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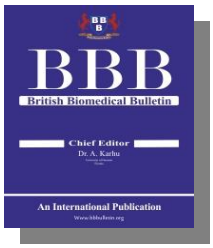
BRITISH BIOMEDICAL BULLETIN



Review Article

Regeneration, Stepping into the Future of Dentistry

Suneeth Shetty*and sunil tejaswi

Dept of conservative dentistry & Endodontics, J.S.S dental college & Hospital, JSS university, Mysore India

ARTICLE INFO

Received 02 April 2016
Received in revised form 16 May 2016
Accepted 25 May 2016

Keywords:
Stem cells,
Scaffold,
Bioreactors.

Corresponding author:
Dept of conservative dentistry & Endodontics, J.S.S dental college & Hospital, JSS university, Mysore India.
E-mail address: s_suneeth@yahoo.com

ABSTRACT

During the last century we have realized that science is the fuel for the engine of technology. Recent advances in scientific and molecular biology have led to a better understanding of biological processes. This is particularly evident when considering the opportunities to understand the etiology, pathogenesis, treatments and outcomes related to dental diseases and disorders. Today, the field of tissue engineering plays a vital role for the development of neotissues for transplantation.

Introduction

Tissue engineering represents the combined efforts of three different fields: the worlds of clinical medicine, engineering and science. Each year billions of dollars are spent on treating Americans suffering some type of tissue loss or end-stage organ failure, these includes 20,000 organ transplants, 500,000 joint replacements, and millions of dental and oral craniofacial procedures, ranging from tooth restorations to major reconstruction of facial soft and mineralized tissues. The regeneration or replacement of oral tissues affected by inherited disorders, trauma, infectious diseases is expected to solve many dental problems. Within the next 25 years, unparalleled advances in dentistry are set to take place, with the availability of artificial teeth, bone, organs, and oral tissues; as well as the ability to stimulate endodontic regeneration.[1,2]

Definition of 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",

MacArthur and Oreffo defined tissue engineering as "understanding the principles of tissue growth, and applying this to

produce functional replacement tissue for clinical use."

Why tissue engineering is necessary?

- Most tissue cannot regenerate when injured or diseased
- Even tissues that regenerate spontaneously may not completely do so in large defects (e.g. bone)
- Replacement of tissue with permanent implant is very limited

Preclinical and clinical accomplishments of tissue engineering in dentistry

It can be categorized into 3 categories –

- Conductive approaches
- Inductive approaches
- Cell transplantation

Conductive approaches

Conductive approaches utilize biomaterials in a passive manner to facilitate the growth or regenerative capacity of existing tissue.

Inductive approaches

This involves activating cells in close proximity to the defect site with specific biological signals. Urist first showed that new bone could be formed at non-mineralizing, or ectopic sites after implantation of powdered bone (bone demineralized and ground into fine particles). Contained within the powdered bone were proteins (BMPs), which turned out to be the key elements for inducing bone formation. These proteins are now available in recombinant forms and produced on a large scale by biotechnology companies.

Cell transplantation

This approach involves direct transplantation of cells grown in the laboratory. The transplantation of cells grown in the laboratory provides another inductive means to engineer new tissues. Cell transplantation is extremely attractive when inductive factors are not known for a specific tissue, when a large tissue mass or organ is needed, or when tissue replacement must be immediate. However, this approach requires the needed cells to be expanded in the laboratory.

Currently literature describes three general approaches. These principles are closely related to each other and may be applied to create new tissues.

These approaches include:

1. Design and grow human tissues *in vitro* for later implantation to repair or replace diseased tissues: The most common example is the skin graft, used for the treatment of burns. Skin graft replacements have been grown in tissue culture and used clinically for more than 10 years – *in vitro*
2. Implantation of cell-containing or cell-free devices that induce the regeneration of functional human tissues: "signal" molecules, e.g. growth factors may be used to assist in biomaterial-guided tissue regeneration. Also novel polymers have been created and assembled into three-dimensional configurations, to which cells attach and grow to reconstitute tissues. An example is the use of a polymer matrix to form cartilage – *In-vivo*
3. The development of external devices containing human tissues designed to replace the function of diseased internal tissues: This approach involves establishing primary cell-lines, placing the cells on or within structural matrices and implanting the new system inside the body. Examples of this approach include repair of bone, muscle, tendon and cartilage, endothelial cell-lined vascular grafts and heart valve substitutes. – *Ex-vivo* (Fig. 2)

Application of tissue engineering in dentistry

The effect that tissue engineering may have in the field of dentistry stems from its wide- spread application to many different types of tissues related to the oral cavity, including bone, cartilage, skin and oral mucosa, dentin and dental pulp, and salivary glands.

