Microscale Technologies For Tooth Tissue Engineering And Regenerative Endodontics

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Abstract

While widespread advances in tissue engineering have occurred over the past decade, many challenges remain in the context of tissue engineering and regeneration of the tooth. The challenges are scaffold vascularization, control of cellular microenvironment, to direct stem cells fate, modifications of scaffold properties. In this regard microscale technologies offer promising approaches to guide vascular formation and create vascular networks. Control of scaffold features at the micro and nano levels presents new opportunities to control the cellular microenvironment and to direct cell fate. Similarly, the high-resolution modification of scaffold properties by incorporation of growth factors, molecules, and cell ligands can also provide other avenues for the control of tissue development. In this review, we will discuss the recent advances in techniques at microscale level to address the current challenges in dental tissue engineering.

INTRODUCTION

While widespread advances in tissue engineering have occurred over the past decade, many challenges remain in the context of tissue engineering and regeneration of the tooth. Tooth tissue engineering have been hindered by a number of scientific and technical challenges including the ability to precisely control the spatial and temporal features of cellular microenvironment, the lack of materials with desired functional properties, the requirement for large sample volumes, low throughput and slow reaction times. To overcome these challenges microscale technologies presents an alternative to conventional tissue engineering approaches.

Microscale techniques can be used to control scaffold features at the micro and nano levels, to spatially pattern and regulate the cellular microenvironment and to direct stem cell fate. Microscale technologies also offer the ability to culture cells in close proximity, facilitating communication and spacial interaction. Microscale technologies can also be used to create large scale, homogenous arrays of stem cell bodies that facilitates the high throughput evaluation of culture conditions to control stem cells differentiation. Microfluidic platforms, surface micropatterning and 3D scaffolds can be used to control the extracellular microenvironment, such as cell-cell, cell-extracellular matrix (ECM), and cell-soluble factor interactions, for basic biology and tissue engineering studies. This review provides a broad overview of the recent developments in the application of microscale technologies to tissue engineering.

TISSUE ENGINEERING

Langer and vacanti defined tissue engineering as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function. The key elements of tissue engineering are stem cells, morphogens, and a scaffold of extracellular matrix.

DENTAL STEM CELLS

To date, four types of human dental stem cells have been isolated and characterized:

(i) Dental pulp stem cells (DPSCs) (4) (ii) Stem cells from exfoliated deciduous teeth (SHED) (5) (iii) Stem cells from apical papilla (SCAP) (6) (iv) Periodontal ligament stem cells (PDLSCs) (7).

Dental stem cells are multipotent mesenchymal type of stem cells that have the future potential to differentiate into a variety of other cell types including cardio myocytes to repair damaged cardiac tissue following a heart attack (8), neuronal to generate nerve and brain tissue (9), myocytes to repair muscle (10), osteocytes to generate bone (11), chondrocytes to generate cartilage and adipocytes to

generate fat.

SCAFFOLDS

The second component of tissue engineering is a physical scaffold. The scaffold provides a physicochemical and biological three-dimensional microenvironment for cell growth and differentiation, promoting cell adhesion, and migration. Scaffold should be effective for transport of nutrients, oxygen, and waste. It should be gradually degraded and replaced by regenerative tissue, retaining the feature of the final tissue structure.

SCAFFOLDS MATERIALS

The material used as scaffold for tooth tissue engineering are Platelet-rich plasma, bone sialoprotein (12), alginate hydrogel, mineral trioxide aggregate (13), synthetic materials include polylactic acid, polyglycolic acid, and polycaprolactone (14).

MORPHOGEN / GROWTH FACTORS

The third component of tissue engineering is morphogen. Morphogens can be used to control stem cell activity, such as by increasing the rate of proliferation, inducing differentiation of the cells into another tissue type, or stimulating stem cells to synthesize and secrete mineralized matrix. A variety of growth factors have successfully been used for dentin-pulp complex regeneration, including Transforming Growth Factors (TGFs) (15), Bone morphogenetic proteins (BMPs) (16), Platelet-derived growth factor (PDGF) (17), Insulin-like growth factor (IGF) (18).

The tissue engineering approach commonly utilizes a cell-seeded scaffold to guide and support tooth formation, while the developmental or “organotype” approach facilitates development of a tooth from a collection of cells resembling the tooth germ. Recent advances in the understanding of tooth development, cellular interaction, and signaling, as well as some extraordinary experimental results, all suggest that the generation of biological tooth replacements may be possible.

