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# **Biotechnology of Tissues and Materials in Dentistry – Future Prospects**

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Andréa Cristina Barbosa da Silva,  
Diego Romário da Silva, Rafael Grotta Grempel,  
Manuel Antonio Gordón-Núñez and  
Gustavo Gomes Agripino

Additional information is available at the end of the chapter

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## **1. Introduction**

Long ago, humanity has sought alternatives to replacing living tissue, mainly due to birth defects, disease and accidents, using synthetic or natural substances as substitutes, best known as biomaterials. Thus, tissue engineering has emerged, a new and challenging field of modern medicine, which aims at recreating tissues and/or healthy organs to replace missing or diseased body parts [1].

Regenerative medicine which used medical devices and grafts underwent some changes in recent years, changing to a more biological approach, with use of specific biodegradable bioactive and supports (scaffolds) with cells and / or biological molecules to create a functional tissue repair in a diseased or damaged site. Thus, some newer and inter-related strategies are being used for the regeneration of tissues such as cell injection, cell induction and cells seeded in scaffolds (cell seeded scaffold) (detailed later in this chapter) [2]. These approaches depend on the use of one or more key elements, such as cells, growth factors and matrix for guiding tissue regeneration [3].

The technique used to obtain tissues (tissue engineering) is the regeneration of organs and living tissues, through recruitment of the patient's own tissue, which are dissociated into cells and cultured on synthetic or biological carriers, known as scaffolds (scaffolds, three-dimensional matrices, structures, etc.) and then being reinserted into the patient. As a multidisciplinary science, the work involves knowledge of the areas of biology, health sciences and engineering and materials science [4, 5].

Thus, one important step for reconstruction of an organ or tissue is the scaffold selection to the cells, which must take into consideration the type, location and extent of injury. The scaffold structure provides mechanical support to the cell growth and allows transport of nutrients, metabolites, growth factors, and other regulatory molecules, both towards the extracellular environment to the cells, as in the opposite direction [6]. When prepared with bioresorbable polymeris, scaffolds, the scaffolds have specific implementation strategies [7].

After a degradable polymer is identified as a possible candidate for applications in tissue engineering, it must be used for manufacturing a porous scaffold [8, 9, 10, 11]. In this case, two methods are required for proper material manufacture: 1) a method that forms the polymer into a bulk material; 2) a method to make porous such material [12]. The optimal method of manufacturing depends in part on the chemical nature of the polymer. Long, saturated and linear polymers such as PLG are typically formed into bulk materials by entangling the individual polymer chains to form a loosely bound polymer network. Polymer chain entanglement is often achieved by casting the polymer within a mold. The advantage to these methods is that they are relatively simple. However, since the material is elastic solid only because of entangled polymer chains, the material is generally lacking significant mechanical strength. This disadvantage is difficult to overcome without altering the chemical structure of the polymer [12].

Another method to form a bulk material from a linear polymer involves forming chemical bonds between polymer chains, known as polymer cross linking [13, 14]. Cross linking is most often performed between unsaturated carbon-carbon double bonds, and thus this moiety, or a similarly reactive one, is required to exist on somewhere along the polymer chain. An initiation system, typically either radical or ionic, is also needed to promote cross-linking. The initiator system is combined with the polymer and, in response to a signal such as heat, light, a chemical accelerant, or simply time, the initiator forms species that propagate cross-linking. As these polymers are formed into bulk materials by covalent cross-linking, they typically posses significant mechanical strength. Furthermore, their ability to cure in response to an applied signal allows these materials to be injected into the defect site and cure in situ. The major disadvantage of crosslinked materials is that the growing complexity of the material, in terms of the number of components and presence of a chemical reaction, often leads to problems with cytotoxicity and biocompatibility [12].

In this context, biomaterials are extremely important for tissue regeneration process, and can be defined as any substance constructed in such a way that, alone or as part of a complex system, is used for driving, through the control of interactions with components a living system, the course of a diagnostic or therapeutic procedure, whether in humans or animals [15].

In recent decades, biomaterials have been used to repair tissue function, such as metal implants, without concern for its effect on local tissues or on the cells. Thus, polymers and other synthetic materials with biological properties were then developed. More recently, degradable and natural scaffolds, considered a breakthrough for regenerative medicine have been used. Thus, there was an evolution of the use of biomaterials that simply replaced the damaged tissue, to others more specific, allowing the development in three dimensions of a tissue regenerated in full operation and structurally acceptable [2].