- Bone –
  - ❖ Replacing bony defects using autografts, allografts, and synthetic biomaterials.
  - ❖ Guided tissue regeneration (GTR) after periodontal surgery
  - ❖ Construction of the entire mandible

- Cartilage –
  - ❖ Cartilage destruction is associated with trauma and a number of diseases including degenerative articular cartilage destruction at the temporomandibular joint.
  - ❖ New cartilaginous tissue with precisely defined sizes and shapes relevant to maxillofacial reconstruction (e.g., nasal septum, temporomandibular joint) can be engineered using appropriate biodegradable scaffolds for transplanting the cells.

- Skin and oral mucosa –
  - ❖ The most successful application of tissue engineering to date is the development of skin equivalents. Skin tissue is needed in adjunctive esthetic treatment of individuals who are severely disfigured following severe burns, in radical resective surgery to treat invasive cancers, and for major trauma wounds.
  - ❖ A similar approach has also been developed for the replacement of oral mucosa, although this procedure has not yet been marketed.
- Salivary glands –
  - ❖ The challenges in this field include replacement of complete organs, and significant progress has been made in efforts to engineer salivary gland function.
  - ❖ One method in treating salivary gland functional deficiencies makes use of an inductive gene therapy approach. The aim in this approach is to make existing non-secretory ductal epithelial cells (following irradiation therapy) into secretory cells capable of fluid movement. (Fig. 2)

Key elements of tissue engineering[4,5]

The key elements of tissue engineering are stem cells, morphogens, and a scaffold of extracellular matrix.

Adult Stem/Progenitor Cells

Adult stem/progenitor cells reside in a variety of tissues. Adult stem cells have unique characteristic;

(a) They are undifferentiated cells and maintain this phenotype by the environment and/or the adjacent cell populations until they are exposed to and respond to the appropriate signals,
(b) They have an ability to self-replicate for prolonged periods,
(c) They maintain their multiple differentiation potential throughout the life of the organism

Progenitor cells retain the differentiation potential and high proliferation capability, but have lost the self-replication property unlike stem cells.

Scaffold

The scaffold provides a physicochemical and biological three-dimensional micro- environment for cell growth and differentiation, promoting cell

adhesion, and migration. They serve as a carrier for morphogen in protein therapy and for cells in cell therapy and 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. They should have biocompatibility, non toxicity, and proper physical and mechanical strength.

Commonly used synthetic materials are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers, poly(lactic-co-glycolic acid) (PLGA). Synthetic hydrogels include poly(ethylene glycol)(PEG) based polymers, and those modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acid (RGD), can improve cell adhesion and matrix synthesis within the three-dimensional network. (Table 1)

Stem cells in dentistry

They are building blocks of the human body. In the embryo they will eventually give rise to every cell, organ and tissue in the fetus's body. Unlike a regular cell, which can only replicate to create more of its own kind of cell, a stem cell is pluripotent i.e. when it divides, it can make any one of the 220 different cells in the human body.[6]

Use of the term *"Stem cell"* dates back at least to William Sedgwick, who used it to describe the regenerative properties of plants in 1886.All tissues originate from stem cells. A stem cell is commonly defined 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.

Properties of stem cells

Stem cells differ from other kinds of cells in the body. All stem cells regardless of their source have three general properties:

i. They are capable of dividing and renewing themselves for long periods
ii. They are unspecialized
iii. They can give rise to specialized cell types

i) Stem cells are capable of dividing and renewing themselves for long periods

Stem cells may replicate many times. They are not like other cells such as muscle cells, blood cells, or nerve cells, which do not replicate themselves. When cells replicate themselves many times over, it is called "Proliferation". If the resulting cells continue to be unspecialized, like the parent stem cells, then these cells are said to be capable of long term self renewal.

ii) Stem cells are unspecialized

Stem cells do not have any tissue-specific structures that allow it to perform specialized functions. A stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell); it cannot carry molecules of oxygen through the bloodstream (like a red blood cell); and it cannot fire electrochemical signals to other cells that allow the body to move or speak (like a nerve cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

iii) Stem cells can give rise to specialized cells

When unspecialized stem cells give rise to specialized cells, this process is called as "Differentiation". Cell differentiation takes place by triggering of two signals:

1. Internal signals
2. External signals

A "Cell genes" control the internal signals.

