Review paper

UDK 616.314-7:66.017/.018
DOI 10.7251/COMEN2301001N

APPLICATIONS OF BIOMATERIALS IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING – CONCEPTS AND PERSPECTIVE

Stevo Najman[1,2*], Sanja Stojanović[1,2], Jelena Živković[1], Jelena Najdanović[1], Milena Radenković[2], Perica Vasiljević[3], Nenad Ignjatović[4]

[1] Department of Biology and Human Genetics, Faculty of Medicine, University of Niš, Niš, Serbia
[2] Department for Cell and Tissue Engineering, Scientific Research Center for Biomedicine, Faculty of Medicine, University of Niš, Niš, Serbia
[3] Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia
[4] Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Belgrade, Serbia
[*] Corresponding author: stevo.najman@medfak.ni.ac.rs

Abstract: Regenerative medicine (RM) exploits the innate potential of the human body to effectively repair and regenerate damaged tissues and organs with the help of various biomaterials. Tissue engineering (TE) makes it possible to replace damaged tissues and organs with new ones. Research in the field of biomaterials has significantly improved the area of RM and TE. Biomaterials are used as orthopedic, dental, cardiovascular implants, medical devices, in the fields of reconstructive and regenerative medicine, among others. Important preconditions for the biomaterial to be used for implantation are its biocompatibility and biofunctionality. Biomaterials should enable adhesion, migration, proliferation and differentiation of cells. The biological properties of biomaterials are a reflection of their physicochemical properties, such as internal architecture, surface characteristics and charge. Biomaterials used in tissue regeneration should mimic the natural structure of the extracellular matrix and represent a physiological microenvironment for normal cellular functions. These biomaterials should also have adequate biodegradability properties to facilitate the formation and growth of new tissue. Biomaterials for use in RM can be of natural or synthetic origin, polymers by structural properties, ceramic and composite type, and based on bioreactivity they can be bioinert or bioactive. In RM and TE, polymers of different classes, natural and synthetic, are used, which can be made as intelligent materials. The structure of hydrogels in the form of a porous network represents a good matrix for cell activity. Ceramic biomaterials based on hydroxyapatite (HAp) are selected for use in RM and TE, especially solid tissues. Properties, such as composition, particle size, material shape, porosity, surface charge, topography, etc., are relevant for the proper use of HAp materials. The properties of HAp allow modification of its structure, surface, particle size design at the micro and nano level, hybridization with polymers, metals, etc. which is very important for its applications. Designed micro-nanohybrid HAp structure is most similar to the bone structure, making the cell environment closest to natural. Bone tissue engineering (BTE) is based on the combined use of cells, osteostimulating (osteoinductive) factors and biomaterials as a scaffolds and carriers for bone regeneration and defects repair. In BTE adipose-derived mesenchymal stem cells (ADSCs) are often used that are induced *in vitro* towards osteogenic cells or endothelial cells, and freshly isolated stromal vascular fraction can also be used. Blood components (PRP, blood plasma or blood clot) can be included in the composition of the BTE construct as a source of osteoinductive factors. *In vitro* models and methods were used to examine the biocompatibility, immunomodulatory and regenerative potential of biomaterials, as well as their influence on cellular functions. After *in vitro* methods, and before clinical studies, various *in vivo* animal models are used to examine the regenerative potential of biomaterials, such as subcutaneous implantations and bone defects in tibia, femur and calvaria in experimental animals (mouse, rat, rabbit).

Keywords: Regenerative medicine, Bone tissue engineering, Biomaterials, Tissue scaffolds, Physicochemical properties, Biomaerials classification, Material bioactivity, Hydrogels, Hydroxyapatite, Adipose-derived mesenchymal stem cells, Macrophages, *In vitro* methods, *In vivo* animal models.

1. INTRODUCTION

Regenerative medicine (RM) is an interdisciplinary field of medical research and clinical practice that deals with therapies and techniques to repair, replace or regenerate damaged or diseased tissues and organs in the body. It utilizes a variety of strategies, including tissue engineering, and components like stem cells, growth factors or gene therapy to exploit the innate potential of the body for healing processes and tissue repair and regeneration. Techniques used in RM often involve biomaterials, which provide the microenvironmental conditions, a scaffold, or a guide for the growth of new tissue. Regenerative medicine research offers a promising approach for treating many diseases and injuries, including heart disease, diabetes, neurological disorders, degenerative joint conditions, skeletal injuries, among others [1].

Tissue engineering (TE) is a field of biomedical engineering that combines engineering principles with biology for the replacement, repair, or regeneration of damaged tissues or organs. TE techniques involve the use of cells, biomaterials, and growth factors to create functional tissues which serve to replace diseased or damaged tissue. Typical procedures and steps that are included totally or partially in tissue engineering are: scaffold preparation, cell isolation and expansion, cell seeding onto the scaffold, growth factor stimulation and cell differentiation (tissue maturation), as well as implantation. These procedures have been used so far to create many tissues and organs such as skin, bones, cartilage, liver, heart and others with the ultimate goal of obtaining functional tissues or organs that can be transplanted into patients to treat disease or injury [2,3].

2. BIOMATERIALS

Nowadays, biomaterials have a wide range of applications in various fields of medicine and dentistry, including orthopedics, oral and maxillofacial surgery, cardiovascular surgery, reconstructive and regenerative medicine, tissue engineering and others. They are used as orthopedic, dental, and other type of implants, cardiovascular and other medical devices, scaffolds for tissue regeneration and repair, and drug delivery systems, among other things [4,5]. Research in the field of biomaterials has greatly improved the development and application of regenerative medicine approaches and tissue engineering. With advances in biomaterials research, there is a growing potential for the development of new and innovative therapies and treatment strategies for a range of medical conditions, as well as diseases and injuries that were previously difficult to treat. It also enables the development of personalized medicine, as biomaterials and their products are created so that they can be adapted to the specific needs of the a patient [4,5].

2.1. Properties of biomaterials as prerequisite and requirement to be used in regenerative medicine and tissue engineering

Important preconditions for the biomaterial to be used in regenerative medicine and tissue engineering are its biocompatibility and biofunctionality. Biomaterial is considered biocompatible if it is non-toxic and allows the body to function without complications or unwanted side effects and to generate the most appropriate beneficial response of cells and tissues [6]. Biocompatibility is an essential precondition for any biomaterial to be used for implantation in the body. Biocompatibility refers to the ability of a material to interact with living tissue or biological systems without causing any harmful effects, an adverse response, or rejection. Biocompatibility ensures that the implantable biomaterial does not cause a negative immune response of the body, toxic effects, or other complications that could threaten the safety and effectiveness of the implant [7]. The biocompatibility of a biomaterial depends on a variety of factors, including its physicochemical characteristics, surface properties, degradation rate, immunogenicity, and bioactivity [6].

Biofunctionality as an essential prerequisite for biomaterials to be used for implantation refers to the biomaterial's ability to perform a specific biological function, such as promoting tissue regeneration, guiding tissue growth, preventing infection, promoting cell adhesion, or delivering therapeutic agents. Biofunctionality is important to ensure that the biomaterial can interact with surrounding tissues and facilitate the desired biological response. The biomaterial biofunctionality is determined by a variety of factors, including its physicochemical characterization, biocompatibility, biodegradability, and characteristics of its interaction with biological systems [8].

The ability to enable cell adhesion, migration, proliferation, and differentiation is a key requirement

for many biomaterials used in regenerative medicine and tissue engineering. These processes are critical for the growth and repair of tissues, and biomaterials that can facilitate or enhance these processes can be useful in a variety of biomedical applications [9]. Adhesion is the process by which cells attach to a surface or substrate, and it is essential for the formation of tissues and organs. Surface properties such as surface chemistry, surface roughness, and surface charge can affect the ability of cells to adhere to the material surface [10]. Cell migrations are important for the integration of the biomaterial with the surrounding tissue. The pore size, porosity, and mechanical properties of the biomaterial can influence cell migration [11]. Biomaterials that promote cell proliferation and differentiation can help to accelerate the growth of new tissue and promote the repair of damaged tissue. Cell proliferation and differentiation, also, depend on physicochemical characteristics of biomaterial [9,12].

2.2. Physicochemical properties of biomaterials

The behavior of biomaterials in biological systems, namely its biological properties, is critically determined by their physicochemical properties, such as chemical composition, degradation properties (biodegradability), surface properties, internal architecture, and mechanical properties [13].

Chemical composition

The chemical composition of biomaterials may have a significant impact on its biocompatibility, degradation rate, as well as on mechanical, surface and other properties, and thus on its application. Biomaterial's molecular weight, as a chemical characteristic, can affect its other properties, such as solubility, viscosity and mechanical strength, so for example, high molecular weight polymers are often used in implantable devices due to their superior mechanical properties [14]. Crystallinity can affect the physical and mechanical properties of biomaterials, such as density, transparency and hardness. Many materials can be prepared in such a way that a mixture of crystalline and amorphous regions is obtained [15,16].