To use a material with the purpose of replacing a part of the body or induce the formation of a given tissue, a range of tests and assessments are necessary to establish the potential benefits and possible adverse effects that the material may have. Thus, biomaterials should have the following characteristics: not inducing thrombus formation as a result of contact between the blood and the biomaterial, not inducing adverse immune response, not being toxic or carcinogenic, not disturbing the blood flow, and not producing chronic or acute inflammatory response that prevents the proper differentiation of adjacent tissues [16].

In other words, the biomaterial must be fully biocompatible, that is, must have the ability to perform its desired function with respect to a medical therapy without inducing any undesirable local or systemic effect to the body; but generating cellular and tissue responses beneficial in that specific situation, and optimizing the clinically relevant responses of that therapy [15]. However, it is worth noting that despite the material having been considered inert for a considerable time, it was suggested that they may induce physical and chemical changes after deployment. Thus, before a biological perspective, no material can be considered in fact inert.

## **2. Strategies for formation and development of tissues**

The strategies employed for tissue engineering can be classified into three main classes: conductive or inductive approaches and cell transplantation.

The conductor/conductive approaches using biomaterials in a passive manner to facilitate the growth or regeneration capacity of existing tissue such as, for example, use of membranes or barriers for applied regeneration, adhesion molecules, growth factors, etc. in cases of periodontal diseases [1, 17, 18] or dental implant itself, which is a relatively simple implementation because the apparatus used does not include the use of living cells or other diffusible biological signals [19]. In the conductive techniques is usually accomplished the neoformation of periodontal complex structures, including cementum and periodontal ligament fibers [1]. The periodontium regeneration is the first engineering technology for dental tissue [17].

In 1965, Urist [20] demonstrated for the first time that the new bone formation could occur in a non-mineralized site after implantation of powder bone. This discovery led to the isolation of the active ingredients (specific growth factors - proteins) from bone powder, and the cloning of the genes encoding these proteins. These concepts have been used by many companies for production and expansion of these factors on a large scale [21]. Another method employed is the induction type or inductive approach, which involves the activation of cells near the defect site with specific biological signals that stimulate proliferation and assist in regeneration and repair of tissues by use of materials such bone morphogenetic proteins (BMPs) [20, 22] with promising results for supplementation therapies and the regeneration and bone repair in cases of fractures and periodontal disease [1].

In other words, an alternative approach is the use of diffusible growth factors, and consists of placing specific extracellular matrix molecules on a scaffold to allow the tissue growth. These molecules have the ability to direct or induce the function of cells already present in this

location, and in consequence, promoting the formation of a tissue type or a particular desired structure at the location [23].

For the tissue induction can be clinically successful, it is necessary that the biologically active factors are delivered properly to the desired location and in the correct dose for the time period necessary. Typically, many such proteins have a short half-life in the body, but must be present for a long time to be effective. Doctors and researchers have shown these concerns so far by offering large doses of protein at the sites of interest [19]. The most recent research involves the development of a controlled release system of these proteins (inducing factors) [24] and, with the advent of genetic engineering in current biotechnology, a somewhat similar approach involves transfection of a gene encoding the inducing factor, instead of delivering the protein itself [19].