The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and through certain molecules in the microenvironment. (Table 2)

Morphogens/ growth factors

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. They can either stimulate cellular division in numerous cell types, or be cell specific. Currently, a variety of growth factors have been identified, with specific functions that can be used as part of stem cell and tissue engineering therapies. They can be used to control stem cell activity, 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. Growth factors, especially those of the transforming growth factor- beta (TGF) family, are important in cellular signaling for odontoblast differentiation and stimulation of dentin matrix secretion.

Fibroblast growth factors(FGF)

Are members of at least 9 related gene products. Named for its general growth-promoting effects on most fibroblastic cell types, it also stimulates angiogenesis, wound healing and cell migration. Studies on the effects of fibroblast growth factor on individual cell types have shown that it can stimulate endothelial cell differentiation of odontoblast and periodontal ligament cell migration and proliferation .*In vivo*, FGF has shown to increase bone formation and rate of fracture repair.

Platelet Rich Plasma (PRP)[9]

The highest concentrations of PDGF and TGF β is found within blood platelets. PDGF and TGF β are present at a concentration of about 50mg/ml of whole blood sequestered within the platelets. Marx and co-workers demonstrated that platelet concentrations could be increased from an average of 232,000 to 785,000. This concentrated gel known as platelet rich plasma (PRP) has been used to treat periodontal and peri-implant defects. It has been used in conjunction with osteoconductive bone substitute, which acts as a carrier.

Advantages

1. The local concentration of cell signaling molecules, including tissue growth factors increased within the wound by use of PRP. This elevation in the growth factors enhances bone formation.
2. The use of autogenous bone graft provides the scaffolding.
3. The use of autogenous bone grafts also increase the population of cells known to be responsive to the PDGF and TGF released from the platelets.

Scaffold

Scaffolds provide a template to introduce the progenitor Mesnchymal Stem cells to the specific site of interest and to provide interim mechanical stability for tissue growth and integration.

The role of the scaffold in tissue engineering is to provide a matrix of special configuration on which seeded cells may grow to produce the desired tissue or organ.

Biomaterials used as scaffolds are broadly classified into two categories – naturally derived and synthetic. Advantages of naturally derived scaffolds include the ability to support cellular invasion and proliferation. Synthetic materials offers ease of processing and mechanical strength.

Biomaterials can also be divided into ceramics and polymers. These biomaterials may be produced into solid blocks, porous sponges or foams or hydrogels.

Over the last decade there has been significant interest in biocompatible biodegradable scaffold materials including synthetic biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), copolymers of PLA and PGA (PLGA), polycaprolactone and natural polymer gels such as hyaluronic acid (HA), fibrin, alginate, collagen and agarose hydrogels.

In addition to the various polymers used as scaffold materials, there are also many different shapes, sizes and structural forms of 3D scaffolds including meshes, foams, and sponges.

Cell seeding

The transfer of cells onto the scaffolds or carriers is done by a process known as cell seeding.

Cell Seeding can be done by various methods

1. Static seeding: cells and scaffolds are brought into direct contact and the cells allowed to grow undisturbed.
2. Spinner flask: commonly used method in which the scaffold is suspended in a stirred suspension of the desired cells. This produces favorable results but has a distinct disadvantage of being time consuming and less efficient at low concentration of cells.
3. Centrifugal seeding: by application of centrifugal force which allows the transfer of cells onto the scaffold. This method has superior seeding efficiency with better cellular distribution within the scaffold and requires lesser time than other methods.

