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Scaffolds for Bone Tissue Regeneration

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Abstract

This work was done within the scope of Seminars in Biomedical Engineering from the Doctoral Program in Biomedical Engineering. It is literature review of the subject "Scaffolds for Bone Tissue Regeneration". Bone is a heterogeneous and complex structure, composed of different cell types,organic and inorganic components with a unique extracellular matrix (ECM). In the human body, bone formation and resorption is an ongoing process with some defects that cause bone diseases. Ultimate goal of bone tissue engineering is to understand these processes and develop new alternative treatment methods for clinics. To be able to do that, bone structure and biology should be well known and correlated with biomaterials. The aim of this review is to focus on the different aspects of the available scaffolds, its characteristics, strengths and flaws.

Contents

1 Tissue Engineering

Tissue Engineering (TE) term was firstly suggested by Professor Yuan-Cheng Fung, from the University of California, San Diego in 1987 [Vacanti & Vacanti, 2014]. Humans have been suffering from acute diseases and traumas which alter cells, tissues and organs [Khademhosseini et al., 2006]. Hence, there has been increasing demand for construction of functional tissues that can mimic architecture and functions of human body including crucial microenvironment [Atala et al., 2012]. Ultimate goal of TE is to develop reliable and sustainable therapies for patients using life sciences and engineering technologies. Basically, TE approaches can be compartmentalized into three different subtopics: isolation of cells, expansion of cells in bioreactor systems to construct functional tissues and integration the final tissue into the living system [Mathew et al., 2016]. However, integration of developed tissue construct into clinics is extremely challenging and there are many criteria to consider. In tissue engineering applications, cells could be autologous, allogeneic and xenogeneic depending on the cell source and cell source is critical for immune acceptance of the final tissue construct. Regarding to that, the immune acceptance and animal sourced contaminant must be concerned [Vats et al., 2005]. Cells can integrated into biomaterials as complementary products for dysfunctional tissue and organs. Biomaterials in TE application rely on two approaches, cell-based and cell-free regeneration [1,]. Cell-based regeneration is integration of engineered device or any kind of product with living cells. Cell-free regeneration is implementation of engineered products into target tissue, which triggers the migration of host cells to inflammation site for healing. For both approaches, stimulation of tissue growth is depending on the physiological, biological, chemical, and mechanical properties of biomaterials which serve as 3D scaffold for cells [Iqbal et al., 2018]. Final and most critical point of TE is integration of this system into living organism. This step mostly starts with long-term animal experiments, and then followed by clinical trials. Even though some diseases are successfully treated using animal models, many methods may not work with humans as a result of foreign body reaction mechanism [Mariani et al., 2019]. To overcome these critical limitations scientists have been researching new alternatives.

2 Bone Tissue Engineering

2.1 Bone Structure and Biology

Bone is a well-organized complex structure and plays a role in mechanical support and protection of organs from injuries. Additionally, it's architecture is highly heterogeneous, composed of different types of cells, organic and inorganic components to form Extracellular Bone Matrix (ECM), mainly composed of type-I collagen [Wang et al., 2016]. Besides type-I collagen, bone matrix also contains other types of collagens, proteins and growth factors such as osteocalcin, fibronectin which promote cell-matrix adherence; TGF-b which regulates bone formation and resorption [Ralston, 2013]. Non-collagenous protein plays an important role in phosphate metabolism and mineralization [Alford et al., 2015]. Hydroxyapatite is one of the major minerals in the bone and modifies tensile strength and elasticity of the bone [Qu et al., 2019]. Bone classified in two according to its architecture: trabecular and cortical bone 1. Trabecular bone, also referred as spongy bone, is highly porous and composed of an interlinked network of trabeculae and bone marrow. Trabecular bone contains osteoblasts, osteocytes, osteoclasts and immune cells in the bone marrow. Trabceular bone is surrounded by cortical bone [Chocholata et al., 2019]. Cortical bone is composed of inorganic minerals,