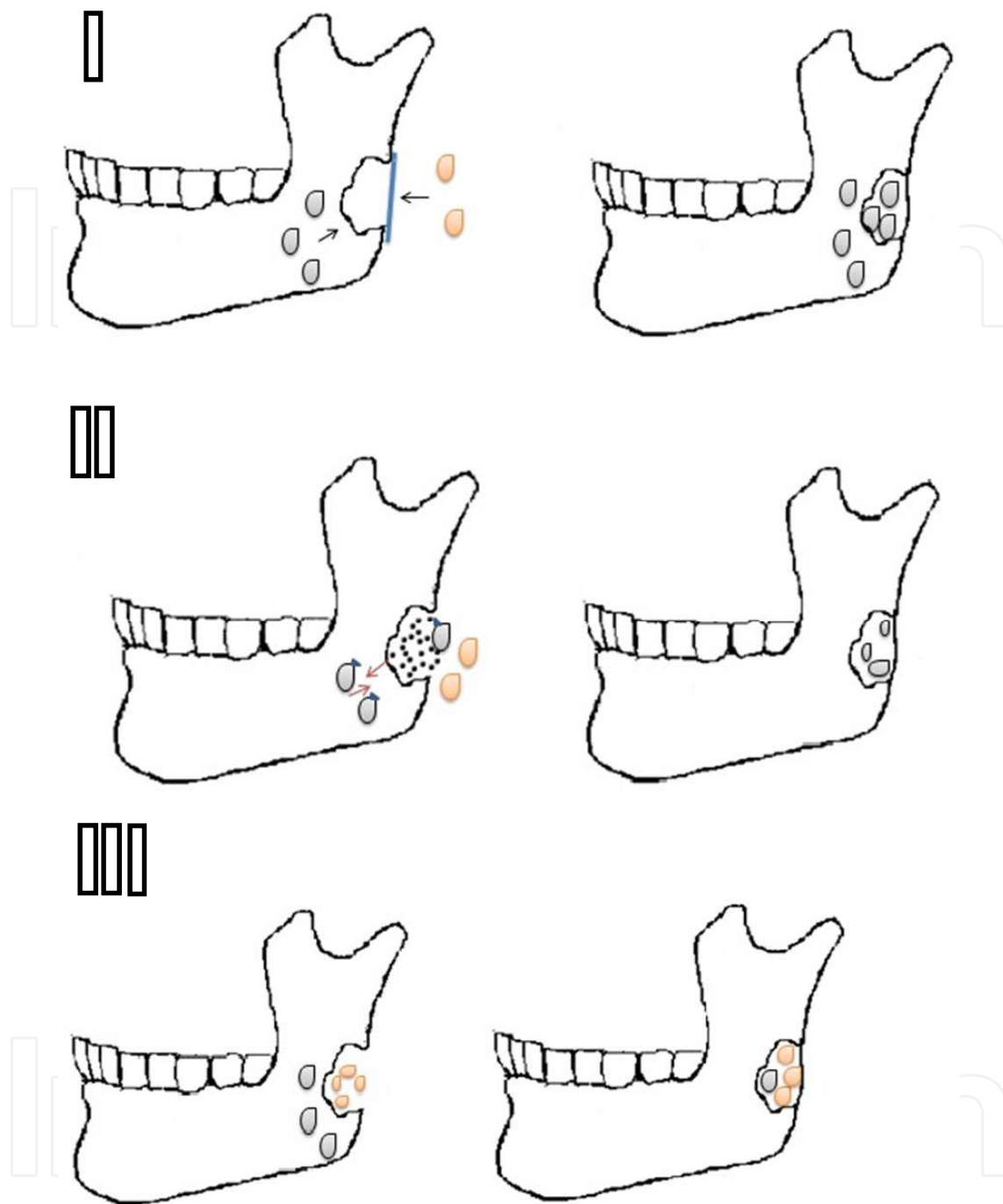
Cell transplantation is the third method, which consists of the direct transplantation of cells grown in the laboratory [25]. This approach is a strategy whose importance is based on the need for a multidisciplinary team for performing tissue engineering, since it requires the physician or surgeon in charge of obtaining tissue samples by biopsy, the bioengineer, who usually participates in manipulating the tissues in bioreactors and prepares the means necessary for placing the cells obtained from biopsy samples, besides cell biologist, who will apply the principles of cell biology required for multiplication and maintenance of cells in the laboratory [1, 18, 26, 27].

Despite having different mechanisms, the three strategies for tissue formation have one characteristic in common: the use of polymeric materials. In conducting approaches, polymer is mainly used as a membrane barrier for exclusion of particular cells that can disturb the regenerative process. In the inductive approaches, these materials act as a carrier for delivery of proteins (e.g., BMP) or the DNA encoding the protein [24, 28]. With regard to approaches used to achieve control of the dose and bioavailability of biodegradable polymer carriers enable localized and sustained release of inductive molecules. The dose rate and the molecule to be delivered are controlled generally by gradual breakdown of the vehicle [24].

These delivery vehicles are often used in cell transplantation approaches. However, in this approach the vehicle serves as a carrier of intact cells and even partial tissues [1].