Biodegradability properties

Biodegradability is an important property of some biomaterials and refers to the ability of biomaterials to break down over time through natural processes in the body and to be absorbed. Biomaterials should have adequate biodegradability properties to facilitate the formation and growth of new tissue. The degradation of the biomaterial should be slow enough to ensure that the scaffold is present for an adequate period of time to support the growth of new tissue. If biomaterial degrades too quickly it may not provide sufficient support for tissue regeneration, while a biomaterial that degrades too slowly may induce a foreign body response and interfere with tissue regeneration [17,18]. The use of biodegradable polymers such as polylactic acid or polyglycolic acid can reduce the risk of long-term foreign body response and improve tissue integration [19].

The rate and mechanism of degradation can affect its biocompatibility and long-term efficacy, and these are influenced by factors such as chemical composition, cross-linking, molecular weight, structure and surface properties. Biodegradable polymers, such as polylactic acid (PLA) and polyglycolic acid (PGA), can be designed to have specific degradation rates by adjusting the molecular weight [20]. These polymers can also be used as a drug delivery systems where controlled release can be accomplished by adjusting the content of the active substance [21]. In addition, incorporation of other materials, such as ceramics or bioglass, can improve the mechanical properties of the scaffold and modify its biodegradability, as well as a tissue response to the biomaterial, stimulating the release of growth factors or other signaling molecules that promote cell proliferation and differentiation [22].

Degradation of biomaterials can result in the release of degradation products that can affect the biocompatibility. Biomaterial degradation products should be non-toxic and easily removed from the body in order to avoid any harmful effects on the surrounding tissues [23]. The toxicity and inflammatory potential of these products depend on the chemical composition and biomaterials' degradation rate [24,25].

Surface properties

Surface properties are important for biomaterial interaction with biological systems, because they have direct contact with host cells, tissues and body fluids. Biomaterials' surfaces have different characteristics such as wettability - hydrophobicity (surface energy), surface textures - roughness (smooth/rough), surface chemistry - chemical composition, functional groups, topographic factors and surface charge. These properties play an important role in

determining biomaterial biocompatibility and functionality, as they can influence cell behavior - adhesion, migration, proliferation and differentiation, as well as its ability to resist healing and stimulate tissue growth and the body's immune response.

The chemical properties of the biomaterials' surface, such as chemical composition, functional groups, charge, polarity and hydrophobicity, can influence its interactions with biomolecules and cells, affecting cell behavior [26]. Surface energy and hydrophobicity (wettability) of biomaterials can affect protein adsorption, which in turn can affect cell adhesion, migration and differentiation. For example, a material with a hydrophilic surface can promote cell adhesion and proliferation, while a material with a hydrophobic surface can inhibit cell growth [27]. Regarding the surface texture, a biomaterial with a rough surface can provide a better substrate for cell adhesion than a smooth surface [28].

The surface charge of biomaterials can have a significant impact on cell behavior and on various cellular processes, such as adhesion, proliferation, differentiation and signaling. This influence can be realized through affecting the adsorption of charged biomolecules, such as proteins (growth factors) and ions [29]. Since the charge of biomaterials can play a significant role in modulating various cellular behaviors and processes, understanding its effects is very important in the design and development of biomaterials for applications in tissue engineering and regenerative medicine [26,29-31].

Surface engineering and modifications, such as plasma treatment or chemical modification, can be used to tailor the surface properties of a biomaterial in order to improve its performance and allow clinicians to have better control over biomaterial's interactions with the living host system [32].

Internal architecture

A biomaterial's internal architecture or microstructure, which includes parameters such as pore size, pore interconnectivity, and surface area, can influence cell adhesion, migration and proliferation, and finally tissue regeneration. Therefore, a scaffold used for tissue engineering should have a porous structure [33]. A biomaterial with a highly porous structure with interconnected pores can facilitate the diffusion of nutrients and waste products, thus providing a large surface area for cell adhesion, migration and proliferation, as well as better support for tissue growth, while a material with small or disconnected pores can inhibit cell growth. Biomaterials can be designed to have specific porosity to promote tissue ingrowth and vascularization which can be controlled by its production process [34,35].

Mechanical properties

Biomaterials must have appropriate mechanical properties, such as strength, stiffness and elasticity, to have the ability to withstand mechanical loads and deformations *in vivo* [36]. These properties are influenced by the composition, atomic and molecular structure and processing of the material. For example, materials used in load-bearing implants must have sufficient strength and stiffness to withstand stresses, like materials used in bone repair [37], while materials used in soft tissue repair must be flexible and elastic to accommodate a movement [38]. In addition, a biomaterial whose stiffness is similar to that of the surrounding tissue can promote cell adhesion and proliferation, while a material that is too stiff or too soft can inhibit cell growth and differentiation [39,40].

Generally, since the biological properties of a biomaterial are a reflection of its physicochemical properties, by carefully controlling the physicochemical properties of biomaterials, researchers can design materials that can interact with biological systems in a way that promotes tissue regeneration and repair and have good potential for use in tissue engineering and application in regenerative medicine.

2.3. Biomaterials used in tissue regeneration mimic extracellular matrix

Biomaterials used in tissue regeneration should mimic the natural structure of the extracellular matrix (ECM) and represent a physiological microenvironment for normal cellular functions [41].

The ECM is a complex network of proteins, glycoproteins, and proteoglycans that plays a critical role in regulating cellular activity, and provides a physical and biochemical microenvironment for cells to perform their normal functions. Biomaterials that mimic the natural structure of the ECM should provide a scaffold for new tissue formation and normal cellular functions. This can be achieved through the design of biomaterials with specific surface properties, as well as appropriate microstructural and mechanical characteristics. A biomaterial that is too stiff

or too soft may affect cell behavior, while a material that lacks the appropriate biochemical cues may not promote cell adhesion and differentiation. Biomaterials composed of natural ECM proteins, such as collagen, fibronectin, hyaluronic acid, or fibrin can promote cell adhesion, migration, proliferation, and differentiation, as well as represent a substrate for biochemical signaling, ultimately leading to tissue regeneration and repair. Alternatively, synthetic biomaterials can be designed to mimic the structure and function of the ECM, for example, by incorporating specific functional groups or by controlling stiffness or porosity [39,40,42,43].

These principles are particularly important for tissue engineering applications, where biomaterials are used to create three-dimensional scaffolds that can support growth and development of new tissue. For example, a biomaterial used in bone regeneration should mimic the mechanical properties of bone tissue, such as its stiffness and elasticity, and also mimic the chemical composition of bone ECM, such as the presence of hydroxyapatite [44].

3. CLASSIFICATIONS OF BIOMATERIALS

3.1. Classification according to origin

Biomaterials for application in regenerative medicine can be of natural or synthetic origin. Naturally-derived biomaterials are obtained from biological sources such as tissues, extracellular matrix and proteins. Examples of naturally-derived biomaterials include collagen, gelatin, fibrin, chitosan, hyaluronic acid, and other extracellular matrix (ECM) components [45]. These materials are attractive for use in tissue engineering and regenerative medicine because they are biocompatible, biodegradable and can mimic the biochemical and mechanical properties of natural tissue and the natural microenvironment of cells. Natural biomaterials can also contain binding sites for cells, growth factors, and other bioactive molecules that can enhance tissue regeneration [9].

Synthetic biomaterials are made from non-biological materials such as polymers, ceramics and metals, are usually derived from chemical synthesis and can be tailored to have specific properties such as mechanical strength, degradation rate and surface chemistry. Examples of synthetic biomaterials include polyesters, polycarbonates, polyurethanes, poly(lactic-co-glycolic) acid (PLGA), polyethylene glycol (PEG), and titanium alloys [46,47]. Synthetic biomaterials can be used alone or in combination with natural biomaterials to create hybrid materials and can be designed with specific properties such as mechanical strength, biodegradability and surface chemistry, which makes them widely applicable in tissue engineering and regenerative medicine [9]. So for instance, a hybrid biomaterial made of natural collagen and synthetic polyethylene glycol (PEG) can combine the biocompatibility and scaffolding properties of collagen with the tunable mechanical properties and degradation rate of PEG [46]. By carefully selecting and designing biomaterials, researchers can create scaffolds that effectively guide tissue regeneration and repair.

3.2. Classification based on bioactivity

Based on bioactivity, biomaterials for application in regenerative medicine and tissue engineering can be classified as bioinert or bioactive. Bioinert biomaterials are characterized by their ability to resist degradation and do not interact with biological systems. They are designed to be stable in the body over a long period of time while maintaining their structural integrity, without causing a significant immune response or inflammation in the surrounding tissue [48]. Examples of bioinert materials include some synthetic polymers and metals. Bioinert biomaterials are often used in applications where the biomaterial will not be resorbed or integrated into the surrounding tissue, such as orthopedic implants [49].

Bioactive biomaterials actively interact with the biological environment and are able to stimulate cell growth and differentiation. These biomaterials stimulate the formation of new blood vessels thus stimulating cell and tissue responses that lead to tissue regeneration and repair. Bioactive materials can also be used as scaffolds to deliver growth factors and other signaling molecules to promote tissue regeneration [50]. Examples of bioactive materials are bone substitutes that include some types of ceramics, such as hydroxyapatite (HAp), and certain types of bioglass and composites [51]. Biomaterials that can be used as bone substitutes are classified into osteoconductive, osteoinductive and osteogenic biomaterials [52]. Osteoconductive biomaterials provide a scaffold for the formation of new bone tissue, osteoinductive biomaterials have the ability to stimulate the differentiation of stem cells into bone-forming

cells, while osteogenic biomaterials have both osteoinductive and osteogenic properties [53,54].

