Tissue Engineering In Endodontics

Anil Dhingra,¹ Shalya Raj²

Introduction

Science has presented us with a hope called ‘stem cell research’ which may provide us with answers which have so long been beyond our grasp. The stem cells are the powerful and unique cells that can multiply several times and depending on the surrounding environment can form specific desired tissue or organ. This has the potential to provide solutions for several incurable diseases or injuries.

Along with the correct concentration of scaffold and growth factors, stem cell can do wonders for the treatment options available for the patient. Tissue engineering in Endodontics is both exciting and promising and the future would only see an increase in this interdisciplinary field.

KEYWORDS: Stem Cells, Tissue engineering, Growth Factors, Endodontics

Abstract

Tissue Engineering is one of the most fascinating areas of contemporary biology. Research in this field continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cells are the cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells. They have the potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive.

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Introduction

Science has presented us with a hope called ‘stem cell research’ which may provide us with answers which have so long been beyond our grasp. The stem cells are the powerful and unique cells that can multiply several times and depending on the surrounding environment can form specific desired tissue or organ. This has the potential to provide solutions for several incurable diseases or injuries.

Stem cell research is directed towards perfecting the art of using one’s own cells to repair that part of the body which has become affected due to a particular disease. The stem cells by virtue of their properties play an important role in the normal development of the organs, the system and normal day to day repair. One of the major sources of stem cells which are not only economical, procedurally safe, controversy free and ease of harvesting is the dental stem cells. The stem cells can now be harvested, made to multiply, stored and utilized whenever required in future. This is similar to the practice of banking where one deposits money, multiplies it, stores it and hence the term ‘stem cell banking’.

The term “tissue engineering” was coined at a National Science Foundation (N.S.F.) bioengineering meeting in Washington D.C., in 1987. At a subsequent N.S.F. sponsored workshop, it was formally defined as “the application of principles and methods of engineering and life sciences, to obtain a fundamental understanding of structural and functional relationships in novel and pathological mammalian tissues, and the development of biological substitutes to restore, maintain or improve tissue function” (Shalak & Fox, 1988).
To successfully achieve this goal, the scientist/researchers need:

Tooth-related cells from each patient, preferably isolated stem cells from the corresponding teeth tissues that are endowed with Odontogenic potential. Culture techniques that permit a fast expansion that would yield the needed cell quantities to condition the microenvironment and allow for cell-cell interactions that would lead to purposeful cell differentiation. An adequate microenvironment supporting 3D tooth-like growth, leading to the production of a genuine tooth-like bud or even a tooth replica.

STEM CELLS

Murray PE et al. (1) in 2007 defined stem cells as, “a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues”.

Bluteau G et al (2) in 2008, defined stem cells as, “a cell that can reproduce unaltered daughters and, furthermore has the ability to generate cells with different and more restricted properties”.

Stem cells differ from other kinds of cells in the body. All stem cells, regardless of their source, have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; they can choose to become one of the many different types of cells present in the body based on signals from their environments. Unspecialized stem cells can give rise to side specialized cells including heart muscle cells, blood cells, or nerve cells. They do this by coordinating their gene expression in an elaborate and complex patterning many generations of cells.

Adult stem cells are quiescent, slow-cycling, undifferentiated cells, which are surrounded by neighboring cells and extracellular matrix. Many adult tissues (such as the bone marrow, brain and gut) contain stem cells. Like ES cells, adult stem cells can make identical copies of themselves for long periods of time (self-renewal). At the same time, they can give rise to mature cell types that have characteristic shapes and side specialized functions. Stem cells typically generate an intermediate cell type(s) before they achieve their fully differentiated state. The intermediate cell is called a progenitor cell. Progenitor cells are partly differentiated cells in the sense that they are committed to a particular cell lineage and, upon division, and give rise to differentiated cells.

PROPERTIES OF THE STEM CELLS

Plasticity:
• 'Plasticity of the stem cells has been defined as the ability to produce cells of different tissues' (1).

• Stem cells are divided into totipotent, pluripotent and multipotent according to their plasticity. Embryonic stem cells show a greater plasticity hence is more valuable for developing new therapies.
• Undifferentiated cells which can respond to appropriate signals.
• Ability to self replicate.
• Maintain the multiple differentiations potential.
• Proliferation properties.

SOURCE OF THE CELLS

1) Autologous cells (the host's own cells)
2) Allogenic cells (cells from a donor)
3) Xenogenic cells (cells from a different species)
4) Stem cells: either allogenic (fetal or adult derived) or autologous (adult derived).