Besides acting as vehicles for the simple delivery of cells, the vehicles also serve as scaffolds to guide new tissue to grow in a predictable way from the interaction between cells or transplanted tissue and host cells. The collagen derived from animal sources, and synthetic polymers of lactic acid and glycolic acid are the main absorbable materials used for tissue repair in three types of approaches. The collagen is degraded by cells in the tissue during its development, whereas the synthetic polymers are degraded into natural metabolites of lactic acid and glycolic acid by the water action at the implant site. From the development and innovation of biotechnology in tissue engineering various new materials are also being developed for these applications, such as injectable materials that enable a minimally invasive delivery of inductive molecules or transplanted cells [1].

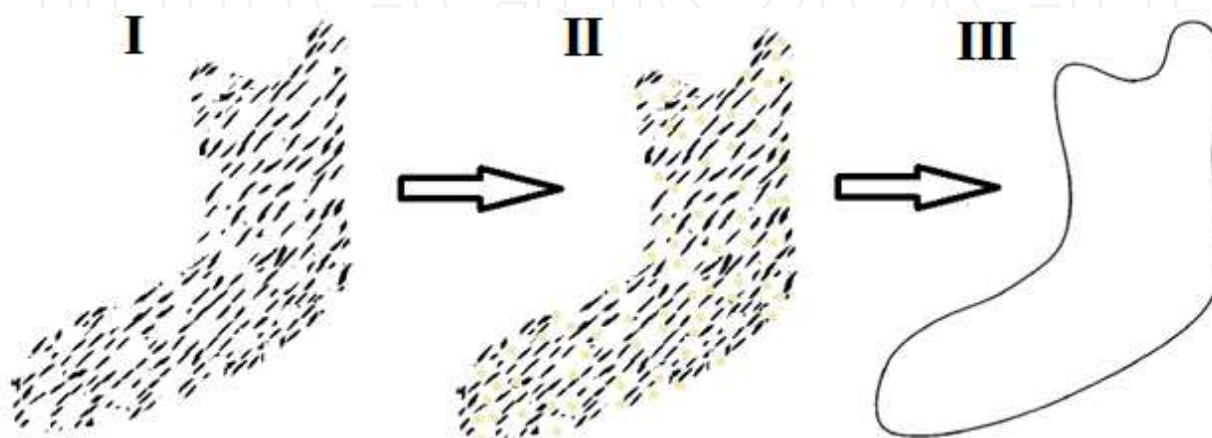
Below (Figure 1), a schematic view of the three types of approaches in tissue engineering:



**Figure 1.** Schematic representation of the three main approaches for tissue rebuilding in tissue engineering in jaw: **I)** by the conductive method where use is made of a barrier that is able to exclude connective tissue cells that may interfere with the regeneration process and at the same time enables the desired host cells to populate the site to be regenerated. **II)** by the inductive method, in which a scaffold of the biodegradable polymer is used as a delivery vehicle for growth factors and / or genes encoding this factor in the desired location. As the polymer is being degraded, the growth factor is being released gradually. **III)** by the strategy of cell transplantation, which uses a delivery vehicle, similar to that used in an inductive approach, with the goal of transplanting cells and partial tissues to the place where we want to regenerate tissue. In this approach can be transplanted only tissues or cells previously formed in the laboratory from scaffolds.



Tissue engineering seeks solutions for the regeneration of various tissues associated with the oral cavity, such as, bones, cartilage, skin and oral mucosa, dentin and dental pulp, and salivary glands. But in fact, this science will probably have its most significant impact in dentistry through bone reconstruction and regeneration. The fact that cell transplantation approaches may offer the possibility of pre-formation of bone structures of large dimensions (for example, full jaw), which may not be possible to use the other two strategies, makes it the most important approach in the engineering scope for bone tissue formation [1] (Figure 2).