Biomaterials can be designed to have both bioinert and bioactive properties. By properly selecting and designing biomaterials, scaffolds can be made that effectively direct tissue regeneration and repair processes [51].

4. TYPES OF BIOMATERIALS ACCORDING TO STRUCTURAL PROPERTIES

In regenerative medicine and tissue engineering, different types of biomaterials are used according to their structural properties, and the most commonly used are polymers, ceramics and composites.

4.1. Polymers

Polymers are popular class of biomaterials due to their versatility, biocompatibility, and adjustable mechanical properties. Polymers can be further classified into synthetic, natural and hybrid polymers [46]. Synthetic polymers include poly(lactic-co-glycolic) acid (PLGA), polyethylene glycol (PEG) and polycaprolactone (PCL), and for regenerative medicine applications can be designed with specific properties such as biodegradability, mechanical strength, and surface chemistry [23,55]. Natural polymers such as collagen, hyaluronic acid and chitosan are derived from biological sources and can provide inherent biocompatibility and biodegradability. Hybrid polymers combine both synthetic and natural polymers to achieve desired properties for applications in tissue regeneration and repair [46].

Hydrogels

Hydrogels are a type of polymers that can absorb and retain a large amount of water or biological fluids due to their hydrophilic nature. They are three-dimensional networks of polymer chains that are cross-linked to form a gel-like structure. The porous network structure of hydrogels can represent a good matrix for cell activity in tissue engineering and regenerative medicine, especially since they can be designed to have a porous structure that closely mimics the extracellular matrix (ECM) of natural tissues [56]. The porous structure of hydrogels allows the diffusion of nutrients, oxygen and signaling molecules, as well as the removal of waste products, in order to support cell growth and function [46]. Pore size and distribution within the hydrogel can also be controlled to create an environment that is suitable for specific cell types and tissue types. Additionally, hydrogels can also be modified by incorporating bioactive molecules such as growth factors, cytokines and extracellular matrix proteins, which can improve cell adhesion, proliferation and differentiation, which can help promote tissue regeneration and repair [57].

According to their origin, hydrogels can be natural or synthetic. Natural hydrogels are derived from natural polymers such as collagen, hyaluronic acid, chitosan, alginate and gelatin. They are biocompatible and biodegradable, and can be used in a variety of applications, including soft and bone tissue engineering, wound healing and drug delivery [56]. Synthetic hydrogels are made from synthetic polymers such as polyethylene glycol (PEG), polyvinyl alcohol (PVA) and polyacrylamide (PAAm). These hydrogels can be designed to have specific mechanical and chemical properties and can be used in applications such as drug delivery, biosensors and tissue engineering [23].

In regenerative medicine and tissue engineering from different classes of polymers, both natural and synthetic, researchers are investigating the development of intelligent biomaterials that can respond to changes in their environment to improve tissue regeneration. Intelligent (smart) biomaterials can be designed to respond to stimuli such as temperature, pH, light, electric fields and mechanical stress [58]. Thanks to such interaction with biological systems, they provide the possibility for controlled release of therapeutic agents, for more precise control over tissue regeneration and repair, and generally enable more efficient and personalized treatments for patients.

4. 2. Ceramics

Ceramics are another class of biomaterials commonly used in TE and regenerative medicine applications. Ceramic biomaterials are for example bone substitutes such as hydroxyapatite (HAp) and tricalcium phosphate (TCP) which are commonly used as scaffolds for bone regeneration due to their ability to integrate with the surrounding bone tissue.

Hydroxyapatite

Ceramic biomaterials based on hydroxyapatite are often chosen for use in regenerative medicine and TE, especially for hard tissues such as bone, teeth,

and cartilage. Hydroxyapatite is the main inorganic component of bone tissue, so it can be used to create scaffolds that closely mimic the structure and composition of natural bone. It has excellent biocompatibility, osteoconductivity and biodegradability, and is also a bioactive material, which means it can stimulate the formation of new bone tissue by promoting the attachment, proliferation and differentiation of osteoblasts, the cells responsible for bone formation. All this makes it an ideal biomaterial for use in bone tissue engineering and bone regeneration [13].

In addition to bone tissue engineering, HAp-based ceramics have also been investigated for applications in cartilage engineering [59], dental implants [60], and drug delivery [61]. HAp can be used alone or in combination with other biomaterials, such as polymers [62] or metals [63], to create composite scaffolds with improved properties. HAp has been shown to promote chondrogenesis (cartilage formation) and can be combined with other materials such as collagen to create scaffolds that support cartilage regeneration [64]. HAp has also been used as a coating on dental implants to improve osseointegration (integration with surrounding bone tissue) and promote long-term stability [60].

Properties, such as composition, particle size and shape, porosity, surface charge, topography, etc., are relevant for the proper use of HAp materials [13]. The size of HAp particles can influence their biological response and interaction with cells, such as ability to be phagocytized by cells, their rate of degradation and their osteoinductive potential. Different material shapes, such as fibers [65], particles [66] or blocks [67], can affect cell adhesion, migration and proliferation, mechanical properties and the way the material interacts with other tissues in the body. The porosity of HAp materials is an important factor for tissue engineering applications, so high porosity can improve cell and blood vessel infiltration and substance diffusion, but may compromise mechanical strength. Porous HAp materials can be designed to have a similar structure to the natural bone, which can improve their ability to integrate with surrounding tissues [68].

The nanoscale characteristics of HAp can also provide a scaffold for cell growth, promoting their differentiation and the formation of new bone tissue [69]. HAp biomaterial can be designed at both micro and nano level to create hierarchical structures that mimic the natural bone structure. Designed micro-nanohybrid HAp structure is most similar to the bone structure, making the cell environment closest to natural one [70-72].

4.3. Composites

Composites are biomaterials that combine the properties of two or more materials, each of which contributes to the unique properties to achieve the desired characteristics to create a scaffold with tailored properties that make it an effective scaffold for tissue regeneration applications. For example, a composite scaffold can be created by combining polymers with ceramics to create a structure that has both mechanical strength and bioactivity. Composites can also be designed to degrade over time, providing a temporary scaffold for tissue growth. Examples of composite biomaterials include PLGA/TCP, collagen/hydroxyapatite, HAp-PLLA, among many others [73].

5. BONE TISSUE ENGINEERING

Bone tissue engineering (BTE) is based on the combined use of cells, osteostimulating (osteoinductive) factors and biomaterials of natural and artificial origin as a scaffolds and carriers for bone regeneration and defects repair [74]. These three components represent a biological triad that imitates the natural environment in order to regenerate bone tissue [2,75].

The scaffold provides a three-dimensional structure that mimics natural bone tissue, allowing cells to adhere, proliferate and differentiate into bone-forming cells and other cell activities [76-78]. They also act as carriers of the second biological triad component - osteoinductive factors, such as growth factors and cytokines, which regulate cellular activities essential for bone formation. Natural biomaterials that can be used as scaffolds in BTE include collagen, fibrin and various components of the extracellular matrix (ECM), whereas synthetic ones are of the ceramic, polymer or composite type [51].

In order to assess the possible impact on the dynamics and outcome of the osteogenic process, numerous studies were conducted on the role of PRP as a source of osteoinductive factors [79]. PRP is a concentrate of platelets and growth factors derived from blood, such as the patient's own blood. These factors can stimulate cell proliferation and differen-

tiation, thereby stimulating tissue regeneration and repair, including bone [80-82]. PRP can be used as a scaffold or carrier for cells and combined with biomaterials as their supplement to increase the osteogenic potential of cells. Several *in vitro* and *in vivo* studies using PRP in BTE constructs have shown promising results for increased bone repair and regeneration [78,82-87]. However, more research is needed to fully understand the mechanisms of PRP-induced osteogenesis and to optimize its use in clinical applications.

5.1. Mesenchymal stem cells in bone tissue engineering

Cells represent the third component of the biological triad in BTE. For this purpose, mesenchymal stem cells (MSCs) that are derived from different sources including bone marrow and adipose tissue [88], as well as induced pluripotent stem cells (iPSCs), can be used [89,90]. MSCs have the ability to differentiate into osteoblasts and form bone tissue [83]. Other cell types, such as osteoblasts, chondrocytes, endothelial cells, macrophages, and others have also been used in BTE.

Stem cells can be obtained from readily available and abundant source – adipose tissue [91]. Adipose-derived mesenchymal stem cells (ADSCs) have been used in several studies for applications in tissue engineering and regenerative medicine, including for bone regeneration purposes. ADSCs have the ability to differentiate into various cell types, including osteoblasts, chondrocytes, endothelial cells, and adipocytes [83,88,91]. This feature makes them a good source of cells for bone regeneration and application in regenerative medicine in general.

The process of isolating ADSCs from adipose tissue usually involves digesting the adipose tissue with enzymes and then centrifugation to separate the stromal vascular fraction (SVF) from the adipocytes [83-85]. The SVF contains a heterogeneous population of cells including mesenchymal stem cells, endothelial progenitor cells, endothelial cells, pericytes, preadipocytes, adipocytes, and immune cells [92,93]. The resulting SVF could be used directly or further processed using various techniques to isolate specific cell populations, such as ADSCs. Further, ADSCs can be expanded in culture and differentiated into different cell types for application in regenerative medicine and tissue engineering.