Bioreactors

Bioreactors may refer to any device or system that supports a biologically active environment

The term “bioreactor” refers to a system in which conditions are closely controlled to permit or induce certain behavior in living cells or tissues. The fact that physical stimuli can modulate cell function and tissue development has motivated the development of biomechanically active simulation systems to recellularise tissues *in vitro* by exposing them to physiologically relevant mechanical and/or hydrodynamic stimulation. The primary objectives of these systems are to establish spatially uniform cell distributions on three dimensional scaffolds, to maintain desired concentrations of gases and nutrients in the culture medium, and to expose developing tissue to appropriate physical stimuli.

The requirements will vary depending on the dimensions, complexity, and physiological environment of the tissue to be engineered. The overall goal is to have systems that reliably and reproducibly form, store, and deliver functional tissues that can sustain function *in vivo*

Tooth regeneration using stem cell therapy

Stem cells are capable of differentiating into several different types, they are attractive for the tissue engineering of complex organs or structures. The concept of using stem cells for dental tissue engineering was explored by Paul Sharpe and his research group. They demonstrated that it is possible to engineer teeth of normal size and structure using stem cells. The same group has also demonstrated that adult stem cells of non-dental origin can be used to engineer teeth.

The discovery of dental stem cells in the pulp tissue of permanent teeth and also in primary teeth raises the exciting

possibility of retrieving these cells, expanding them in culture and seeding them in biodegradable scaffolds. These cells have 2 characteristics that make them attractive for dental tissue engineering –

- Viability of dental pulps from patients who need teeth replacement. Stem cells can be retrieved and isolated from pulp with relatively minimal morbidity, especially in primary teeth.
- Dental pulp stem cells are pluripotent cells, i.e., they have the capability of differentiating into most, if not all, cells that give rise to tooth structures.

Human embryonic stem cell lines (hESC) approved for use by the National Institutes of Health, adult mesenchymal stem cells and human epithelial stem cells (hEpSC) would be surveyed with the goal of identifying the optimal stem cell population for production of the regenerated tooth.

However the challenges in the field are-

- The identification and characterization of suitable dental progenitor cell populations that can be obtained easily and used for autologous regenerating procedures.
- 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.

Harvesting teeth created by tissue engineering[7,8]

The ability to create in the laboratory teeth that can be harvested and implanted into patients to restore extracted or lost teeth long has been a goal for dental research. The future creation of replacing teeth for patients involve a chair-side technology with the following process:

- The first step is to create a computer-aided biomodel of the oral cavity and analyze the aesthetics of existing teeth.
- The second step is to use a database of tooth sizes, shapes, and aesthetics as a blueprint for designing a replacement tooth.
- The third step is to biomanufacture the tooth using a scaffold and three-dimensional cell pattern printing and deposition methods. Slabs of biosynthetic enamel and dentin are cut into the shape of the tooth.
- The forth step is to implant the tooth surgically into the patient and reconnect blood flow, nerves, and periodontal ligaments.

Much of this technology already exists or is close to development, and the goal for dental researchers is to put the technology together and make it work reliably.

There are four stem cells which are of interest they are SHED, adult dental pulp stem cells (DPSCs), stem cells from apical papilla (SCAP) and periodontal ligament stem cells (PDLSCs).[14,16]

Regenerative dentistry: Making dentin

It is known that the currently available materials do not mimic all the physical, mechanical and esthetic properties of the enamel and dentin. Whenever the restorations are done, patients frequently return either with restorative or tooth fracture that may lead to the need for tooth extraction. Such patients might benefit from dentin regeneration and the strengthening of tooth structure, if such a therapeutic strategy were available. Much research has been done in the area of biological inducers of dentin mineralization, and the following is a brief summary of the work of many investigators. Early work on the biological induction of dentin was inspired by a

seminal paper by Urist, which demonstrated, for the first time, that demineralized bone powder had inductive potential and led to ectopic bone formation.[12,13]

Regenerative dentistry: Making Enamel[10]

If there is pulp exposure, the undifferentiated mesenchyme from the pulp differentiate and forms odontoblasts and form dentin. In contrast, amelobalsts are no longer present in teeth with complete crown development. Therefore, an in situ cell-based strategy to regenerate enamel is not feasible.

