

Progress in both biodegradable polymer and cell culture technology has also greatly contributed to the success of artificially engineered tissue. Biodegradable polymers, which are dissolved in the bodily environment by hydrolysis or enzymes, play the role of structure replacement in artificial tissue construction. Synthetic poly lactic acid (PLA) and poly glycolic acid (PGA) are representative scaffold materials utilized in tissue engineering, due to the ease with which their dissolving rate and physical properties may be controlled, and hence volumetric harmonization between the gradual degradation of scaffold polymer and the replacement tissue is achievable.[13]

The discovery of cytokines, which are small molecular peptides, in extracellular matrix has accelerated research in controlling the cell life cycle. In particular, research into the application of growth factors (GFs) during cell culture stimulated the *in vitro* cell ex-

pansion technology.[14] The birth of molecular biology in the late 1970s, provided an enormously valuable contribution to the comprehension of living substances, especially in defining peptide sequences. Gene cloning technology helped biomaterial researchers to modulate cells by replacing DNA in order to provide cellular affinity with the host tissue.[15]

Artificial tissue made by modulated cells cultured *in vitro* or *in vivo,* in or on an extracellular matrix hybridized biodegradable polymer scaffold, with growth factors is currently the state of the art in the production of engineered biomaterial for tissue replacement.

Because the engineered tissue consists of cells and extracellular matrix, preserving it in a ready-to-use condition while still maintaining cellular viability is also a critical problem. Cryopreservation, which is primarily designed to store cellular order, has also been applied to natural or engineered tissues. In general, cells demonstrates no metabolic activity below $-150^{\circ}C$, and they are usually cryopreserved at $-196^{\circ}C$. After thawing, the cells recovers metabolic activity, and this technology has made it possible to supply viable cells and tissues for transplantation.[16]

## TISSUE REGENERATION

Restoration and replacement of dysfunctional tissue have greatly progressed and contributed significantly to surgery in the 20th century. Especially, tissue replacement by engineered tissue is still one field of important research, since the goal of producing perfect artificial tissue with complete physiological function has not been achieved.

However, many researchers have been trying to expand cell manipulation engineering techniques into the field of internal medicine by utilizing the active cell inductive characteristics of growth factors to give self regeneration of damaged tissue, through the deliverance of GFs to the necessary tissue. GFs are frequently used in *in vitro* cell cultures to accelerate cell differentiation without concern for the future of cultured cell, but in the *in vivo* condition, the delivered GF has the potential for promoting not only the normal but also the neoplasmic cells.[17] To avoid this hazard and also to maintain the intensity of GF in tissue, an effective material that conjugates with GF to become a carrier is necessary, and various hybridized biodegradable polymers with extracellular matrix components are under investigation.[18]

Cultured host cell delivery has also been studied, but the difficulty of obtaining cells with differentiation potential has lead researchers to consider allogenic or xenogenic cell delivery. Cell encapsulation by semi-permeable synthetic membrane designed to protect against attack by host immune substances but to permit the transportation of metabolic substances has been investigated.[19] Further, following the first success of embryonic stem (ES) cell culture in 1981, various mice with recombinant genes have been produced.[20,21] Trials in host gene transfection to allogenic or xenogenic cells have been pursued after the historical birth of the gene duplicated sheep "Dolly" in 1997 at the Roslin Institute in Scotland.[22] Dolly demonstrates that researchers have the technology to duplicate an individual by using already differentiated and matured cells. In 1998, Thomson reported a success with human ES cell culture, and this lead to a new method for the regeneration of tissue by delivering cells of multi-differentiation potential with the lowest rate of host immune rejection, in spite of an on-going ethical debate on the morals of harvesting embryo or fetus.[23]

Stem cells have an endless self-renewal and differentiation potential to specific cells in accordance with varying signals. The cells appear during the embryonic and fetal organogenesis process, and even exist in specific adult tissues like bone marrow and epidermis that continuously reproduce cells. Fertilized ovum is a totipotent stem cell that is ready to form the embryo, fetus and placenta. The inner cell mass (ICM) of the blastocyst that later converts into the complete body is called the pluripotent stem cell mass, and the cultured ICM cells are defined as the embryonic stem (ES) cells that can be differentiated into any kind of cells. The pluripotency of the ES cells is evaluated by inserting the cells into the other blastocyst. If the delivered cell is well mixed with the transferred ICM, and presently differentiated to all kinds of tissue cells afterward, it is defined as an ES cell. As the ES cells are free from host immune reaction, because the immune system has not yet been established at that stage, they are recognized as ideal cells for transplantation. But using ES cells is illegal in the field of cell therapy for tissue regeneration due to the ethics of human rights. Therefore, researchers are examining the multipotent stem

cells that are converted from ES cells, that are ready to differentiate to specific tissue cells, and that even appear in adult tissue where continuous new cell supply is necessary. Hematopoietic stem (HS) cell is a progenitor cell of blood cells and lymphocytes, Mesenchymal stem (MS) cell is differentiated to osteoblast, chondrocyte, myocyte, keratinocyte and connective tissue to form stroma.[24] And the recently found neural stem (NS) cells is a progenitor cell of the central nervous system that has the potential to become a neuron or glial cell. Actually, it is preferable to use multipotent stem cells rather than ES cells in the field of cell therapy, because the future of the cells can be predicted, and they can be collected from adult hosts.[25]

However, host stem cell delivery is not practical at present, because large numbers of stem cells are required for transplantation. Therefore, researches is continuing about immortalizing cell lines using retrovirus mediated human gene transfer to porcine stem cell.[26] With the outstandingly rapid advancement of molecular biology in the late 1990s, even the chromosomes released the secret of their gene arrangements in 2000. Understanding the structure of individual genes will certainly promote the development of various ways to achieve the complete self-regeneration of host tissue in the coming 21st century.

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