One possibility for application of ADSCs in BTE is to use them as freshly isolated stromal vascular fraction of adipose tissue [85,94,95]. In this case, potential of the SVF for the bone regeneration is tested without previously undergoing *in vitro* expansion process. By using this approach, encouraging results were obtained in an ectopic mouse bone formation model. Specifically, it was found that simulating an intraoperative procedure with freshly isolated SVF cells from adipose tissue combined with platelet-rich plasma (PRP) delivered on a bone mineral matrix (BMM) carrier rapidly initiated osteogenesis [85]. However, the use of SVF has some limitations, such as the lower yield of ADSCs compared to culture-expanded cells, the potential presence of unwanted cells, and the lack of standardization in the isolation procedure [96]. Therefore, the application of freshly isolated SVF in bone regenerative medicine requires more research for further optimization.

In some studies, researchers have used ADSCs *in vitro* induced towards osteogenic cells and/or endothelial cells in order to facilitate bone regeneration and repair of bone defects [83,84,86]. ADSCs can be induced *in vitro* into osteogenic cells, through induction of osteogenic markers gene expression, which can be achieved by using various growth factors and other osteogenic factors [97,98]. ADSCs can be seeded onto biomaterial scaffolds, cultivated *in vitro*, which allows them to differentiate into osteogenic cells and form new bone tissue, and then be implanted ectopically [99]. Another approach is to induce ADSCs into osteogenic cells *in vitro*, and afterwards to construct the implants out of differentiated cells, as a source of growth factors and a biomaterial-carrier as a scaffold. This combination triggered advanced and well-balanced osteogenic process when implanted ectopically [83].

Blood vessels formation is necessary condition for bone formation [100]. Endothelial cells are a primary cell type found in blood vessels lining their inner surface and are responsible for maintaining vascular integrity and regulating blood flow [101]. The ability to generate functional endothelial cells from stem cells is important for tissue engineering applications that require the formation of new blood vessels [86]. To induce ADSCs to differentiate into endothelial cells, researchers used a combination of growth factors and culture conditions that mimic the *in vivo* environment of endothelial cells [84,102]. Some commonly used growth factors include vas-

cular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and angiopoietin-1 (Ang-1), among others [103-105]. These growth factors can stimulate the expression of genes and proteins involved in the development and function of endothelial cells.

ADSCs can also be *in vitro* induced into endothelial cells and then used for prevascularization of scaffolds. Further, ADSCs induced into osteoblasts can be added to prevascularized scaffolds and such constructs can be implanted in order to repair critical-sized bone defects [106]. Also, ADSCs *in vitro* induced into endothelial cells and ADSCs *in vitro* induced into osteoblasts can be combined and then seeded onto biomaterial-carrier enriched with a potent source of growth factors such as PRP [86]. Both approaches led to the improvement of vascularity, and subsequently to the appearance of signs of the osteogenic process.

5.2. Macrophages in bone tissue engineering

Macrophages are immune cells that play an important role in the inflammatory process, in the regulation of the immune response, and during tissue repair and regeneration. They are involved in the removal of dead cells and debris, and they also secrete various cytokines and growth factors that can influence cell behavior and tissue regeneration. Macrophages secrete cytokines and osteoinductive factors that promote osteogenesis and are involved in bone remodeling and regeneration, such as transforming growth factor-beta (TGF-β) and bone morphogenetic proteins (BMPs). The position and roles of macrophages in bone tissue engineering are complex and depend on factors such as their phenotype, state of activation and interactions with other cells and biomaterials in the microenvironment. Studies have shown that macrophages can have both positive and negative effects on bone regeneration. For example, M2 macrophages, which are involved in tissue repair and remodeling, secrete growth factors and cytokines that promote osteogenesis and angiogenesis, while on the other hand, M1 macrophages, which are involved in the inflammatory response, produce proinflammatory cytokines that inhibit bone regeneration [32,107].

In bone tissue regeneration, macrophages are recruited to the site of injury or incorporated into a scaffold to enhance the osteogenic process, and in each approach they interact with other cells and thus affect the regenerative process. In one study, macrophages were co-cultured with bone marrow-derived mesenchymal stem cells (BMSCs) on a hydroxyapatite scaffold, and the resulting construct showed increased osteogenic differentiation and mineralization compared to BMSCs cultured alone [108].

Studies have suggested that modulating the phenotype and behavior of macrophages may be a promising approach to enhance bone regeneration. Research has been carried out on the use of biomaterials that can modulate the behavior of macrophages in BTE constructs. For example, developed biomaterials with surface charge and chemical functional groups can influence the polarization of macrophages towards an anti-inflammatory phenotype. These materials have been shown to improve bone regeneration in animal models [109].

Inclusion of macrophages in the implants based on hydroxyapatite and in combination with a blood clot has a beneficial effect on the osteogenic process *in vivo*. This result was demonstrated in the case of both resident and inflammatory macrophages included in subcutaneous implants, unlike implants without these cells. In this way, it was shown that macrophages, adapting to the environment in which they are located, positively act on angiogenesis, collagen production, and maturation of osteoblast-like cells, probably through secretion of various molecules. Their combination with blood clot and hydroxyapatite aimed to mimic the situation at the site of a bone fracture in macrophage-enriched environment. The results of these studies showed that there is a great potential for the application of macrophages in bone tissue engineering and orthopedic surgery [110,111].

The specific role of macrophages in the context of BTE is still an active area of research and more studies are needed to fully understand their effect on the osteogenic process and to optimize the design and development of scaffolds and therapies for bone repair and regeneration.

6. TESTING BIOMATERIALS FOR USE IN REGENERATIVE MEDICINE

6.1. *In vitro* models and methods for testing biomaterials

In vitro models and methods have been used to examine the biocompatibility, immunomodulatory and regenerative potential of biomaterials, as well as their influence on cellular functions.

In vitro cell models enable investigation of specific cellular and molecular interactions that may not be possible *in vivo*, but can provide preliminary data for selecting biomaterials for further *in vivo testing*. Using *in vitro* cell models, researchers can test the effects of biomaterials on different cell types, tissues and biological processes under precisely controlled conditions and observe cellular responses and functions over time allowing a better understanding of the molecular mechanisms underlying the observed effects. These models can be simple 2D cell cultures or complex 3D tissue engineering models that simulate the *in vivo* microenvironment. Cells are directly exposed to biomaterials *in vitro* by being grown on or within the biomaterials, or the biomaterials are tested indirectly via their extracts [112-115].

In vitro tests can be performed to evaluate various cellular functions, activities and responses, such as cell adhesion, migration, proliferation, differentiation, gene expression, which are important factors and parameters for assessing the ability of biomaterials to support tissue regeneration and repair. Cytotoxic and genotoxic effects of biomaterials and their decomposition products are tested *in vitro*, which is important for assessing their safety in medical applications [112,116]. Morphology is also studied and morphometry of living cells is performed, as well as examination of various functions and activities such as various products secretion. *In vitro* methods can also be used to study the interaction between biomaterials and immune cells, such as macrophages or dendritic cells, to determine whether the material can induce an immune response or has immunomodulatory effects [116-119]. Additionaly, wound healing effects of biomaterials intended for wound management can be studied *in vitro* on cell models as well [21,117].

In vitro studies can help guide the development of new biomaterials for applications in regenerative medicine and tissue engineering and can help researchers identify optimal biomaterials for specific applications. However, it is important to keep in mind that results obtained from *in vitro* studies cannot always be translated to *in vivo* situations, as the *in vivo* environment is much more complex and dynamic. Therefore, *in vitro* studies are often followed by *in vivo* studies in animal models to confirm the results.

6.2. *In vivo* experimental models for testing biomaterials

Various *in vivo* animal models of implantation are used in research for the examination of the regenerative potential of biomaterials. *In vivo*, experimental models have been used to assess the biocompatibility, safety, and efficacy of biomaterials in a more clinically relevant context before proceeding to human clinical trials [120]. The usage of animal models with orthotopic or ectopic implantations is important because it allows researchers to examine the regenerative potential of biomaterials in a more complex and dynamic environment than *in vitro* models [121,122]. *In vivo* models with bone defects provide a controlled environment for tracking the effects of biomaterials on various factors, such as immune response, vascularization, new bone formation, integration of the implanted biomaterial with the surrounding tissue, and mechanical stability, which are key to successful bone regeneration and repair.

Bone tissue injuries are often examined in models of the defects of calvaria, jaw bones, and long bones (most commonly the tibia or femur) [120-122]. Bone defect models typically involve surgically removing a section of bone and creating critical-sized bone defects in the bone of experimental animals (mouse, rat or rabbit). Afterward, the biomaterial is implanted into the defect site to assess its ability to promote bone regeneration and repair. The choice of animal model and the location and size of the bone defect are important considerations in these experiments. For example, the calvarial defect model is commonly used to study bone regeneration, because the skull is easily accessible and the defect can be created in a reproducible manner. On the other hand, femoral or tibial defect models are used to study larger bone defects and the ability of biomaterials to promote healing in load-bearing bones. Additionally, the calvaria has a poor vascular network, while biomaterial in the full-depth defects in the long bone has direct contact with the bone marrow. In intraosseous implantation models, polymers as a collagen can be implanted alone [123], or in combination with bone substitutes [124,125]. The choice of bone defect model to be used for testing biomaterials *in vivo* depends on the biomaterial characteristics and its potential application as well.