However with the use of tissue engineering and nanotechnology the scientists have been able to develop synthetic enamel with fluoride. Non-carious enamel is a highly organized structure made of enamel prisms consisting of bundles of nanorod-like calcium hydroxyapatite crystals arranged roughly parallel to each other. The prisms can be considered micro architectural units of enamel. Surfactants which may mimic the biological action of enamel proteins in enamel development can be used as reverse micelles or microemulsions to synthesize nanoscale structures that may self-assemble into "one-dimensional building blocks. The resulting hydroxyapatite nanorods are similar in size and composition to natural enamel crystals.

Regenerative dentistry: Making dental pulp.[19]

Maintaining dental pulp vitality is an underlying goal of most restorative procedures. The pulp necrosis in immature teeth makes endodontic procedures quite challenging. In addition, these teeth present very large chambers and incomplete lateral formation of root structure. The current treatment for these teeth is induction of the apical closure followed by conventional endodontic treatment. But the results are unpredictable with frequent failures.

A tissue engineering-based approach that results in new pulp tissue could potentially allow for the completion of vertical and lateral root development and perhaps prevent the premature loss of these teeth.

The key elements for dental pulp engineering are –

- Molecular signals which induce the differentiation of cells that constitute dental pulps
- Cells that will respond to the signals
- Scaffolds that will either carry or attract these cells and provide an environment where they can proliferate, differentiate and develop a tissue with the characteristics and function of normal pulp.

Conclusion

Even though the problems of introducing these new technologies are immense the benefits can be overwhelming - a cure for oral cancer, correction of congenital defects, and the regeneration of teeth and tissues to restore oral functions.

Regenerating tooth structures is a complex proposition. The question the field faces are: Can we do it in a way that is predictable, clinically feasible and practical, high quality research and effective collaborations between basic scientists and clinicians is the way to move this field towards its ultimate goal of regenerating either individual tooth structures or the entire tooth itself.

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Table 1: Ideal structural parameters of a tissue engineering scaffold

SCAFFOLD FUNCTION	SCAFFOLD DESIGN PARAMETER
Not to provoke inflammatory response or toxicity *in vivo*.	Must be biocompatible, non-toxic and non-carcinogenic.
To assist in the growth of three-dimensional tissue and organs.	Three-dimensional scaffold of specific shape.
Give way to a uniform high cell seeding density.	High porosity and high interconnectivity between pores.
To provide the appropriate surface for cell attachment, proliferation and differentiation of function.	Optimum polymer surface chemistry and topography
To allow significant cell surface interactions such as cellular attachment.	High surface-area to volume ratio.
To promote cell proliferation and migration leading to tissue growth throughout the scaffold.	Optimum pore size to allow for cell penetration, with high porosity and interconnectivity between pores.
To direct the orientation of cells, ECM and new tissue.	Correct fibre orientation within the scaffold.
To allow for the movement of nutrients and waste in and out of the scaffold.	High porosity and interconnectivity between pores.
The scaffold may degrade to leave only natural tissue.	Rate of degradation to match rate of tissue formation. Polymer degradation products must not be toxic or promote inflammation *in vivo*.
Possess sufficient structural integrity to retain shape *in vivo*, with enough mechanical strength to support developing tissue and withstand *in vivo* forces.	Scaffold should equal mechanical properties of developing tissue.

Table 2: Types of stem cells

Stem cell type	Cell Plasticity	Source of stem cell
Totipotent	Each cell can develop into a new individual	Cells from early (1–3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5–14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and postnatal stem cells including dental pulp stem cells

