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TISSUE ENGINEERING AND APPLICATION OF SYNTHETIC BIOMATERIALS

TKIVNI INŽENJERING I PRIMENA SINTETIČKIH BIOMATERIJALA

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Abstract

This review presents some key issues in the current tissue engineering research with focus on design and processing of synthetic scaffolding biomaterials. The main strategy in tissue engineering involves tissue regeneration by basic, tissue-specific cells that are seeded into specially designed polymeric matrices - scaffolds. The main guiding principle in scaffold development is that the scaffolding material should resemble the natural extra-cellular matrix of the target tissue. The scaffold should biologically degrade over time leaving engineered functional tissue without toxic effects from degradation products. The state of the art polymer engineering can provide design routes for macromolecular networks with molecular-scale control over polymer structure with tailored biologically-responsive properties, such as hydrophilicity, mechanical strength, surface chemistry/morphology and biological degradation profile. Some important future aspects of tissue engineering are also presented here.

Key words
Tissue engineering, scaffolds, biomaterials, synthetic polymers, polyester elastomers.

Ključne reči
tkivni inženjering, matrice, biomaterijali, sintetički polimeri, poliestarski elastomeri

GENERAL STRATEGY AND LIMITATIONS OF SCAFFOLD-GUIDED TISSUE ENGINEERING

Tissue engineering is defined as "an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function" [1]. The main goal of tissue engineering is to produce new tissue where it is needed. Therefore, knowledge of the structure and functional limits of the regenerated tissue is essential. The ultimate goal in tissue engineering is to discover and optimize processes which produce completely biocompatible substitutes that eventually would be used to replace damaged or diseased tissues in reconstructive surgery. One of the main strategies in tissue engineering today is to produce artificial matrices called scaffolds that have the appropriate physical, chemical and mechanical properties to enable optimal conditions for tissue growth. Different tissue types normally require scaffolds with specific properties and constructions that would mimic natural environment for the relevant cells [2]. Ideally, the scaffold material should be able to support the initial cell growth and further proliferation that is necessary for regeneration of the desired tissue. The chosen material should have the ability to biologically degrade over time while leaving a reproduced functional tissue [3]. In terms of scaffold implantation, the applied synthetic material should exhibit minimal immunological response from the host metabolism. Biomaterials in tissue-engineered substitutes serve as a structural component and provide the proper three-dimensional (3D) architecture of the construct. The scaffold provides a 3D matrix for guided cell proliferation and controls the shape of the bioartificial device. Principally, a scaffold should have high porosity and have suitable pore sizes, and the pores should be interconnected [4]. Scaffolds designed for tissue engineering should mimic the site where they will be implanted as closely as possible, and they should support cell growth. Several criteria define the ideal material for tissue-engineering scaffolds. The material should be biocompatible, absorbable, and easily and reproducibly processable, and the surface of the material should interact with cells and tissues. The material should not transfer antigens [4, 5]. The most commonly used scaffold materials are the natural polymers (such as chitosan, collagen, and hyaluronic acid with its derivatives), ceramics such as hydroxyapatite and transformed coral, and synthetic bioabsorbable polymers (of these, polylactide - PLA, polyglycolide - PGA and their copolymers - PLGA have been the most studied). Due to high sensitivity of the material-body interaction, careful design of scaffolding biomaterials plays a crucial role for successful tissue engineering.

CHOICE OF SCAFFOLDING BIOMATERIAL

The scaffolds have been produced in a variety of forms such as solid porous scaffolds [6], nano-fibrous materials [7, 8], microspheres [9, 10] and hydrogels [11, 12], foils, foams, membranes, and

capillary membranes, non-wovens and other textiles, tubes, beads, porous blocks, and specialized 3D shapes [5, 13-18] for generation of functional tissue both in vivo and in vitro. Porosity made by leaching salts or porosity made by fibrous structure has been achieved for polymer scaffolds. Other methods applied have included non-woven technology, freeze drying, rapid prototyping, 3D printing, and phase separation [13-18]. However, production of fully functional human tissue is still one of the major problems for tissue engineers. The versatility of the scaffolds is often counterbalanced by a lack of crucial parameters of engineered tissue such as mechanical strength and thickness, content of collagen [6, 12] and other non-living components produced by seeded cells and cell distribution and proliferation within engineered extra-cellular matrix (ECM). In addition, the relationship between tissue regeneration and physical/chemical integrity of polymeric scaffolds is still poorly understood.