blood vessels and osteocytes. Cortical bone is more abundant in bone skeleton however, distribution of cortical and trabecular bone varies depending on the bone type and its region [Ralston, 2013]. Detailed information in bone formation is necessary to be able to understand bone structure clearly. Bone formation is classified in two phases: primary and secondary, also known as osteogenesis [Olszta et al., 2007]. In primary phase, chondrogenic formation occurs as type-I collagen fibrils organized into hydroxyapatite form within the proteoglycan matrix [Bonucci & Gomez, 2012].This phase is followed by the secondary stage, where collagen molecules covalently bind each other to form larger collagen fibrils. Mainly osteoblasts and other bone lining cells in the endosteum excrete these collagen fibrils, which provide tensile strength to the bone [Chocholata et al., 2019]. Then osteoblasts differentiate into osteocytes. Osteocytes in the cambium layer of periosteum regulates mechanical loading of the bone and phosphate metabolism [Ralston, 2013], [Chocholata et al., 2019]. Afterwards, fibers form aligned lamellar structure [CAMERON, 1963]. Then, intrafibrillar crystals may occur on the surface of collagen fibrils and between gaps of the aligned structure [Olszta et al., 2007]. Bone formation and bone resorbing continuously take place during life and sometimes there might be bone fractures. Thus, comprehension of bone structure and biology is critical to develop suitable scaffolds to promote bone remodelling in patients.

2.2 General aim of BTE

Although Bone is one of the major tissues capable of regeneration, its healing abilities are limited by defects with a so-called "Critical Size", leading to a process of scarring instead of regeneration in large wounds, as mentioned by [Petite et al., 2000]. As a way of tackling these problems, several approaches were already developed such as the grafting of Autologous Tissue, the current "Gold Standard" and one of the most traditional. Each one of those methods, however, present several restrictions, as summarized in Table 1 besides all of them being generally constrained by diffusion and bone resorption rates ,risk of contamination and the "non-controllable" mechanical properties of the final tissue.

Bone Grafts	Advantages	Disadvantages
Autologous	No immune system response. Harvesting is time consuming	Limitations regarding the shape and the amount Usually linked to a high morbidity of the donor tissue, pain and infection.
Allogeneic	"Off-the-Shelf" availability Large amounts	High risk of contamination Bone inducing factors may not be active
Vascularized	Reduce the diffusion rate limitation as it is already vascularized	Requires complex and quite invasive surgeries
Bone Fillers	—	—

Table 1: Advantages and disadvantages of different bone grafts materials.

Bone Tissue Engineering, in that way, serves as an alternative proposal to address such impediments, allowing the development of new procedures and techniques. Albeit the relative novelty of the field, having its debut on a major paper published in 1993 [Langer & Vacanti, 1993], the technological development of the previous decades have permitted a humongous improvement in the field, settling it as the "Future Goal of regenerative Medicine". Defined as "a combination of multidisciplinary approaches to improve or replace biological tissue" [Qu et al., 2019]. Tissue engineering revolve around three major aspects, the resorbable scaffold in which the cells will grow; its Growth Factors and the Stems Cells, that combined, aim to induce reparation and/or regeneration of new tissue in a structure, commonly referenced as the "Tissue Engineering Triangle" such as exemplified in Figure 1 [Acri et al., 2018].

For this paper review, the goal of the group is to briefly focus on the different aspects of the available

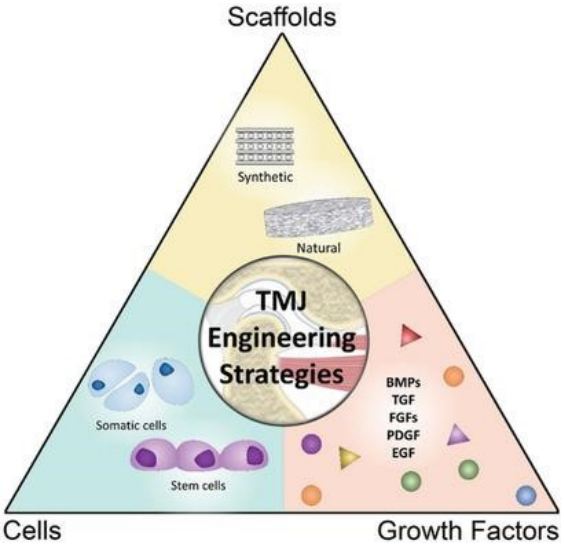

Figure 1: Tissue Engineering Triangle.

scaffolds, its characteristics, strengths and flaws.