It is often assumed that the use of autologous cells implies minimal manipulation and maximum safety for the host because of the use of the host's own cells. This is not entirely correct as culture processes and reagents can alter cells, regardless of their origin.

When allogenic cells are used, the potential for immune response and disease transmission must be considered.

Xenogenic stem cells have been isolated by Johara K et al. (3) in 2006 and Cheng PH et al. (4) in 2008 from porcine and chimpanzees respectively.

Association of Epithelial and Mesenchymal Stem Cells

Since teeth are formed from two different tissues, building a tooth logically requires the association/cooperation of odontogenic mesenchymal and epithelial cells. The recombination of dissociated dental epithelial and mesenchymal tissues leads to tooth formation both in vitro and in vivo.

SCAFFOLDS

Tissue engineering inherently involves recreation of a 3-dimensional(3-D) tissue structure from a source of cells derived from an endogenous source in the patient (e.g. bone wound healing) or from an exogenous source like a donor (e.g. skin). Biomaterials (scaffolds) are used to guide the organization, growth and differentiation of cells in the process of forming functional tissue and provide both physical and chemical signals. The interaction of the scaffold with stem cell population is of great importance in guiding the size shape and differentiation of bioengineered dental tissues.

These provide a physiochemical and biological three dimensional micro environments for cell growth and differentiation, promoting cell adhesion and migration and serve as a carrier for morphogens and delivery vehicle to the specific sites in the body. It should be effective for transport of nutrients, oxygen and waste products. A Scaffold matrix should be highly porous with
an adequate pore size to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients and should be as biocompatible as much possible to reduce tissue toxicity. Biodegradability is an essential requirement as it should not require surgical excision. It should gradually degrade and be replaced by regenerative tissue, retaining features of the final tissue structure. The rate of degradation of scaffold matrix and new tissue formation should coincide as much as possible to achieve successful regeneration.

Scaffolds should be able to support and guide the cell growth and the development of new tissues. The matrix should be able to withstand forces and maintain a potential space for tissue development and should provide a controlled vehicle for gene and protein delivery. A large surface area to volume ratio is desirable to allow delivery of high density cells. It should encourage cell migration and differentiation. The matrix should allow rapid colonization by cells of the desired phenotype and also neovascularisation once implanted into the defect. The matrix can also serve as a regulator of cell function through its interaction with other cell receptors. These may include antibiotics to prevent bacterial growth.

GROWTH FACTORS

Growth factors have been described by Murray PE, Gracia-Godoy F and Hargreaves KM in 2007(1) as proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. Many growth factors are quite versatile, simulating cellular division in numerous cell types, while others are more cell specific.

The growth factors that play an important role in endodontic regeneration are:

- Platelet derived growth factor (P.D.G.F.),
- Insulin-like growth factor (I.G.F.),
- Transforming Growth Factor- β (T.G.F.-β),
- Fibroblast Growth Factor,
- Bone Morphogenetic Proteins (B.M.P.s).

1) PLATELET DERIVED GROWTH FACTOR (PDGF):

FORMS:Consequently, PDGF can exist either as a heterodimer (AB) or as a homodimer (AA, AB). These 3 isoforms of PDGF have unique binding properties for PDGF receptor sub-units, α and β, found on the cell membrane.

EFFECTS: The primary effect of PDGF is that of a mitogen- initiating cell division. Thus, it has been characterized as a competence factor, i.e. a growth factor that makes a cell competent for cell division. A progression factor, such as I.G.F.-1, is then necessary to induce mitosis. PDGF also causes replication of endothelial cells, causing budding of new capillaries (angiogenesis).

2) INSULIN-LIKE GROWTH FACTORS (IGF-I, II):

- They are peptide growth factors with biochemical and functional similarities to insulin.
- Bone cells produce and respond to IGF’s, and bone is a storage house for these factors in their inactive form.
- They are mitogens, and in fibroblastic systems appear as progression factors. In bone cell systems, they stimulate both proliferation of pre-osteoblasts, as well as the differentiation of osteoblasts, including Type I collagen synthesis. Thus, IGF increases both the number of cells synthesizing bone, as well as amount of extracellular matrix deposited by each cell.