There are two general approaches in selection of scaffolding material: natural derived polymers such as collagen and elastin and synthetic polymers produced out of non-toxic monomers. Authors often describe the advantage of synthetic over natural scaffolding materials as that biologically responsive material properties of synthetic materials can be finely tuned to suit requirements for tissue-specific cells [19-22]. This review focuses only on synthetic biomaterials. One of the most cited synthetic biomaterials for fabrication of tissue engineering scaffolds are polylactic acid (PLA), polyglycolic acid (PGA) and their co-polymer polylactic co-glycolic acid (PLGA) (Fig. 1). Extensive research on those particular polymers led to approval from Federal Drug Authority (FDA) in USA as the material that can be used in clinical applications.

Polymeric scaffolds are often fabricated into porous three-dimensional matrices (Fig. 1) by first developed technique reported as "solvent casting/particulate leaching" method for scaffolds fabrication with controlled pore size and distribution [1, 24]. This method is consistent of three technical steps: *Step1*: the biocompatible polymer is dissolved and the solution is mixed with porogen (NaCl) of pre-determined particle sizes. *Step 2*: after mixing and casting the polymer solution/salt composite into desirable shape and size, the mixture is immediately placed in oven in order to evaporate the solvent out of the system. The product of such process is a solid block of thoroughly mixed polymer with porogen NaCl. *Step 3*: the NaCl particles are leached out of the system by soaking the polymer/salt composite in water. The product is a polymer porous structure with well defined pore sizes and optimised porosity. One of the commercial scaffolds, Osteofoam™ produced from PLGA for bone tissue engineering is presented in Fig. 1.

Apart from PLGA, synthetic scaffolds have been produced from various polyurethanes, polyesters and number of co-polymers specifically designed to be compatible with cells of interest. Recently a group of novel polyesters synthesised from non-toxic and metabolic components, such as citric acid (CA) and sebacic acid (SA) have attracted attention due to their excellent performance in comparison to approved biomaterials for tissue engineering applications.

NOVEL SEBACIC ACID AND CITRIC ACID-BASED ELASTIC POLYESTER BIOMATERIALS

In recent years, catalyst free synthesis has emerged as a potential route to synthesise elastic polyesters with appropriate mechanical integrity, suitable hydration characteristics and compatibility for fabrication of tissue engineering scaffolds [25]. Polyester elastomers, synthesized so far from low-cost and non-toxic precursors such as sebacic acid (SA), glycerol, citric acid (CA), and 1,8-octanediol (OD) represent a new generation of advanced biocompatible and biodegradable synthetic materials with potential biomedical applications [25-45]. The main advantage of polyglycerol sebacate (PGS) and polyoctanediol citrate (POC) polymers is that their synthesis involves low-cost monomers, SA and CA, both considered to be biocompatible due to their roles in metabolic cycles in humans [25, 35]. Both the polymers exhibit superior performance over conventional PLA, PGA and PLGA materials. Furthermore, their physical properties closely resemble that of the ECM from most tissues, which is tough, elastic and highly hydrated polymer network that provides a natural support to all tissues and organs [25]. Chemical structure of POC and presentation of developed POC scaffold is displayed in Figure 2.

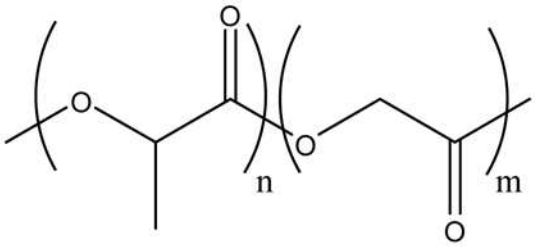
The first work on sebacate biocompatible polyester elastomeric material PGS was reported and discussed by Wang et al. [25]. This work was followed by the research on PGS with various cells and tissue types, mainly focused on soft tissue regeneration [26-34]. POC elastomer was first reported in 2004 [35], followed by some interesting results on its applications for tissue engineering of soft tissue (similar to PGS) [25-27], cartilage tissue [39] and fabrication of orthopaedic devices [38].

PGS and POC are consistent of pendant hydrophilic functionalities (hydroxyl and carboxyl groups) that are most likely present on the materials' surface making PGS and POC hydrophilic in contrast to hydrophobic PLA, PGA or PLGA which often require additional surface treatments in order to impart hydrophilicity required for tissue engineering applications [25, 35]. Physico-chemical characteristics of biomaterials are just one of the important parameters in bringing about specific interactions in biological systems. Materials behaviour is closely related to their chemical structure and therefore, understanding of biomaterials chemistry is of significance, if not the most important knowledge required for developing tissue engineering templates.