2.3 Classification of scaffolds

The development of new Bone Tissue Engineering Techniques is, thus , intrinsically related to the uprising of the "Biomaterials". According to [Burny et al., 1995], the two decades following the end of the II World War were crucial for exploring the effects of long time exposure of different materials to the harsh internal environment of the human body, consequently leading to the consolidation of fields such as Biomechanics and Biomaterials [Burny et al., 1995]. Those fields followed specifics "trends" that would reflect the way of thinking the human body and their available technology of its time, allowing us to classify it in different generations:

I. First: Materials designed to endure the maximum possible time, presenting zero to no toxicity and keeping the performance of the Tissue to be replaced. They were known as Bioinert Materials and included some Metals(Ti or Ti-Alloys), Synthetic Polymers(PMMA or PEEK) and some Ceramics(Al,Zi).

II. Second: The idea of the second generation is that these materials no longer have to be isolated from the body, but, on the contrary, they would interact with the surrounding tissue and, in some cases, be completely biodegradable. Named Bioactive Materials, this "class" refers to both Synthetic and Natural Polymers (Collagen) and other Ceramics (Calcium Phosphates and Bioactive Glasses)

III. Third: The recent approach is to develop materials capable of induce some specific biological response according to the need of the patient and the occasion. This is generally achieved by adding instructive substances (such as Growth Factors) in the structure of the scaffold.

Desirable qualities of a bone tissue-engineering scaffold	
Available to surgeon on short notice	Promotes bone ingrowth
Absorbs in predictable manner in concert with bone growth	Does not induce soft tissue growth at bone/implant interface
Adaptable to irregular wound site, malleable	Average pore sizes approximately 200–400 μm
Maximal bone growth through osteoinduction and/or osteoconduction	No detrimental effects to surrounding tissue due to processing
Correct mechanical and physical properties for application	Sterilizable without loss of properties
Good bony apposition	Absorbable with biocompatible components

Figure 2: Desired qualities and characteristics of the optimal Scaffold. Figure from [Burg et al., 2000]

It is important to say that the different types of materials mentioned can also be used in association with each other to improve both biological interaction and mechanical properties. This is generally done in approaches such as Composites or by Coating a material with another, for example. Currently, the

desired qualities and characteristics of the optimal Scaffold are well defined and are summarized in the Figure 2 [Burg et al., 2000].

Regarding future advents, [Du et al., 2018] present an overview on different techniques of using 3D Bioprinting to achieve precise control of the ceramic materials' properties, analysing its accuracy and related costs [Du et al., 2018].

3 Polymer based scaffold

Polymers are organic materials, which have repeating units of covalently bonded molecules. Polymers can be one, two and three-dimensional properties depending on the configuration of covalent bonding [Koons et al., 2020]. Additionally, polymers can classified natural and synthetic based on their sources. Both type of polymers have been commonly used for bone tissue engineering applications due to their specific biocompatibility, biodegradability and being bioresorbable [Hutmacher, 2000]. Most common natural polymer on the bone tissue engineering market are collagen, gelatin, alginate and chitosan; synthetic polymers are Polycaprolactone (PCL), aliphatic polyesters, Poly(vinyl)alcohol (PVA) and Polyethylene Glycol (PEG) [Qu et al., 2019].

3.1 Natural Polymers

3.1.1 Collagen

Collagen is the most abundant protein in the body with a complex hierarchical conformation within the ECM. It is preferred in bone tissue engineering applications since it is the main component of bone, cartilage, ligament and tendon. Collagen is highly hydrophilic, biodegradable, biocompatible and easily crosslinkable polymer with low mechanical stability [Hutmacher, 2000]. Additionally, microporous structure of collagen scaffolds promote cell survival, adhesion, differentiation, proliferation more specifically mineralization for osteoinductive [Koons et al., 2020]. It has been demonstrated that collagen composites with glycosaminoglycan, a polysaccharide formed collagen-GAG scaffold, which is promising for mimicking GAG formation in the bone [O'Brien, 2011]. Beside the advantages of natural polymers, they have some limitations too. Natural polymers bring with batch varieties problems and they have limited mechanical strength due to their porous and hydrophilic structure [Koons et al., 2020]. For those reasons, synthetic polymeric scaffolds or their combination with natural polymeric has leading the scaffold technologies in bone tissue engineering.