The subcutaneous implantation model is a common method for rapid assessment of biocompatibility and basic biological properties of biomateri-

als, so this model can be used for initial screening of biomaterials after which potential clinical applications can be considered [126-128].

The site of implantation and tissue surrounding the implanted biomaterial is usually examined, thus providing insight into the host's response to the implanted material, including inflammation, cellular infiltration, fibrosis, foreign body reaction, adverse reactions, and possible toxic effects [126-130].

The effectiveness of biomaterials in stimulating bone regeneration can be evaluated by different methods. Imaging techniques such as X-ray imaging, computed tomography (CT), and magnetic resonance imaging (MRI) can provide information on the degree of new bone tissue formation and the integration of biomaterials with the surrounding tissue [131]. Histological analysis involves examining tissue samples from the defect site to assess cellular and molecular changes associated with bone regeneration, including the presence of new bone tissue, blood vessels, and other components of the extracellular matrix [126-130].

Mechanical testing can be used to assess the strength and stiffness of the regenerated tissue, providing an indication of its suitability for load-bearing applications.

In general, *in vivo* experimental models provide a valuable tool to assess the regenerative potential of biomaterials in a biologically relevant context and potential clinical application, and guide the development of new materials with improved regenerative properties. However, it should be noted that the use of animal models requires prior careful ethical and practical considerations.

7. CONCLUSION

Regenerative medicine as a field of medicine that deals with therapies, strategies and techniques for repairing, replacing or regenerating damaged or diseased tissues and organs in the body today offers a promising approach in the treatment of many diseases and injuries, and in the future even more. Tissue engineering methods have so far created many tissues with the ultimate goal and perspective of obtaining functional tissues or organs that can be transplanted into patients to treat disease or injury. Research in the field of biomaterials has significantly improved the field of RM and TE. Important prerequisites for the biomaterial to be used for implantation are its biocompatibility and biofunctionality. Biomaterials should also enable adhesion, migration, proliferation and differentiation of cells.

Since the biological properties of a biomaterial are a reflection of its physicochemical properties, by carefully controlling the physicochemical properties of biomaterials, researchers can design materials that can interact with biological systems in a way that promotes tissue regeneration and repair and has good potential for application in RM and TE. The choice of natural or synthetic, bioactive or bioinert, and biomaterials of a certain structure (polymeric, ceramic, composite) for use in RM and TE depends on the specific application, as well as on the desired properties, scaffold characteristics and functions of the biomaterial. By designing hydrogels with specific properties, researchers can create scaffolds that closely mimic the ECM of native tissues and provide an environment that supports cell growth, function, and regeneration, making them an attractive material for tissue engineering and regenerative medicine applications. Ceramic biomaterials of the calcium phosphate type are used as bone substitutes, because they can act as scaffolds for bone regeneration due to their ability to integrate with the surrounding bone tissue. Understanding and controlling the properties of HAp materials is critical to determining their suitability for use in regenerative medicine and tissue engineering applications and to promote and create new tissue and support cell growth and function in the body. The nanoscale characteristics of HAp can also provide a scaffold for cell growth and new bone tissue formation, and the design of micro-nano hybrid HAp structures is an important area of research because it closely mimics the structure of natural bone.

BTE is based on the combined use of cells, osteostimulating (osteoinductive) factors and biomaterials as scaffolds and carriers for bone regeneration and defect repair. Researchers have used ADSCs induced in vitro towards osteogenic cells or endothelial cells, although freshly isolated stromal vascular fraction of adipose tissue can also be used for regenerative purposes. Studies have suggested that modulating the phenotype and behavior of macrophages may be a promising approach to enhance bone regeneration. It has been shown that the incorporation of a blood clot into BTE constructs as well as the use of PRP can stimulate angiogenesis, the wound healing process, bone repair and regeneration, as they can be a source of osteoinductive factors.

In vitro studies can help in guiding the development of new biomaterials for regenerative medicine and tissue engineering applications and may help researchers identify optimal biomaterials for specific applications, but *in vitro* studies are often followed by *in vivo* studies in animal models to confirm the results. *In vivo* experimental models provide a valuable tool to assess the regenerative potential of biomaterials in a biologically relevant context for potential clinical application and guide the development of new materials with improved regenerative properties.

8. ACKNOWLEDGMENT

The authors would like to thank the Ministry of Education, Science and Technological Development and Ministry of Science, Technological Development and Innovation of the Republic of Serbia for financial support through Grants [numbers: III 41017, 451-03-68/2020-14/200113, 451-03-9/2021-14/200113 and 451-03-68/2022-14/200113 and 451-03-68/2023-14/200113].

9. REFERENCES

[1] A. S. Mao, D. J. Mooney, *Regenerative medicine: Current therapies and future directions*, Proc Natl Acad Sci U S A, Vol. 112-47 (2015) 14452-14459.

[2] J. G. Najdanović, et al., *Effects of bone tissue engineering triad components on vascularization process: comparative gene expression and histological evaluation in an ectopic bone-forming model,* Biotechnology & Biotechnological Equipment, Vol. 30-6 (2016) 1122-1131

[3] S. Caddeo, et al., *Tissue Engineering Approaches in the Design of Healthy and Pathological In Vitro Tissue Models,* Frontiers in bioengineering and biotechnology, Vol. 5-40 (2017) 1-22.

[4] M. Rahmati, et al*., Biomaterials for Regenerative Medicine: Historical Perspectives and Current Trends,* Adv Exp Med Biol, Vol. 1119 (2018) 1-19.

[5] R. Agrawal, et al., *Biomaterial types, properties, medical applications, and other factors: a recent review*, J Zhejiang Univ Sci A, (2023) 1-16.

[6] J. M. Anderson, *Biological Responses to Materials*, Annual Review of Materials Research, Vol. 31 (2001) 81–110.

[7] D. F. Williams, *On the mechanisms of biocompatibility*, Biomaterials, Vol. 29–20 (2008) 2941–2953.

[8] C. J. Kirkpatrick, et al., *Current trends in biocompatibility testing*, Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine, Vol. 212-2 (1998) 75–84.

[9] E. J. Lee, et al., *Biomaterials for Tissue Engineering*, Annals of Biomedical Engineering, Vol. 42-2 (2014) 323–337.

[10] A. Khalili, M. Ahmad, *A Review of Cell Adhesion Studies for Biomedical and Biological Applications*, International Journal of Molecular Sciences, Vol. 16-8 (2015) 18149–18184.

[11] H. S. Kim, et al., *Biomaterial-directed cell behavior for tissue engineering*, Current Opinion in Biomedical Engineering, Vol. 17 (2021) 100260.

[12] H.-K. Jang and B.-S. Kim, *Modulation of Stem Cell Differentiation with Biomaterials*, International Journal of Stem Cells, Vol. 3-2 (2010) 80–84.

[13] J. M. Živković, N. Ignjatović, S. Najman, *Properties of Hydroxyapatite-Based Biomaterials Important for Interactions with Cells and Tissues*, In: S. Najman,V. Mitić, T. Groth, M. Barbeck, P-Y. Chen, Z. Sun, B. Ranđelović (eds), *Bioceramics, Biomimetic and Other Compatible Materials Features for Medical Applications*, Cham: Springer International Publishing, 2023, 115-135.

[14] M. Hussain, et al., *Ultra-High-Molecular-Weight-Polyethylene (UHMWPE) as a Promising Polymer Material for Biomedical Applications: A Concise Review*, Polymers (Basel), Vol. 12-2 (2020) 323.

[15] N. Ignjatović, et al., *Synthesis and properties of hydroxyapatite/poly-L-lactide composite biomaterials*, Biomaterials, Vol. 20-9 (1999) 809-816.

[16] E. Capuana, et al*., Poly-l-Lactic Acid (PLLA)-Based Biomaterials for Regenerative Medicine: A Review on Processing and Applications,* Polymers (Basel), Vol. 14-6 (2022) 1153.

[17] S. Wei, et al., *Biodegradable materials for bone defect repair*, Military Med Res, Vol. 7-1 (2020) 54.

[18] R. Geevarghese, et al., *Biodegradable and Non-Biodegradable Biomaterials and Their Effect on Cell Differentiation*, International Journal of Molecular Sciences, Vol. 23(24) (2022) 16185.

[19] K. A. Athanasiou, et al., *Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers*, Biomaterials, Vol. 17(2) (1996) 93-102.

[20] S. Najman, et al., *Biological evaluation of hydroxyapatite/poly-L-lactide (HAp/PLLA) composite biomaterials with poly-L-lactide of different molecular weights intraperitoneally implanted into mice*, Bio-medical materials and engineering, Vol. 14.1 (2004) 61-70.

[21] I. Gajić, et al., *Electrospun Poly(lactide) Fibers as Carriers for Controlled Release of Biochanin A*, Pharmaceutics, Vol. 14-3 (2022) 528.