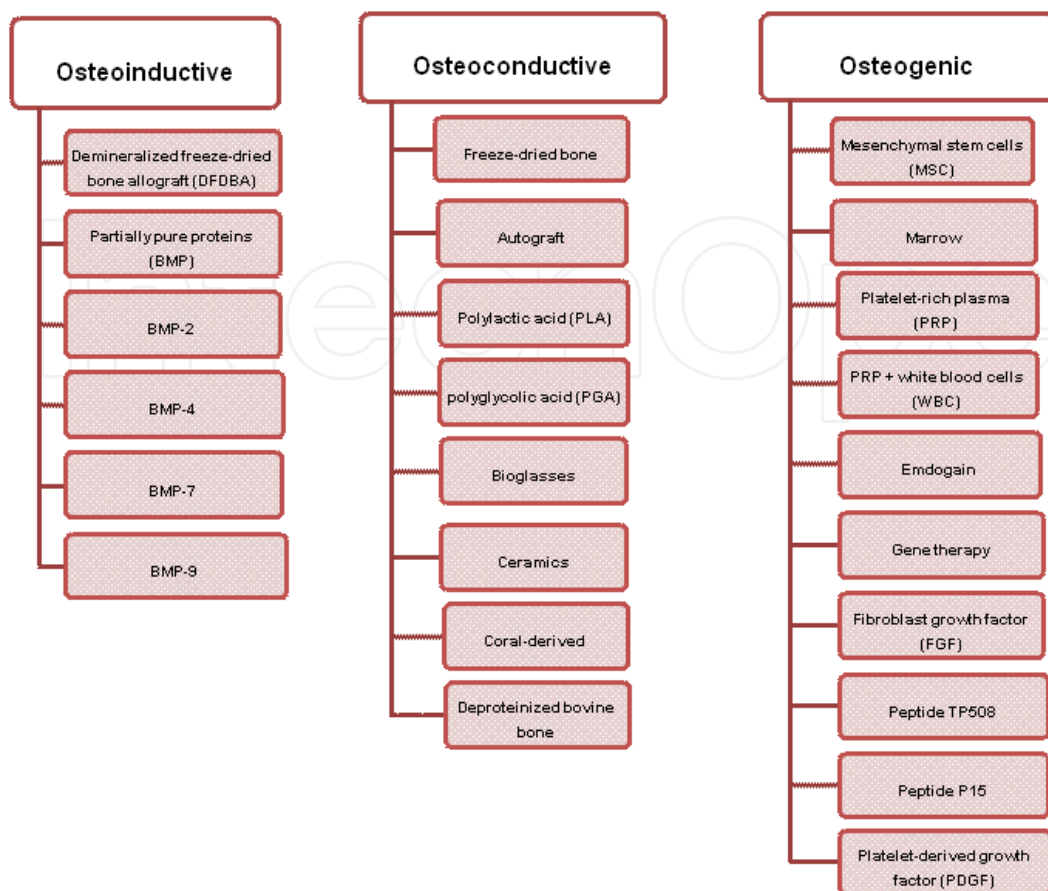


**Figure 2.** Schematic representation of the advances in tissue engineering to regenerate part of the jaw by means of cell transplantation. A scaffold consisting of biodegradable polymer in the shape of half of the jaw is built (I). Thereafter, bone precursor cells are seeded on the polymer (beige dots) and stimulated to grow in a bioreactor (II). The scaffold will then be gradually degraded, while facilitating growth of jaw-shaped bone (III) (Scheme adapted from [1]).

Thus, the tissue repair from the in vitro tissue engineering requires the use of cells to completion and production of similar matrix to the native tissue. The main successful developments in this field have been using the transplant of primary cells taken from patient and used in combination with scaffolds to produce the required tissue to re-implant. However, this strategy has limitations due to the invasive nature of how the cells are removed. Thus, attention has turned to the use of stem cells, including embryonic stem cells and mesenchymal cells derived from bone marrow. In addition to being able to turn into all body tissues, these cells have the capability and advantage of being maintained in culture for long periods, thus having the potential to obtaining large amounts of cells to tissue. The extraordinary ability of these pluripotent cells is linked to their ability to form teratoma [29]. Besides the potential to differentiate into osteoblasts, the possibility of rejection of these cells is greatly reduced.

In cell transplantation, these units can be directly transplanted to the desired location or they may be cultured in the laboratory on scaffolding. In this case, those cells are stimulated to lay the groundwork matrix to produce a tissue for transplantation [29].

Currently, several products can be used to achieve tissue regeneration or reconstruction. These options are divided according to the approach to be used (Inducing, conductive or cell transplantation) as shown in the scheme below (Figure 3) adapted from [19].



**Figure 3.** Products used for bone tissue repair in different types of approaches (Inducing, or conductive cell transplantation) (Adapted from de Kumar, Mukhtar-Un-Nisar and Zia, 2011) [19].