3.1.2 Gelatin

Gelatin is denaturized form of collagen and it categorized in two: gelatin A and gelatin B depending on the preparation process [Lee & Mooney, 2001]. Gelatin A formulated by acidic cure while gelatin B formulated by alkaline cure. Gelatin-based hydrogel scaffolds are bioactive and tailorable due to their easy crosslinking processes. In addition these properties, gelatin based scaffolds promote cellular behaviours through perfusion capacity of growth factors and other supplements for cells [Lee & Mooney, 2001]. Furthermore, gelatin provides better solubility and less antigenicity compared to collagen. Various architectures with heterogeneous subunits of cartilage tissue can be fabricated by direct printing of gelatin based bio inks.

3.1.3 Alginate

Alginate is a natural polymer extracted from algea. Alginate based hydrogel scaffolds have been used in drug delivery, wound dressing, dental impression, immobilization matrix and chondrocyte grafts owing to its biocompatibility, low toxicity and effortless crosslinking methods [Lee & Mooney, 2001].

However, alginate is highly degradable material and degradation capacity of alginate hydrogels is controlled depending on the crosslinking concentration. Another drawback of alginate is low cell adhesion capacity. It has been reported that bonding of RGD peptide to alginate enhanced cell adhesion, proliferation and differentiation of skeletal muscle cells [Lee & Mooney, 2001].

3.1.4 Chitosan

Chitosan is hydrophobic polymer obtained from a polysaccharide (chitin). Chitosan hydrogel formed by chemical crosslinking of glutaraldehyde using derivatives of chitosan, especially , methylpyrrolidinone-derivated chitosan supports bone formation [Lee & Mooney, 2001]. Functionality of chitosan also can be customized by incorporating other natural polymers such as gelatin and collagen to promote cell adhesion and proliferation. It has been demonstrated that chitosan based scaffolds in presence of bone morphogenetic proteins such as BMP2, BMP-7 or transforming growth factor (TGF-β1) activate osteogenesis pathway and leads to increase bone formation [Aguilar et al., 2019]. Chitosan scaffolds also promotes chondrogenic differentiation and osteoblast cell proliferation [Martins et al., 2007].

3.2 Synthetic Polymers

3.2.1 Polycaprolactone (PCL)

Polycaprolactone (PCL) is Food and Drug Administration (FDA) approved polymer for tissue engineering applications [Lee & Mooney, 2001]. PCL has also contains some growth factors, minerals and ions to promote cellular behaviours [Martins et al., 2007]. PCL based scaffolds have been used as drug carrier to provide controlled release of drug and as surgical implants owing to it's desirable mechanical stability for mimicking cartilage and bone. It has been demonstrated that PCL-HAp composite promotes osteogenic differentiation [Park et al., 2016], [Yang et al., 2015]. Another study showed that PCL scaffolds promotes osteogenic differentiation of Mesenchymal Stem Cells (MSCs), expression of type I collagen and mineralization as well as chondrogenic differentiation [Martins et al., 2007].

3.2.2 Linear Aliphatic Polyesters

Polyglycolic Acid (PGA), Polylactic Acid (PLA) and their co-polymers Polylactic Co-Glycolic Acid (PLGA) were classified as polyesters [Gregor et al., 2017]. All of these polymers commercially available and FDA approved materials in clinics owing to their biocompatibility, highly porous structures with high mechanical stability [Qu et al., 2019]. It has been indicated that high porosity of PLA scaffolds promotes expression of osteoblastic markers, viability, proliferation and metabolic activity of cells comparing to lower porous structure [Gregor et al., 2017]. PLGA has been used to mimic periosteum in the bone [Koons et al., 2020] and nerve formation into lumen owing to its perfusion capacity [Martins et al., 2007].