Our own research lead to a novel class of p(OCS) polymers developed by polyesterification between CA, SA and OD monomers [46]. The versatility of the p(OCS) materials was established through various processing techniques such as: complex film design; porous scaffold fabrication (Fig. 2); and thin-film coating [46-48]. The novel citrate/sebacate copolyesters are elastomeric in nature and their characteristics, such as strength, elasticity, hydration and hydrolytic degradation, are strongly influenced by their structure which can be elegantly controlled by means of chemical synthesis [49]. The p(OCS) polymers in their coating form, tested in vitro, support the growth and proliferation of human-derived bone forming cells (MG63). The cell culture tests showed optimal performance of all the three p(OCS) polymer compositions displaying cellular attachment and healthy phenotype. The cell culture results clearly showed that alteration of the p(OCS) composition influenced cellular growth on the polymer surface, a feature advantageous for synthetic tissue engineering materials [48]. Future work will involve porous scaffold design and investigation of their performance both in vitro and in vivo.

METHODS FOR STRUCTURAL CHARACTERIZATION OF SYNTHETIC BIOMATERIALS AND FOR STUDY OF CELLULAR CONTACT WITH MATERIAL

Microscopy can be utilized to follow the structure of any scaffold used and to determine whether it is broken down. In addition to examining how the cells interact with it, the scaffold can be examined to establish whether they adhere successfully, which molecules are involved, and whether the cells go on to produce any matrix and if so, whether it replaces the original scaffold or integrates with it and builds on it. Different microscopic techniques are best-suited to answer different questions. For example, molecular biology applications such as in situ hybridization (ISH), will indicate the cell's gene expression and thus synthetic capabilities,



PLGA

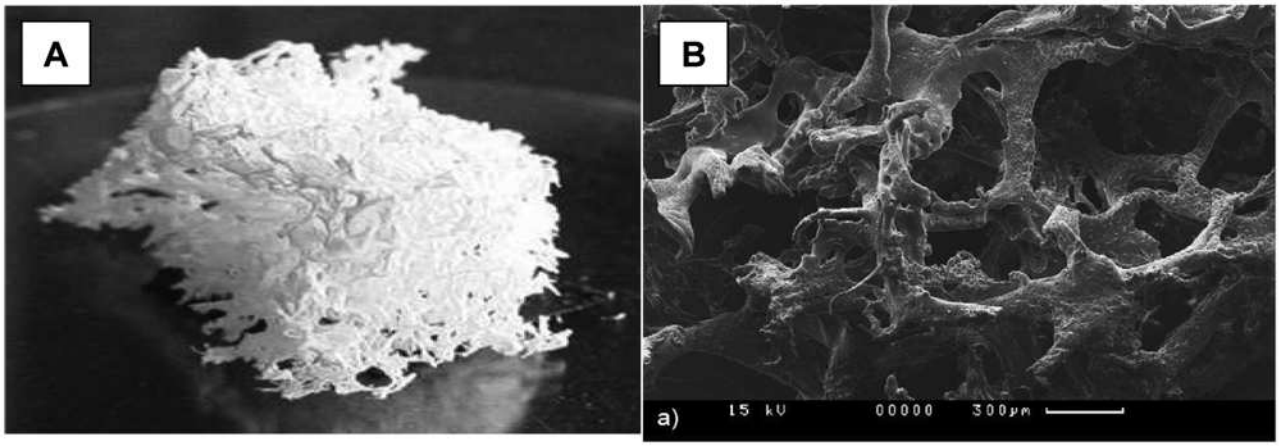
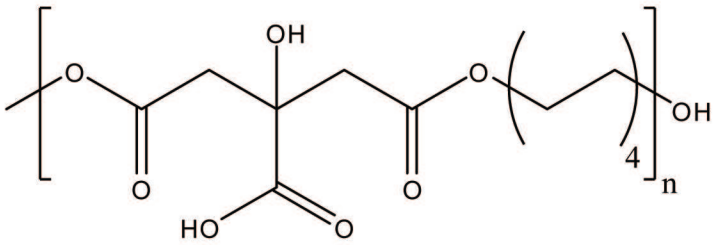


Figure 1. (Above) Chemical structure of PLGA co-polymer ("n" and "m" indexes represent a number of lactic acid and glycolic acid monomer units respectively); (A) macroscopic view of commercial PLGA scaffold Osteofoam™ produced by solvent casting/particulate leaching method; (B) scanning electron microscopy (SEM) image of untreated Osteofoam™ scaffold (scale bar = 300 µm)[23].