[22] M. Barbeck, et al., *Analysis of the in vitro degradation and the in vivo tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components – Guidance of the inflammatory response as basis for osteochondral regeneration*, Bioactive Materials, Vol. 2-4 (2017) 208–223.

[23] L. S. Nair and C. T. Laurencin, *Biodegradable polymers as biomaterials*, Progress in Polymer Science, Vol. 32-8–9 (2007) 762–798.

[24] C. Ruan, et al., *The interfacial pH of acidic degradable polymeric biomaterials and its effects on osteoblast behavior*, Scientific Reports, Vol. 7 (2017) 6794.

[25] S. Ye, et al., *Evaluating platelet activation related to the degradation of biomaterials using molecular markers*, Colloids and Surfaces B: Biointerfaces, Vol. 184 (2019) 110516.

[26] S. Metwally, et al., *Surface potential and charges impact on cell responses on biomaterials interfaces for medical applications*, Materials Science & Engineering C-Materials for Biological Applications, Vol. 104 (2019) 109883.

[27] V. M. Villapun Puzas, et al., *Surface Free Energy Dominates the Biological Interactions of Postprocessed Additively Manufactured Ti-6Al-4V*. ACS Biomaterials Science & Engineering, Vol. 8-10 (2022) 4311-4326.

[28] G. Yang, et al., *Osteoblast response to the surface topography of hydroxyapatite two-dimensional films*. Journal of Biomedical Materials Research Part A, Vol. 105-4 (2017) 991–999.

[29] M. G. L. Olthof, et al., *Effect of Biomaterial Electrical Charge on Bone Morphogenetic Protein-2-Induced In Vivo Bone Formation*, Tissue Engineering Part A, Vol. 25(13-14) (2019) 1037-1052.

[30] J. A. Hunt, et al., *Effect of biomaterial surface charge on the inflammatory response: evaluation of cellular infiltration and TNF alpha production*, Journal of Biomedical Materials Research, Vol. 31-1 (1996) 139-144.

[31] J. Yan, et al., *The effect of surface charge on the cytotoxicity and uptake of carbon quantum dots in human umbilical cord derived mesenchymal stem cells*, Colloids and Surfaces B: Biointerfaces, Vol. 171 (2018) 241-249.

[32] P. J. Vasiljević, J. Živković, M. Vukelić-Nikolić, S. Najman, *Determining the Biological Properties of Biomaterials In Vivo*, In: F. Živić, S. Affatato, M. Trajanović, M. Schnabelrauch, N. Grujovic, K. Choy (eds), *Biomaterials in Clinical Practice*, Springer, Cham 2017, 477-499.

[33] L. Polo-Corrales, et al., *Scaffold design for bone regeneration*, Journal of Nanoscience and Nanotechnology, Vol. 14-1 (2014) 15–56.

[34] M. Mastrogiacomo, et al., *Role of scaffold internal structure on in vivo bone formation in macroporous calcium phosphate bioceramics*, Biomaterials, Vol. 27-17 (2006) 3230–3237.

[35] Y. Reinwald, et al., *Interconnectivity and permeability of supercritical fluid-foamed scaffolds and the effect of their structural properties on cell distribution*, Polymer, Vol. 55 (2014) 435–444.

[36] Y. Niu, et al., *Biomechanical Characteristics and Analysis Approaches of Bone and Bone Substitute Materials*, Journal of Functional Biomaterials, Vol. 14-4 (2023) 212.

[37] R. Agarwal, et al., *Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair*, Advanced Drug Delivery Reviews, Vol. 94 (2015) 53-62.

[38] A. Béduer, et al., *Design of an elastic porous injectable biomaterial for tissue regeneration and volume retention*, Acta Biomaterialia, Vol. 142 (2022) 73-84.

[39] H. Lv, et al., *Biomaterial stiffness determines stem cell fate*, Life Sciences, Vol. 178 (2017) 42-48.

[40] B. Yi, et al., *An overview of substrate stiffness guided cellular response and its applications in tissue regeneration*, Bioactive Materials, Vol. 15 (2021) 82-102.

[41] S. Amorim, et al., *Extracellular Matrix Mimics Using Hyaluronan-Based Biomaterials*, Trends in Biotechnology, Vol. 39-1 (2021) 90-104.

[42] M. G. Haugh, et al., *Investigating the interplay between substrate stiffness and ligand chemistry in directing mesenchymal stem cell differentiation within 3D macro-porous substrates*, Biomaterials, Vol. 171 (2018) 23-33.

[43] H. Xing, et al., *Extracellular matrix-derived biomaterials in engineering cell function*, Biotechnology Advances. Vol. 42 (2020) 107421.

[44] H. D. Kim, et al., *Biomimetic Materials and Fabrication Approaches for Bone Tissue Engineering*, Advanced Healthcare Materials, Vol. 6-23 (2017) 1700612.

[45] M. Brovold, et al., *Naturally-Derived Biomaterials for Tissue Engineering Applications*, Advances in Experimental Medicine and Biology, (2018) 421–449.

[46] A. Sionkowska, *Current research on the blends of natural and synthetic polymers as new biomaterials: Review*, Progress in Polymer Science, Vol. 36-9 (2011) 1254–1276.

[47] A. E. Eldeeb, et al., *Biomaterials for Tissue Engineering Applications and Current Updates in the Field: A Comprehensive Review*, AAPS PharmSciTech, Vol. 23(7) (2022) 267.

[48] L. L. Hench, *Biomaterials*, Science, Vol. 208(4446) (1980) 826–831.

[49] M. Bove, et al., *Tissue engineering in musculoskeletal tissue: a review of the literature,* Surgeries, Vol. 2(1) (2021) 58-82.

[50] M. Navarro, et al., *Biomaterials in orthopaedics.* Journal of the Royal Society, Vol. 5(27) (2008) 1137–1158.

[51] J. G. Najdanović, J. Rajković, S. J. Najman. *Bioactive biomaterials: potential for application in bone regenerative medicine*, in: F. Živić, S. Affatato, M. Trajanović, M. Schnabelrauch, N. Grujović, K. Choy (eds) *Biomaterials in Clinical Practice*, Springer, Cham 2018, 336-360.

[52] V. M. Goldberg and S. Akhavan, *Biology of bone grafts*, in: J. R. Lieberman, G. E. Friedlaender (eds), *Bone Regeneration and Repair*, Humana press 2005, 57–65

[53] M. M. Stevens, *Biomaterials for bone tissue engineering*, Materials Today, 11(5) (2008) 18–25.

[54] A. Kaur, et al., *Bone graft materials and substitutes*, Integrative Journal of Orthopaedics and Traumatology, Vol. 6(1) (2023) 1–8.

[55] F. Oberdiek, et al., *Ex Vivo and In Vivo Analyses of Novel 3D-Printed Bone Substitute Scaffolds Incorporating Biphasic Calcium Phosphate Granules for Bone Regeneration*, International Journal of Molecular Sciences, Vol. 22-7 (2021) 3588.

[56] T.C. Ho, *et al.*, *Hydrogels: Properties and Applications in Biomedicine,* Molecules, Vol. 27-9 (2022) 2902.

[57] A. Pröhl, et al., *In Vivo Analysis of the Biocompatibility and Bone Healing Capacity of a Novel Bone Grafting Material Combined with Hyaluronic Acid*, International Journal of Molecular Sciences, Vol. 22-9 (2021) 4818.

[58] S. Ilić-Stojanović, et al., *Intelligent Poly(N-Isopropylmethacrylamide) Hydrogels: Synthesis, Structure Characterization, Stimuli-Responsive Swelling Properties, and Their Radiation Decomposition*, Polymers, Vol. 12-5 (2020) 1112.

[59] T. Kumai, et al., *A novel, self-assembled artificial cartilage-hydroxyapatite conjugate for combined articular cartilage and subchondral bone repair: histopathological analysis of cartilage tissue engineering in rat knee joints*, International Journal of Nanomedicine, Vol. 14 (2019) 1283-1298.

[60] J. L. Ong, et al., *Hydroxyapatite and their use as coatings in dental implants: a review*, Critical Reviews in Biomedical Engineering, Vol. 28(5-6) (2000) 667-707.

[61] O. Geuli, et al., *Synthesis, coating, and drug-release of hydroxyapatite nanoparticles loaded with antibiotics*, Journal of Materials Chemistry B, Vol. 5(38) (2017) 7819-7830.

[62] N. Ramesh, et al., *Hydroxyapatite-polymer biocomposites for bone regeneration: A review of current trends*, Journal of Biomedical Materials Research – Part B: Applied Biomaterials, Vol. 106(5) (2018) 2046-2057.

[63] N. Ignjatović, et al., *Enhanced Osteogenesis of Nanosized Cobalt-substituted Hydroxyapatite*, Journal of Bionic Engineering, Vol. 12 (2015) 604–612.

[64] Y. Ohyabu, et al., *A collagen sponge incorporating a hydroxyapatite/chondroitinsulfate composite as a scaffold for cartilage tissue engineering*, Journal of Biomaterials Science, Polymer Edition, Vol. 20(13) (2009) 1861-1874.