3.2.3 Poly(vinyl)alcohol (PVA)

PVA is a hydrophilic polymer, containing hydrogen bonds between polar hydroxyl groups which gives PVA water solubility and ability to adhere [Ma et al., 2017]. PVA-based hydrogels can be crosslinked through physically, chemically or combination of these two methods [Kobayashi & Hyu, 2010]. PVA based hydrogels are also biocompatible, highly porous and not degradable which makes it suitable as long-term scaffold. Despite these well outstandings, PVA has limited cell adhesion capacity. However, it is reported that addition of some natural polymers such as gelatin and heparin to PVA enhance cell attachment and porosity of the scaffold

[Ma et al., 2017]. It has been reported that foams with bioactive glasses promote osteoblast proliferation, collagen production and mineralization and formation of bone [Costa et al., 2007]. However, the foams decrease the mechanical stability of bioactive glasses. [Costa et al., 2007] showed that PVA composite with bioactive glass improved the toughness and tensile strength of the foams. Another studies showed that PVA composites with HAp, major natural inorganic component of bone with high osteoconductivity capacity, increase the cell adhesion, proliferation [Chocholata et al., 2020] and functional activity of osteoblast [Enayati et al., 2018], as well as improved the mechanical properties of the scaffold.

3.2.4 Polyethylene Glycol (PEG)

PEG is one of the most well-known natural polymer and molecular structure of PEG is PEG hydrogels can be formed by crosslinking either via physically or chemically interactions. PEG hydrogels commonly synthesized by photo-polymerization method, which allows encapsulation of cells. PEG hydrogels do not promote cell adhesion however; encapsulation method of cells eliminates this limitation. It has been indicated that PEG/PLA fibrous scaffolds [Enayati et al., 2018] and PEG/PLA-HAp scaffolds [Liao et al., 2014] promotes cellular behaviours and osteogenic differentiation of MSCs.

4 Ceramic based scaffold

Due to its chemical structure, biocompatibility and relatively high durability, the Ceramic Materials answer for most of the materials designed for Bone Scaffolding.

With mechanical properties similar to the original tissue, as well as the capability to form a Hydroxyapatite layer that interact with the surrounding environment, Bio-Ceramics promote both Osteoconduction and Osteoinduction, key-factors in Bone Tissue Formation [Kokubo, 2008].

Apart from the chemical composition, Joseph R. Woordada et. al. (2007) emphasize the role of the Scaffold's Architecture regarding the ingrowth and development of Osteoblasts and Endothelial Cells, namely the pore size and the interconnectivity. There are two major 'classes' of pores, Macroporos - related to the transportation of both cells and nutrients - and Microporosity - increasing the surface area for a better protein adsorption and, consequently, improving its biocompatibility at the same time that provides more sites for cells attachment.

That being said, the Ceramic Based Scaffolds can be classified in three different groups: Calcium Phosphate; Inert Ceramics and Inert Ceramics.

4.1 Calcium Phosphate

Considering the bone mineral structure as described before in subsection 2.1, Calcium Phosphates are, consequently, the most common types of bioceramics used in Bone Tissue Engineering, especially as injectable bone cements or coatings to other materials, as elucidated [Koons et al., 2020].

Those materials can be grouped as: HAp; TCP; BCPs, each one formed by different sintering processes crucial to define their mechanical properties.

HAp based materials present the bigger similarity with the original bone tissue and, therefore, are known to be extremely biocompatible and thermally stable. However, they also struggle with their slow resorption rates as well as low osteoconductivity properties when compared to autografts [Ishikawa et al., 2018].

TCP are materials with a specific structure, being characterized by its Ca/P ratio of 1:5. Due to its stability and easier production methods, β - TCP is the most common type used. When compared to HAp, those ceramics present a higher resorption rate

[Ishikawa et al., 2018].

BCPs are, then, a mixture of the two previously mentioned materials with the goal of retaining both HAp's stability and TCP's solubility. The effect of the Ratio HAp/TCP on osteoblasts due to the release of ions is discussed by some scientists [Bouler et al., 2017], [Liu et al., 2009], [Maeno et al., 2005] and some results show a better solubility and osteoconductivity at 25/75% [Kokubo, 2008].

4.2 Inert Ceramics

When it comes to implants in regions that suffer high loads such as the Hip, the britille characteristic of the Calcium phosphates mentioned earlier makes them an impractical solution to act as a Bone Tissue scaffold. Therefore, Inert Ceramics such as Alumina and Zirconia appear as one possible solution. Those do not interact with its surrounding tissue and are able to bear the corrosion effect without producing high amounts of what is called Wear Debris.