MICROSCALE TECHNOLOGIES FOR TOOTH TISSUE ENGINEERING

In typical tissue engineering approaches, cells are seaded onto a 3D biodegradable scaffold. As cells deposit their own matrix, the scaffold degrades, resulting in the formation of a biological tissue construct. Critical limitations with present tissue engineering techniques include the inability to create vascularized tissue constructs, the insufficient mechanical strength of engineered tissues and lack of suitable source of functional cells that are immunologically compatible with the host.

One of the most significant obstacles to overcome in creating replacement pulp tissue for use in regenerative endodontics is to obtain progenitor pulp cells that will continually divide and produce cells or pulp tissues that can be implanted into root canal systems. Possibilities are the development of an autogenous human pulp stem cell line that is disease and pathogen free. Microscale technologies can be used to produce relatively reproducible stem cell aggregates for evaluation (19). This is particularly desirable, because the development of artificial stem cell environments or ‘niches’ may be an effective means to differentiate stem cells efficiently and reproducibly into a variety of lineages for tissue engineering (20, 21). Fabrication of micro-bioreactor array that provide myriad functionalities to monitor and control cell growth are technologies that will likely advance the field (22). Also, reproducible cellular patterning, patterned co-cultures and control of the microenvironment over large areas permit arrays of cell constructs to be assessed in a high-throughput manner (23, 24).

Microscale technologies are becoming increasingly used as tools for the development and investigation of tissue regeneration, where spatial control of cells is of primary interest (25). Cell-laden, microfabricated scaffolds provide the means to bring cells, potentially of different origins, together so that they can communicate and interact during tissue formation and maturation, much as they would during embryonic development. Such cell-cell interactions and repeated temporal signaling are known to be important for the development and maturation of a tooth, making such approaches of interest to tissue engineering (26).

Tooth-like tissues have been generated by the seeding of different cell types on biodegradable scaffolds (27). While promising, the overall size of most tissue-engineered constructs is small (1–2 mm) and does not mimic the 3D complexity of the adult human tooth. The reason was that with non-vascularized scaffold structures, diffusion of nutrients and metabolites is generally limited to the periphery. Microscale technologies that support the development of suitable in vitro environments and scaffolds with appropriate microstructures to facilitate vascularization may be of benefit for both the in vivo and in vitro development of sizeable tooth-like structures

Microfabrication has been increasingly used to fabricate tissue-engineered scaffolds with micro-engineered capillary beds (28). Encapsulated cells in such structures remain viable by diffusion of oxygen and nutrients from micro- and nanochannels thus providing evidence that microfluidic channels can support cells in tissue-engineered constructs (29). Also, collagen scaffolds reinforced with biomimetic hydroxyapatite crystals with micro-channels have been fabricated. The ability to pattern scaffolds and create microchannels in the construct permits the development of 3D structures with the potential for rapid vascularization or fluid exchange.

Photolithography is technique used to create microscale features in scaffolds. In this approach, a light-sensitive solution is selectively exposed to light by means of a photomask. The exposed solution undergoes a polymerization or crosslinking reaction, and the unpolymerized solution can subsequently be washed away. Such approaches can be used to pattern substrates in 2D or can be layered to achieve structures with a 3D architecture, useful for the generation of tissue-engineered scaffolds or micro-channels to support vascular ingress (30, 31). Soft lithography has been used to “print” or mold surfaces with chemical and topographical patterns (at resolutions as low as tens of nanometers), as well as to pattern cells selectively rapidly, and inexpensively (32).

Another microscale technique is a three-dimensional cell printing technique (33). This technique can be used to precisely position cells, and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. In this technique, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The subsequent stacking and printing layer recreates the full structure of the desired object.

SUMMARY

The application of microscale technologies will likely help to advance the technology and knowledge associated with dental tissue regeneration. Microscale technologies are likely to advance scaffold development and increase stem cell sources for dental tissue regeneration. High-throughput tools have been developed to facilitate the rapid screening and optimization of biomaterials for dental tissue regeneration. Similarly, high-throughput techniques have been used to evaluate stem cells and their responses to numerous conditions in a manner directly applicable to the regeneration of dental tissues.

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