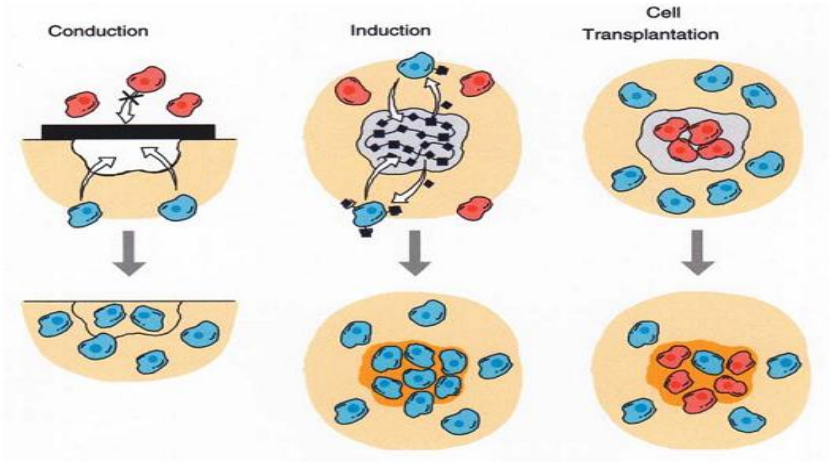

Fig. 1. Tissue engineering strategies

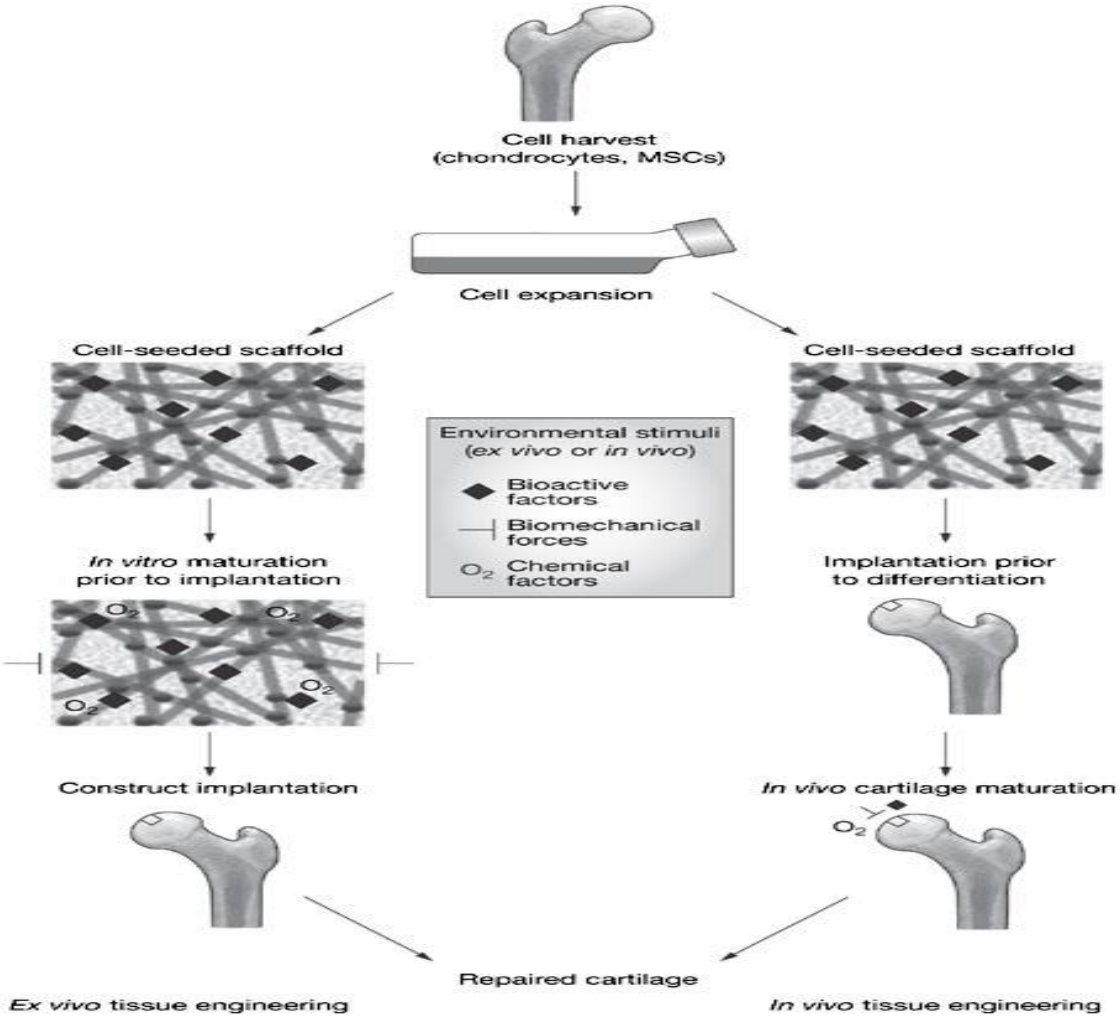

Fig. 2. Tissue engineering a multidisciplinary approach