Alumina	Formation of a Lubricious layer. [Kokubo, 2008]	Probable aseptic loosening of the material due to gradual decrease of the resistance to wear. (delayed fracture). [Affatato et al., 2011], [Boyer et al., 2010]
Zirconia	Toughening Mechanism allows it to absorb the load uniformly and increases the resistance to crack propagation [Muñoz et al., 2017]	Suffers from ageing due to the propagation of micro-fractures during its volume expansion [Muñoz et al., 2017]

Table 2: Inert Ceramics characteristics.

4.3 Glass Ceramics/Bioactive Glasses

Although there are several types of Glass Ceramics, Bioglass® is, by far, the most well known for the scientific community. Discovered during the years of 1969-1971, and having a fixed structure composed by 45% SiO2, 24.5% Na2O, 24.5% CaO and 6% P2O5, the in vitro tests showed that the material is able to produce a HAp layer even when in solutions without both Calcium or Phosphate ions, besides that, the HAp crystals formed were strongly attached to the collagen fibrin layer from the osteoblasts, resulting in a product extremely bonded to the host tissue. Results presented in [Hench, 2006] prove that the strength of the Bone-BioGlass® "was equal or greater than the strength of the host bone". The same paper provides an extensive review on both the history and development of this ceramic material [Hench, 2006]. Nevertheless, BioGlass® is still limited by its high concentration of Na , that could change the pH of the environment turning it into a cytotoxic one, as well as its relatively poor "sintering ability", which makes the process of producing a porous 3D scaffold harder [Fernandes et al., 2018].

5 Metal based scaffolds

The use of metals in scaffold production has been limited to hard tissue engineering for dental and orthopedic surgery. They are frequently used as implant materials for bone replacement or as a healing baseline. In order to introduce metals as scaffold materials some surface modifications or coating must be performed in order to increase biocompatibility retaining mechanical properties. Metals can also release toxic ions or particles from corrosion that may lead to some unwanted reactions [Alvarez & Nakajima, 2009].

5.1 Tantalum

With a similar elasticity to bone Tantalum (Ta) is widely used in bone tissue engineering and knee replacement surgeries [Ghassemi et al., 2018]. As a porous biomaterial with a high-volume porosity it allows rapid bone ingrowth [Alvarez & Nakajima, 2009]. Several studies [Bobyn et al., 1999], [Tanzer et al., 2019] show that Ta has very desirable characteristics for bone

ingrowth and a great potential for reconstructive orthopaedics surgeries.

5.2 Magnesium

Magnesium is naturally found in the human body and an essential mineral. As a lightweight and degradable metal it is very used in load bearing orthopedic implants retaining mechanical properties for 12-18 weeks. In the physiological pH range however it corrodes rapidly losing mechanical properties and releasing hydrogen gas. This has led to the abandoning of magnesium as a detriment for stainless steel. There are however techniques to reduce corrosion rate such as using alloying materials and protective coatings [Staiger et al., 2006].

5.3 Titanium

When used as implants or scaffolds for bone ingrowth Titanium (Ti) and it's alloys have shown poor surface interactivity with the surrounding medium [Das et al., 2008]. In order to improve biocompatibility several techniques have been developed such as oxidation (TiO_2), surface of modification and combination chrome-cobalt ($Cr - Co$) alloys and stainless steel with Ti alloys [Ghassemi et al., 2018]. Molybdenum (Mo), Zirconium (Zr) or Niobium (Nb) have also been used in the second generation Ti alloys increasing biocompatibility [Ghassemi et al., 2018]. Nickel (Ni) and Ti alloys have also been developed but have revealed high allergic response and toxicity [Ghassemi et al., 2018]. Although great advantages in metal scaffolds their poor osseointegration make them more of implants than scaffolds. There isn't yet an optimal scaffold for bone tissue regeneration and work seems to be more directed to composite materials with the integration of biomolecules. The perfect scaffold would be a mix of all biocompatible material for example a ceramic coated biocompatible metal.

6 Composites

The best solution at present are composite scaffolds, a combination of ceramics and polymer, or of synthetic polymers with natural polymers. Composite materials include a polymer phase with toughness and compressive strength and an inorganic phase with bioactivity, which improves the mechanical properties and degradation rate.