[65] R. C. Thomson, et al., *Hydroxyapatite fiber reinforced poly(alpha-hydroxy ester) foams for bone regeneration*, Biomaterials, Vol. 19(21) (1998) 1935-1943.

[66] D. A. Florea, et al., *Hydroxyapatite Particles—Directing the Cellular Activity in Bone Regeneration Processes: An Up-To-Date Review,* Applied Sciences, Vol. 10(10) (2020) 3483.

[67] A. Scarano, et al., *Hydroxyapatite Block Produced by Sponge Replica Method: Mechanical, Clinical and Histologic Observations*, Materials, Vol. 12(19) (2019) 3079.

[68] I. Sopyan et al., *Porous hydroxyapatite for artificial bone applications*, Science and Technology of Advanced Materials, Vol. 8(1-2) (2007) 116-123.

[69] T. J. Webster, et al., *Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics*, Journal of Biomedical Materials Research, Vol. 51(3) (2000) 475–483.

[70] K. Lin, et al., *Tailoring the nanostructured surfaces of hydroxyapatite bioceramics to promote protein adsorption, osteoblast growth, and osteogenic differentiation*, ACS Applied Materials & Interfaces, Vol. 5(16) (2013) 8008–8017.

[71] F. Shi, et al., *The synergistic effect of micro/nano-structured and Cu2+-doped hydroxyapatite particles to promote osteoblast viability and antibacterial activity*, Biomedical Materials, Vol. 12(3) (2017) 035006.

[72] S. Jiang, et al., *Synergistic Effect of Micro-Nano-Hybrid Surfaces and Sr Doping on the Osteogenic and Angiogenic Capacity of Hydroxyapatite Bioceramics Scaffolds*, International Journal of Nanomedicine, Vol. 17 (2022) 783-797.

[73] N. Ignjatović, et al., *A study of HAp/PLLA composite as a substitute for bone powder, using FT-IR spectroscopy*, Biomaterials, 22(6) (2001) 571-5.

[74] K. E. Healy and R. E. Guldberg, *Bone tissue engineering*. Journal of Musculoskeletal and neuronal interactions, Vol. 7(4) (2007) 328–330.

[75] S. Partap, F. Lyons, F. J. O'Brien, *IV.1. Scaffolds & Surfaces*, Studies in health technology and informatics, Vol. 152 (2010) 187-201.

[76] C. M. Cowan, et al., *Adipose-derived adult stromal cells heal critical-size mouse calvarial defects*, Nature biotechnololgy, Vol. 22(5) (2004) 560–567.

[77] G. Vunjak-Novakovic, S. A. Goldstein, *Biomechanical principles of cartilage and bone tissue engineering*, in: V. C. Mow, R. Huiskes (eds), *Basic orthopaedic biomechanics and mechano-biology, 3rd edition*, Lippincott Williams and Wilkins, Philadelphia 2005, 343–408.

[78] Y. Liu, et al. *Injectable tissue- engineered bone composed of human adipose-derived stromal cells and platelet-rich plasma*, Biomaterials, Vol. 29(23) (2008) 3338–3345.

[79] G. Intini, *The use of platelet-rich plasma in bone reconstruction therapy.* Biomaterials, Vol. 30(28) (2009) 4956–4966.

[80] B. H. Choi, et al., *Effect of platelet-rich plasma on bone regeneration in autogenous bone graft,* International journal of oral and maxillofacial surgery, Vol. 33(1) (2004) 56–59.

[81] I. A. Rodriguez, et al., *Platelet-rich plasma in bone regeneration: engineering the delivery for improved clinical efficacy*, BioMed research international,Vol. 2014 (2014) 392398.

[82] G. Fernandes, S. Yang, *Application of platelet-rich plasma with stem cells in bone and periodontal tissue engineering,* Bone research, Vol. 4 (2016) 16036.

[83] V. J. Cvetković, et al., *Osteogenic potential of in vitro osteo-induced adipose-derived mesenchymal stem cells combined with platelet-rich plasma in an ectopic model*, International Orthopaedics, Vol. 39(11) (2015) 2173-2180.

[84] J. G. Najdanović, et al*., The influence of adipose-derived stem cells induced into endothelial cells on ectopic vasculogenesis and osteogenesis*, Cellular and Molecular Bioengineering, Vol. 8(4) (2015) 577-590.

[85] S. J. Najman, et al., *Ectopic osteogenic capacity of freshly isolated adipose-derived stromal vascular fraction cells supported with platelet-rich plasma: a simulation of intraoperative procedure*, Journal of Cranio-maxillo-facial surgery, Vol. 44(10) (2016) 1750-1760.

[86] J. G. Najdanović, et al., *Vascularization and osteogenesis in ectopically implanted bone tissue-engineered constructs with endothelial and osteogenic differentiated adipose-derived stem cells*, World journal of stem cells, Vol. 13(1) (2021) 91-114.

[87] M. Đ. Vukelić-Nikolić, et al., *Osteogenic capacity of diluted platelet-rich plasma in ectopic bone-forming model: Benefits for bone regeneration*, Journal of cranio-maxillo-facial surgery, Vol. 46(11) (2018) 1911–1918.

[88] J. M. Seong, et al., *Stem cells in bone tissue engineering*, Biomedical Materials, Vol. 5(6) (2010) 062001.

[89] J. Xie, et al., *Osteogenic differentiation and bone regeneration of iPSC-MSCs supported by a biomimetic nanofibrous scaffold*, Acta Biomaterialia, Vol. 29 (2016) 365–379.

[90] H. Kato, et al., *Bone regeneration of induced pluripotent stem cells derived from peripheral blood cells in collagen sponge scaffolds*, Jornal of applied oral science, Vol. 30 (2022) e20210491.

[91] Fraser JK, et al., *Fat tissue: an underappreciated source of stem cells for biotechnology,* Trends Biotechnol, Vol. 24(4) (2006) 150-154.

[92] V. Planat-Benard, et al., *Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives*, Circulation, Vol. 109(5) (2004) 656–663.

[93] A. Scherberich, et al., *Adipose tissue-derived progenitors for engineering osteogenic and vasculogenic grafts*, Journal of cellular physiology, Vol. 225(2) (2010) 348-353.

[94] A. M. Müller, et al., *Towards an intraoperative engineering of osteogenic and vasculogenic grafts from the stromal vascular fraction of human adipose tissue,* European cells & materials, Vol. 19 (2010) 127-135.

[95] A. Mehrkens, et al., *Intraoperative engineering of osteogenic grafts combining freshly harvested, human adipose-derived cells and physiological doses of bone morphogenetic protein-2,* European cells & materials, Vol. 24 (2012) 308–319.

[96] S. J., Najman, J. G. Najdanović, V. J. Cvetković, *Application of adipose-derived stem cells in treatment of bone tissue defects,* in: M. Barbeck, N. Rosenberg, P. Rider, Ž. Perić Kačarević, O. Jung, *Clinical implementation of bone regeneration and maintenance*, IntechOpen 2021.

[97] O. Hayashi, et al., *Comparison of osteogenic ability of rat mesenchymal stem cells from bone marrow, periosteum, and adipose tissue*, Calcified tissue international, Vol. 82(3) (2008) 238-247.

[98] S. Stojanović, et al., *Stem cells derived from lipoma and adipose tissue-similar mesenchymal phenotype but different differentiation capacity governed by distinct molecular signature*, Cells, Vol. *7*(12) (2018) 260.

[99] H. Hattori, et al., *Bone formation using human adipose tissue-derived stromal cells and a biodegradable scaffold*, Journal of biomedical materials research. Part B, Applied biomaterials, Vol. 76(1) (2006) 230-239.

[100] J.M. Kanczler, R. O. Oreffo, *Osteogenesis and angiogenesis: the potential for engineering bone*, European cells & materials, Vol. 15 (2008) 100-114.

[101] B. E. Sumpio, et al., *Cells in focus: endothelial cell*, The international journal of biochemistry & cell biology, Vol. 34(12) (2002) 1508–1512.

[102] R. Madonna, R. De Caterina, *In vitro neovasculogenic potential of resident adipose tissue precursors*, American journal of physiology. Cell physiology, Vol. 295(5) (2008) C1271–C1280.

[103] Z. S. Patel, et al., *Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model*, Bone, Vol. 43(5) (2008) 931–940.

[104] S. M. Anderson, et al. *The effect of vascular endothelial growth factor (VEGF) presentation within fibrin matrices on endothelial cell branching*. Biomaterials Vol. 32(30) (2011) 7432–7443.

[105] G. Sun, et al., *Functional neovascularization of biodegradable dextran hydrogels with multiple angiogenic growth factors*, Biomaterials, Vol. 32(1) (2011) 95–106.

[106] J. Du, et al., *Time-Phase Sequential Utilization of Adipose-Derived Mesenchymal Stem Cells on Mesoporous Bioactive Glass for Restoration of Critical Size Bone Defects,* ACS applied materials & interfaces, Vol. 10(34) (2018) 28340–28350.

[107] J. M. Živković, et al., *Interactions between skeletal system and macrophages in homeostasis and bone injury*, Facta Universitatis: Series Medicine and Biology, Vol. 18(1) (2016) 6-11.

[108] X. Zhou, et al., *nHA-loaded gelatin/alginate hydrogel with combined physical and bioactive features for maxillofacial bone repair*, Carbohydr Polym, Vol. 298 (2022) 120127.