### 3. Importance of tissues for maxillofacial complex

The maxillofacial complex can be subjected to processes of physical, chemical and biological nature, which usually determine from minor tissue losses to the involvement of large areas of structures of this complex. In this context, dentistry has been explored new technologies in order to change this reality, adapting to new concepts, scientific innovations that include research on stem cells, tissue engineering, and molecular biology techniques, as tools to stimulate regeneration or replacement of damaged tissue by tissue engineering.

Considering the scenario of new technologies, however, still in 2001 it was asked: "What impact could have this engineering in dentistry?" And "What maxillofacial tissues have potential or are important for that engineering?" According to Kaigler and Mooney (2001) [1], at that time the answer to the first question was still being formulated, since the engineering probably would have a revolutionary effect on the field of Dentistry, once almost all types of tissues in the maxillofacial complex could have potential for engineering. Currently, reality has changed significantly due to which the tissue engineering has wide application to many different tissue



types associated with the oral cavity, including bone, cartilage, skin, oral mucosa, dentin and dental pulp, and salivary glands.

As previously mentioned, inductive, conductive and cell transplantation strategies, which represent the most used techniques in tissue engineering, are of importance to typically use different material components in order to achieve the goal of regeneration and / or replacement of damaged tissues.

Absolutely, all tissues of the maxillofacial complex are important for its proper functioning, playing a crucial role also in facial aesthetics. Thus, some comments are required about the major oral tissues and their importance for tissue engineering.

With respect to bone, it can be said that tissue engineering has had a greater impact in dentistry, particularly with regard to bone regeneration. Bone loss associated with trauma, diseases or disorders can currently be handled through the use of biomaterials for auto-grafts, allografts or synthetic, morphogenetic proteins (BMPs) and growth factors. It is reported that even though these biomaterials stimulate, replace and / or restore the stability and function of tissues in a reasonably sufficient manner, there are still limitations in their use, which is of importance for research is increasingly carried out using the three main strategies of tissue engineering in order to optimize the mechanisms of regeneration in bone areas compromised by various damaging agents [1, 28, 30].

The importance of cartilage tissue to tissue engineering of structures of the maxillofacial complex lies in the possibility of reconstruction of craniofacial chondromatous structures, the design of polymeric structures with defined mechanical and degradative properties that can serve as a support structures for cartilage cell proliferation of temporomandibular or intranasal joints if compromised by trauma or degenerative diseases. One of the limitations of the use of cartilage tissue in tissue engineering is due to its limited capacity for regeneration and lack of inductive molecules to the proliferation of their cells; thus it is one of the tissues of great interest among researchers to develop envisaging bioengineering techniques for transplanting of cartilage cells [1, 31, 32].

Researches have been and continue to be focused on the production of dentin and dental pulp by the use of tissue engineering strategies. The importance of these tissues for this engineering is associated with the possibility to replace material lost by carious processes. There is evidence that odontoblasts, even lost due to caries, it would be possible to induce the formation of new pulp tissue cells by tissue engineering based on the use of certain biomolecules stimulating or inducing odontoblast proliferation and / or nerve cells, and these new odontoblasts, in turn, could synthesize new dentin material. Furthermore, it is suggested that the tissue engineering of the dental pulp itself may be possible by using techniques of cultured fibroblasts in synthetic polymer matrices [33, 34, 35, 36, 37].

One of the most exploited tissues in research of tissue engineering in dentistry is the epithelial lining of the oral mucosa with significant advances in the use of these tissues in regeneration and / or replacement of structures of the oral mucosa damaged by various aggressors. Recently, the introduction of 3D reconstruction of the oral mucosa has significantly impacted the

approaches to biocompatibility evaluation of tissues and materials to replace and / or regenerate oral soft tissues [2, 38, 39, 40].

One of the most challenging areas of genetic engineering applied to the structures of the maxillofacial complex is the replace of function of salivary glands, since these tissues play important roles in mastication, phonation and protection of hard and soft tissues of the mouth by saliva production. In this context, we study the possibility of salivary gland cells transplantation or creating a replacement for compromised glandular structures through the use of artificial salivary glands consisting of a polymer tube coated with salivary epithelial cells [41]. The success importance of future tissue engineering for these tissues might represent the possibility of new and more effective approaches to the treatment of conditions associated with loss of function of the salivary glands, including dysphagia, dysgeusia, rampant caries and mucosal infections [1].