6.1 Calcium Phosphate Bioceramics and Polymers

Modern chemistry advances have made it possible to manipulate and customize molecular structures. Adding phosphorus to a polymer increases biocompatibility by enhancing tissue contact. The form of these polymers can be linear, cross-linked or interpenetrated polymer networks [Puska et al., 2011]. Although very good as a potential bone substitute they lack bioactive function. To overcome this issue they are combined with calcium phosphate bioceramics. This combination is widely used in bone repair and regeneration.

Hydroxyapatite (HAp) main inorganic component of bones. Combined with many different natural or synthetic polymers and/or growth factors/cells to imitate the natural structure of bone achieves bone formation and regeneration by either enhancing its osteoconductivity, osteoinductivity, or both [Bal et al., 2019].

Tricalcium Phosphate (TCP) less stable than HAp but with a faster degradation rate and higher solubility. It presents a high resorption rate, promotes osteoblasts proliferation and bone marrow stromal cells. Biphasic materials have been developed combining the characteristics of TCP and HAp. They were found to have microporous struc-

tures that influenced cell growth and vascularization [Jeong et al., 2019].

6.2 Bioactive Glass and Polymers

Bioactive Glasses (BGs) are amorphous silicate-based materials that are compatible with the human body, bond to bone, and can stimulate new bone growth while dissolving over time. Combining BGs with biodegradable polymers can turn these composites into great candidates as a bone filler material or 3D scaffolds. As filler material for bone defects and cavities treatment, BGs lack cohesiveness in bulk [Floroian et al., 2011] .A typical application is to incorporate silicate BGs nanoparticles into polymer matrices thus providing an appropriate environment for cell proliferation and a significant improvement of the mechanical properties compared to the polymer alone [Kargozar et al., 2018].

6.3 Silicate Bioceramics and Polymers

The third generation of orthopaedic biomaterials matching surface, chemistry, biological and mechanical properties of natural bone. However a high bioactive ceramic powder ratio is needed reducing the advantages, flexibility and formability of the polymer matrix. Silicon (Si) is an essential element in human body participating in the biosynthesis of collagen and essential for the formation of cytoskeleton [Zhou et al., 2015]. In the implantation process degradation occurs due to the apatite formation resulting from the ionic dissolution products such as Ca^{2+}, Mg^{2+} and Si^{4+} from silicate-based ceramic triggering osteogenesis leading to bone formation and growth [Ahmadipour et al., 2020]. The inclusion of the ceramic phase or coating in the formability of polymers in a controlled-volume fraction can produce a mechanical reinforcement and higher bioactivity of the surface [Ahmadipour et al., 2020].

7 Scaffold Tissue Interaction

Engineering scaffold design with requisite microstructure, topography and surface chemistry can effectively regulate bioactivity and cell growth [Chang & Wang, 2011]. Attributes such as nano and microscale surface topography, architecture, and nature of scaffold play important roles in providing the microenvironment affecting cell responses. Mechanical properties such as stiffness, softness, and elasticity of scaffolds influence cell behaviour. Scaffold surface chemistry and topography are affected by the roughness of the surface, response to wettability, and its mechanical attributes [O'Brien et al., 2005].

Topographical attributes can guide cell alignment and confluence. For vascular tissue engineering, nano and microsized aligned fibers affect confluence of endothelial cells in electrospunpoly (ϵ-caprolactone) scaffolds fabricated with different topographies [Bacakova & Others, 2018]. Macro roughness increases the anchorage of implants on natural tissues and does not restrict their attachment or spreading [Peyton,], [Li et al., 2018].

Polymers conjugated with nano-dimension materials produce favourable outcomes on surface roughness. Interestingly, novel porous scaffolds increased roughness of poly (butylene succinate) matrix contributed towards hydrophilicity of the surface. The increased LAP content of scaffold promoted mineralized apatite formation which in turn favored cellular activity [Tang, 2018].

Mesenchymal Stem Cells (MSCs) sense matrix elasticity and used this information for lineage specification. Thus, matrix elasticity and stiffness can also regulate cell growth. It was observed that stem cell differentiation can get affected by the microenvironment. Research has shown that the elasticity of the matrix exhibits a relationship between substrate stiffness and its impact on the MSC differentiation [Engler et al., 2006].