[109] F. Batool, et al., *Modulation of immune-inflammatory responses through surface modifications of biomaterials to promote bone healing and regeneration*, Journal of Tissue Engineering, Vol. 12 (2021) 20417314211041428.

[110] J. M. Živković, et al., *Osteogenic effect of inflammatory macrophages loaded onto mineral bone substitute in subcutaneous implants*, Archive of Biological Sciences, Vol. 67(1) (2015) 173-186.

[111] J. M. Živković, et al., *Macrophages' contribution to ectopic osteogenesis in combination with blood clot and bone substitute: possibility for application in bone regeneration strategies*, International Orthopaedics (SICOT), Vol. 45(4) (2021) 1087-1095.

[112] D. Takić Miladinov, et al., *Synthesis, Swelling properties and evaluation of genotoxicity of hydrogels based on (meth) acrylates and itaconic acid*, Materials Research, Vol. 19 (2016) 1070-1079.

[113] J. S. Vuković, et al., *In vitro cytotoxicity assessment of intelligent acrylate based hydrogels with incorporated copper in wound management*, Materials Chemistry and Physics, Vol. 175 (2016) 158-163.

[114] B. D. Krezović, et al., *Structural, thermal, mechanical, swelling, drug release, antibacterial and cytotoxic properties of P (HEA/IA)/PVP semi-IPN hydrogels*, Chemical Engineering Research and Design, Vol. 121 (2017) 368-380.

[115] P. Janošević, et al., *Comparative In Vitro Biocompatibility Study of the Two Orthodontic Bonding Materials of Different Types*, Polymers, Vol. 14.22 (2022) 4998.

[116] V. J. Cvetković, D. Takić Miladinov, and S. Stojanović, *Genotoxicity and mutagenicity testing of biomaterials*, *Biomaterials in Clinical Practice: Advances in Clinical Research and Medical Devices*, 2018, 501-527.

[117] S. Stojanović, and S. Najman, *The effect of conditioned media of stem cells derived from lipoma and adipose tissue on macrophages' response and wound healing in indirect co-culture system in vitro*, International journal of molecular sciences, Vol. 20.7 (2019) 1671.

[118] H. AlKhoury, et al., *Study on the potential mechanism of anti-inflammatory activity of covalently immobilized hyaluronan and heparin*, Journal of Biomedical Materials Research Part A, Vol. 108.5 (2020) 1099-1111.

[119] H. Alkhoury, et al., *Studies on the mechanisms of anti-inflammatory activity of heparin-and hyaluronan-containing multilayer coatings—targeting NF-κB signalling pathway*, International Journal of Molecular Sciences, Vol. 21.10 (2020) 3724.

[120] J. A. McGovern, et al., *Animal models for bone tissue engineering and modelling disease*, Disease Models & Mechanisms, Vol. XI-4 (2018) dmm033084.

[121] W. Xu, G. Spilker, *Methodological Consideration of Various Intraosseous and Heterotopic Bone Grafts Implantation in Animal Models*, Journal of Tissue Science & Engineering, Vol. 6-3 (2015) 1000161.

[122] M. Peric, et al., *The rational use of animal models in the evaluation of novel bone regenerative therapies*, Bone, Vol. 70 (2015) 73–86.

[123] H. Schliephake, et al., *Use of a mineralized collagen membrane to enhance repair of calvarial defects in rats*, Clinical Oral Implants Research, Vol. 15-1 (2004) 112–118.

[124] Y. Z. An, et al., D*ehydrothermally Cross-Linked Collagen Membrane with a Bone Graft Improves Bone Regeneration in a Rat Calvarial Defect Model*, Materials, Vol. 10-8 (2017) 927.

[125] D. Sieger, et al., *The Addition of High Doses of Hyaluronic Acid to a Biphasic Bone Substitute Decreases the Proinflammatory Tissue Response*, International Journal of Molecular Sciences, Vol. 20-8 (2019) 1969.

[126] S. Ghanaati, et al., *Evaluation of the tissue reaction to a new bilayered collagen matrix in vivo and its translation to the clinic*, Biomedical Materials, Vol. VI-1 (2011) 015010.

[127] S. Ghanaati, *Non-cross-linked porcine-based collagen I–III membranes do not require high vascularization rates for their integration within the implantation bed: A paradigm shift*, Acta Biomaterialia, Vol. 8-8 (2012) 3061–3072.

[128] M. Barbeck, et al., *Multinucleated giant cells in the implant bed of bone substitutes are foreign body giant cells-New insights into the material-mediated healing process*, J Biomed Mater Res A, Vol. 105(4) (2017) 1105-1111.

[129] I. Willershausen, et al., *Non-cross-linked collagen type I/III materials enhance cell proliferation: in vitro and in vivo evidence*, Journal of Applied Oral Science, Vol. 22-1 (2014) 29–37.

[130] S. Al-Maawi, et al., *In vivo cellular reactions to different biomaterials—Physiological and pathological aspects and their consequences*, Seminars in Immunology, Vol. 29 (2017) 49–61.

[131] E. A. Fragogeorgi, et al., *In vivo imaging techniques for bone tissue engineering*, Journal of Tissue Engineering, Vol. 10 (2019) 204173141985458.

ПРИМЕНА БИОМАТЕРИЈАЛА У РЕГЕНЕРАТИВНОЈ МЕДИЦИНИ И ТКИВНОМ ИНЖЕЊЕРСТВУ - КОНЦЕПТИ И ПЕРСПЕКТИВЕ

Сажетак: Регенеративна медицина (РМ) користи урођени потенцијал људског тела да ефикасно поправи и регенерише оштећена ткива и органе уз помоћ различитих биоматеријала. Ткивно инжењерство (ТЕ) омогућава замену оштећених ткива и органа новим. Истраживања у области биоматеријала значајно су унапредила област РМ и ТЕ. Биоматеријали се користе као ортопедски, стоматолошки и кардиоваскуларни имплантати, као медицински уређаји и у разним областима реконструктивне и регенеративне медицине. Важни предуслови да се биоматеријал користи за имплантацију су његова биокомпатибилност и биофункционалност. Биоматеријали треба да омогуће адхезију, миграцију, пролиферацију и диференцијацију ћелија. Биолошка својства биоматеријала су одраз њихових физичко-хемијских особина, као што су унутрашња архитектура, карактеристике површине и наелектрисање. Биоматеријали који се користе у регенерацији ткива треба да опонашају природну структуру екстрацелуларног матрикса и представљају физиолошку микросредину за нормалне ћелијске функције. Ови биоматеријали такође треба да имају адекватна својства биоразградивости како би се олакшало формирање и раст новог ткива. Биоматеријали за употребу у РМ и ТЕ могу бити природног или синтетског порекла, по структурним особинама полимерни, керамички и композитни тип, а на основу биореактивности могу бити биоинертни или биоактивни. У РМ и ТЕ се користе полимери различитих типова, природни и синтетски, који се могу направити и као интелигентни материјали. Хидрогелови имају структуру порозне мреже представљајући тако добру матрицу за активност ћелија. Керамички биоматеријали на бази ХАп се користе у РМ и ТЕ, посебно чврстих ткива. Својства, као што су састав, величина честица, облик материјала, порозност, површинско наелектрисање, топографија и сл, релевантни су за правилну употребу ХАп материјала. Важна каракеристика ХАп-а су могућности модификације његове структуре, површине, дизајна величине честица на микро и нано нивоу, хибридизација са полимерима, металима итд, што је веома важно за његове примене. Дизајнирана микро-нано-хибридна ХАп структура је најсличнија структури костију, чинећи ћелијско окружење најближим природном. Ткивно инжењерство кости (КТЕ) се заснива на комбинованој употреби ћелија, остеостимулирајућих (остеоиндуктивних) фактора и биоматеријала као скафолда и носача за регенерацију костију и поправку дефеката. У КТЕ се често користе мезенхимске матичне ћелије добијене из масног ткива које се индукују *in vitro* према остеогеним или ендотелним ћелијама, а може се применити и свеже изолована стромална васкуларна фракција. У састав КТЕ конструкта могу се укључивати компоненте крви као извор остеоиндуктивних фактора (ПРП, krvna plazma или угрушак), а и макрофаги као модулатори остеогеног процеса. *In vitro* модели и методе се користе за испитивање биокомпатибилности, имуномодулаторног и регенеративног потенцијала биоматеријала, као и њиховог утицаја на ћелијске функције. После *in vitro* метода, а пре клиничких студија, за испитивање регенеративног потенцијала биоматеријала користе се различити *in vivo* животињски модели, као што су поткожне имплантације и дефекти костију тибије, бутне кости и калварије код експерименталних животиња (миш, пацов, зец).

Кључне ријечи: Регенеративна медицина, Коштано ткивно инжењерство, Биоматеријали, Ткивне скеле, Физичко-хемијска својства, Класификација биоматеријала, Биоактивност материјала, Хидрогелови, Хидроксиапатит, Мезенхимске матичне ћелије из масног ткива, Макрофаги, Методе *in vitro*, *In vivo* животињски модели.

Paper received: 22 May 2023 **Paper accepted: 15 June 2023**