Regarding the possibility of reproducing teeth, there are numerous growth factors involved in the development of dental organs and biological processes involved in odontogenesis are quite complex, reason why we still cannot form a complete tooth; however, some studies have shown the enamel and dentin formation from stem cells isolated from dental pulp [42, 43]. The replacement of missing teeth by tissue engineering in humans is still being researched, but with a real possibility of application in the future.

#### **4. Biomaterials used in craniofacial tissue regeneration**

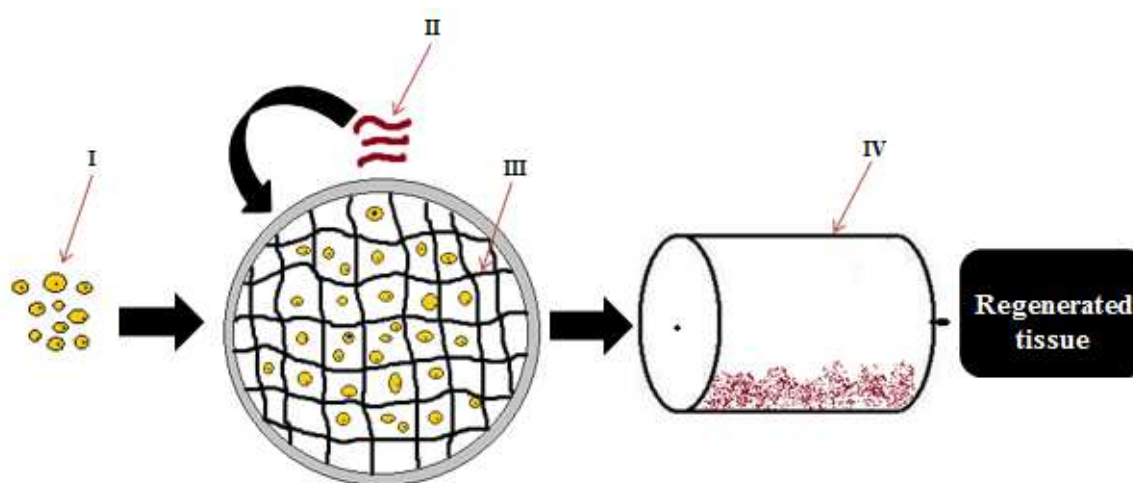
Biomaterials play a crucial role in tissue engineering. They are used for the manufacture of supports or matrices which allow a suitable microenvironment for optimal cell regeneration.

Biomaterials for constructing scaffolds can be natural/synthetic and rigid/non rigid. Natural biomaterials offer good cellular compatibility i.e. ability to support cell survival and function thereby enhancing the cells' performance, and biocompatibility. Their disadvantages include source variability, immunogenicity, if not pure, limited range of mechanical properties and lack of control over pore size. Unlike natural biomaterials, synthetic biomaterials can be manufactured in unlimited supply under controlled conditions, are cheaper and can be tailored to obtain desired shape, cell differentiation properties and mechanical and chemical properties especially the strength, pore characteristics and degradation rate suited for intended applications. However, synthetic biomaterials lack cell adhesion sites and require chemical modifications to improve cell adhesion

During the last century, various natural or synthetic biomaterials have been used for the manufacture of supports for tissue engineering (fabrication of tissue engineering scaffolds) such as metals, ceramics and polymers. However, metals and ceramics are not biodegradable and its processing is limited, which prevents their application as effective supports (scaffolds) for tissue regeneration. Thus, the polymers has been the most commonly used because they have some important characteristics for tissue regeneration such as biodegradability, porosity, large surface area and ease of processing, among others [44, 45].

There are two types of polymers: natural and synthetic [46, 47]. The main biodegradable synthetic polymers include polyesters, polyanhydride, polyfumarate, polycaprolactone, polycarbonate and polyorthoester [7, 48]. The polyesters such as poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and their copolymer of poly [lactic-co-(glycolic acid)] (PLGA) are most commonly used for tissue engineering. The natural polymers include proteins of natural extracellular matrices such as glycosaminoglycan, collagen, alginic acid and chitosan etc [49, 50]. These polymers of natural origin are biodegradable and possess known cell-binding sites. However, they have some disadvantages such as the level of immunogenicity and speed of degradation.