Surface charge affects adsorbed protein and protein cell interactions, and thus plays a vital role in cell attachment. Enhanced cell attachments and spreading of fibroblasts and osteoblasts have been observed on hydrogels combined with positive charges in comparison with neutral, negative charges or Arginine-glycine-aspartic acid (RGD) grafted ligands [Schneider, 2004].

The Extracellular Bone Matrix (ECM) serves as a microenvironment niche for cells in a pool of polysaccharides and proteins. This natural scaffold is locally secreted to support cellular adhesion, growth, proliferation, differentiation, migration and cell death. The ECM comprises polysaccharides (glycosaminoglycan) and fibrous protein (collagen, elastin, fibronectin, and laminin), along with minerals and water. The extracellular portion of integrins binds to various ECM proteins collagen, laminin, fibronectin, and biomaterial scaffolds to mimic these ECM functions [Arnaout et al., 2005].

Transplantation of cells or biomaterials in vivo can cause immune reactions which can lead to adsorption of proteins with activation of leukocytes, and release of cytokines with fibrous encapsulation. This inflammatory cascade reaction restricts the effective integration of biomaterial in tissues [Bridges et al., 2008]. The exposure of scaffolds to immune cells can be avoided by selecting materials which are inherently inert. Bridges et al. demonstrated an approach to reduce adsorption of protein along with reduced adhesion of monocytes by coating hydrogels with microparticles over the polymeric substrates, and after implantation this setup resulted in reduced cytokines [Bridges et al., 2008].

8 Cell types and their role in bone regeneration

MSCs, play an essential role in organ development and postnatal repair [Bianco & Others, 2013], [Frenette et al., 2013]. In addition to differentiation potential, MSCs also regulate the function of other cells [Wang et al., 2014], [Sui & Others, 2017].

BMMSCs were first discovered by Friedenstein et al [Friedenstein et al., 1976]. BMMSCs have become the most extensively studied MSCs for bone regeneration due to their intimate involvement in bone physiology and pathology [Fernandes & Yang, 2016], [Liu & Others, 2011]. BMMSCs modulated bone homoeostasis via differentiation into osteoblasts and regulating osteoclasts activities but of note, act as potent microenvironmental modulators that exert enormous anti-inflammatory effects after systemic transplantation, which benefit diverse tissues/organs, including bone tissue engineering [Ren & Others,].

Since their discovery by [Zuk et al., 2001], ADMSCs have been increasingly demonstrated to hold great promises in regenerative medicine. Similar to BMMSCs, ADMSCs display steady growth kinetics in vitro and are able to differentiate into various cell types, including osteocytes, chondrocytes and adipocytes. In addition, with a prevalence of lipoaspirates and less morbidity to the host during procurement, ADMSCs are, to some extent, more advantageous than BMMSCs due to easy accessibility and abundant supply [Grottkau & Lin, 2013], [Beane et al., 2014].

Despite the enormous efforts devoted to exogenous MSC transplantation for tissue regeneration, an alternative therapeutic strategy is to take advantage of endogenous MSCs, which reside within specific tissues and are able to self-renew and produce specific cell types.

Exogenous MSCs through different routes has been widely explored in bone and dental regenerative medicine. A promising strategy is the systemic application via primarily intravenous infusion and intraperitoneal delivery, which exerts therapeutic effects on various disorders, including osteoporosis, bone fracture, osteoarthritis [Sui & Others, 2016]. In

this regard, genetic or pharmacological approaches that enhance the homing of MSCs could strengthen their therapeutic efficacy on bone loss and defects

Acronyms

BCP Biphasic Calcium Phosphate.

BGs Bioactive Glasses.

ECM Extracellular Bone Matrix.

FDA Food and Drug Administration.

HAp Hydroxyapatite.

Mo Molybdenum.

MSCs Mesenchymal Stem Cells.

Nb Niobium.

Ni Nickel.

PCL Polycaprolactone.

PEG Polyethylene Glycol.

PGA Polyglycolic Acid.

PLA Polylactic Acid.

PLGA Polylactic Co-Glycolic Acid.

PVA Poly(vinyl)alcohol.

RGD Arginine-glycine-aspartic acid.

Si Silicon.

Ta Tantalum.

TCP Tricalcium Phosphate.

TE Tissue Engineering.

Ti Titanium.

Zr Zirconium.

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