3) TRANSFORMING GROWTH FACTOR-β:

- It is a multifactorial growth factor, structurally related to B.M.P.s, but functionally quite different. - It has been shown to be chemotactic for bone cells, and may increase or decrease their proliferation depending upon the differentiation state of the cells, culture conditions and concentration of TGF-β applied.- In-vivo, it produces new cartilage and / or bone, if injected in proximity to bone; however, it does not induce new bone formation when implanted away from a bony site.- In spite of its effects on the augmentation of bone, no positive data have been reported on in-vivo healing in a periodontal setting.

4) FIBROBLAST GROWTH FACTORS:

- They are family of at least 9 related gene products of which 2 major members are acidic FGF (α-FGF or FGF-1) and basic FGF (b-FGF or FGF-2).
- It can stimulate endothelial cells and periodontal ligament cell migration and proliferation, as well as stimulation of bone cell replication.- b-FGF is more potent than α-FGF and may act via stimulation of other growth factors like TGF-β.

5) BONE MORPHOGENETIC PROTEINS (BMPs):

Bone morphogenic proteins are secreted by the oral epithelium layer (oral ectoderm) during early stages of odontogenesis. (Dental lamina).

HURDLES

Manufacturing concerns: For tissue engineering to help alleviate clinical problems, it is necessary for tissue-engineered products to be manufactured reliably. This need is almost self-evident, but worthy of emphasis.

Ethical concerns: There are at least two major ethical concerns related to tissue-engineered products. The first, tissue procurement, for many tissue-engineered products (such as skin equivalents and bioartificial organs), viable cells are an essential component. Unless a patient’s own cells can be amplified in an adequate and timely manner, enabling them to be used in the tissue-engineered device (that is, a cell autograft), then cells must be derived from another tissue.
Another risk is that an animal (in this case, porcine) virus might successfully overcome the human species barrier, perhaps mutate, and result in a serious human disease.

Later Yelick and Vacanti in 2006(5) classified the existing challenges into two broad categories, Identification and characterization of suitable dental progenitor cell populations and the development of methods to reproducibly manipulate dental progenitor cells to bioengineer dental tissues and whole teeth of predetermined size and shape in a timely fashion. Secondly the formation of the root of a bioengineered tooth crown.

Nor JE in 2006 (6) stated that the several challenges before a successful functional dental pulp can be effectively and safely engineered in patients:

1. A clinically feasible way of delivering the scaffold (with or without cells) needs to be found, along with morphogenic signals to the root canal and pulp chamber.
2. A strategy for inducing angiogenesis will need to be developed and characterized. The engineered pulp will have to be vascularized to allow the survival of the cells and sustainability of the pulp tissue over time.
3. The correct morphogenic signals, as well as the timing and sequence of their use, will have to be identified.

Nakashima M and Akamine A in 2005 (7) described the importance and need for vascularization and a good innervations for the tissue engineered or even a healthy pulp for that matter. They explained that the vascular system in the dental pulp plays a role in nutrition, oxygen supply and as a conduit for removal of metabolic waste. The innervation of pulp plays a critical role in the homeostasis of the dental pulp. Invasion of immune and inflammatory cells into sites of injury in the pulp is stimulated by sensory nerves. Growth factors like bone morphogenic protein and vascular endothelial growth factor have shown encouraging results in the nervous regeneration and angiogenesis. The increasing interest in tissue engineering of tooth must take in account neuro-pulpal interactions and nerve regenerations.

CONCLUSION

Stem cell research and scaffolding are now the buzz words in the basic science pulp researches. The endodontic community needs to thus enhance its clinical understanding of the vital pulp and dentin and embrace new treatment modalities.

Several regenerative techniques have been described, proposed therapies involving stem cells, growth factors and scaffolds. Each technique has its own advantages, disadvantages, some techniques still in a hypothetical stage or in an early stage of development. The regenerative therapies will revolutionize the future endodontics with the synergistic confluence of advances in signaling pathways underlying morphogenesis and lineage stem/ progenitor cells by morphogens such as BMPs and synthetic scaffolds.

As we enter an exciting and a promising new era where the diverse fields of tissue engineering, material science, nanotechnology and stem cell biology have converged synergistically to provide unprecedented opportunities to characterize and manipulate signaling cascades regulating tissue and organ regeneration.

References

1. Murray PE, Godoy-Gracia F. The outlook for implants and endodontics: A review of the tissue engineering strategies to create replacement teeth for patients. DCNA 2006 Apr;50:299-315


5. Yelick PC, Vacanti JP. Bioengineered teeth from tooth bud cells. DCNA 2006 Apr;50:191-203.