The tissue regeneration from cells transplanted into a polymer scaffold is summarized in Figure 4.



**Figure 4.** Schematic figure illustrating the steps performed in the laboratory for tissue regeneration from the use of transplanted cells stimulated to grow on biomaterials. It is necessary to understand the importance of biomaterial to perform this technique. It can be natural or synthetic and should meet the requirements of biocompatibility and other features already mentioned in this chapter. It is also important to realize the multidisciplinary involved in this process. The physician is needed in order to perform the tissue biopsy to remove the cells (I). This tissue/cell is then taken to the laboratory to be multiplied several times. Thereafter, the use of principles of cell biology, such as growth factors (II) to stimulate the cells to grow and maintain their functions will be necessary. It is also required the involvement of engineers for manufacturing matrices of biodegradable polymers (III) and the bioreactor (IV). When cells grow in appropriate number, they are seeded on the polymer scaffold. The tissue is then allowed to grow in the bioreactor until the time of transplantation by clinical surgeon. Biomaterials can be used to stimulate the growth of several types of tissues, e.g. bone, cartilage or skin. After the appropriate development, the tissue is transplanted and the area is regenerated.

Other extracellular matrices used as scaffolds include fibrin and fibrinogen. [51, 52, 53]. According to some studies, both can induce angiogenesis during tissue regeneration [54, 55, 56]. Chitosan is a derivative of chitin, a natural biopolymer which is biocompatible, biodegradable, antimicrobial and possesses tissue healing and osteoinductive effects. It has the ability to bind to growth factors, glycosaminoglycans and DNA and can be easily processed into membranes, gels, nanofibres, beads, scaffolds and sponges. Because of these properties, chitosan gel alone or in combination with demineralized bone matrix/collagenous membrane is quite promising in periodontal regeneration [57].

Considering the bone tissue engineering, porous scaffolds are designed to support the migration, proliferation, and differentiation of osteo-progenitor cells and aid in the organization of these cells in three dimensions. These scaffolds may be made from a wide variety of both natural and synthetic materials. The naturally derived materials include cornstarch-based polymers, [58] chitosan [59, 60] collagen, [61] and coral [62, 63]. Among these materials, the coral has been shown to be an effective clinical alternative to autogenous and allogeneous bone grafts [64, 65].

Examples of synthetic materials include calcium phosphates [66, 67] and organic materials such as poly (phosphazenes), [68] poly (tyrosine carbonates), [69] poly (caprolactones) [70], poly (propylene fumarates) [71], and poly ( $\alpha$ -hydroxy acids) [72, 73]. Composites of inorganic and organic materials have also been successfully used to create scaffolds for bone grafts [74, 75]. Poly ( $\alpha$ -hydroxy acids) are the most commonly used polymeric materials for the creation of tissue-engineering scaffolds for bone. The most common of the poly ( $\alpha$ -hydroxy acids) are poly (glycolic acid), poly (lactic acid) (PLA), and copolymers of poly (lactic-co-glycolic acid) (PLGA). These materials are readily metabolized and excreted when degraded by the body [44].

## 5. Challenges and future prospects

Tissue engineering is an emerging technology with potential application in various medical fields. The main focus of recent research is the development of techniques for manipulating stem cells, aiming at the achievement of restorative treatments of injured and/or lost tissues and organs. Apart from stem cells, bioengineering requires the presence of factors that allow their proliferation in a microenvironment closer to tissue reality, including the extracellular matrix and growth factors. The biomaterials, in turn, are necessary for serving as porous scaffold upon which tissue regeneration is set. As knowledge is acquired with respect to stem cells and biomaterials, the potential for treating diseases may extend beyond the craniofacial region of the body. However, the mechanisms of action of these biotechnologies are not yet fully understood and offer a promising future, so that research is needed to apply them clinically.

### Author details

Andréa Cristina Barbosa da Silva\*, Diego Romário da Silva, Rafael Grotta Grempe, Manuel Antonio Gordón-Núñez and Gustavo Gomes Agripino

\*Address all correspondence to: [andreacbsilva@gmail.com](mailto:andreacbsilva@gmail.com)

Graduate Program in Dentistry, Center of Sciences, Technology and Health, State University of Paraíba, UEPB, Araruna, Paraíba, Brazil

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