POC

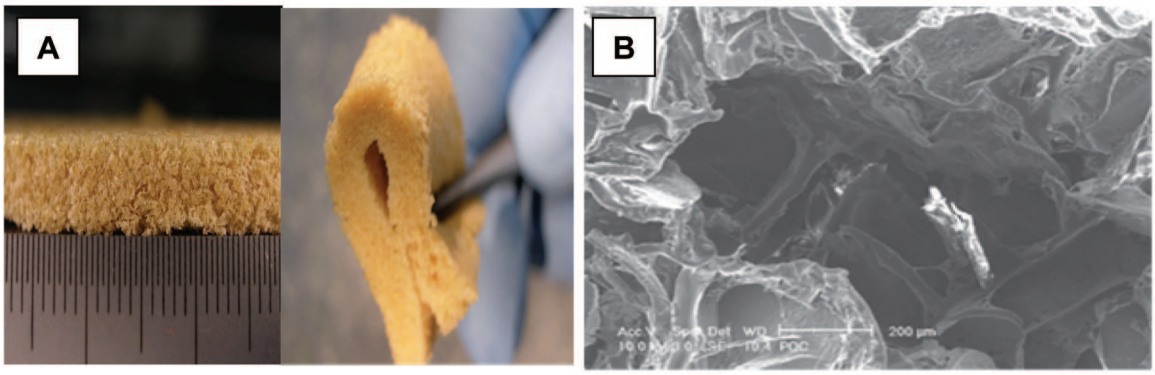


Figure 2. (Above) Chemical structure of POC polyester elastomer; (A) macroscopic view of POC scaffold; (B) SEM image POC scaffold (scale bar = 200 µm) [46].

whereas immunohistochemistry demonstrates whether this is put into practice and the cell has actually synthesized specific molecules. Variations of these techniques can be used to undertake cell biology studies in tissue-engineered constructs. Staining techniques have been used with light microscopes, including transmitted, reflected, fluorescence, or laser-scanning confocal microscopy [50]. However, all of these techniques are limited to describing relatively gross structural features, and they give little insight into the ultrastructural relationships between cells and biomaterials within a construct. Other visualization instruments and the potential of techniques such as transmission electron microscopy (TEM), SEM, and atomic force microscopy (AFM) have a greater potential in this

respect. SEM has been applied to the study of constructs [51, 52], as well as TEM to study the ultrastructure of cell-polymer constructs. Analysis of gene expression at the mRNA level may be used [53], together with other structural and functional evaluations, to characterize the quality of engineered tissues. In turn, engineered tissues may be used as model systems of developing tissues to investigate how mRNA gene expression is modulated by a variety of factors, including structural (e.g., type of threedimensional [3D] scaffold), biochemical (e.g., combination of bioactive molecules), and physical (e.g., regime of bioreactor cultivation).

SURFACE OPTIMISATION AND BIOLOGICAL ACTIVATION OF SYNTHETIC BIOMATERIALS

Characterisation of polymeric biomaterials surface and the nature and density of inherent functional groups present on those surfaces is crucial for improving biomaterials function in tissue engineering applications [54]. It is well known that the biological response to a given surface depends critically on these characteristics. In particular, research results have shown that the free surface carboxyl (–COOH) groups would interact with different cell types in either inhibitory or encouraging fashion. Li et al. reported that –COOH groups have an inhibitory effect on cell attachment and differentiation of neural cells [55]. On the other hand, keratinocytes and osteoblast-like cells exhibited strong affinity towards substrates with –COOH surface groups as a result of improved hydrophilicity [56]. Another important feature of such functionalised surfaces is possibility for activation with biologically active substances. For example, the –COOH groups can be used for covalent attachment of ECM proteins, tissue growth factors and protein sequences that provide tissue guidance during the regeneration process [54]. Numerous chemical processes have been developed to achieve covalent attachment of biomolecules (such as proteins or peptide sequences) onto biologically inert polymer surfaces in order to improve biocompatibility of synthetic materials. These methods are highlighted in a recent review paper by Goddard and Hotchkiss summarising the techniques usually employed for introducing functional groups on polymer surfaces (such as –OH, –COOH and –NH2) and protein attachment via numerous cross-linking strategies [54]. It is important to mention that the optimisation of functional group surface density is more important than their maximisation. For example, too many functional groups on a biomaterial surface can lead to overcrowding of proteins attached thus causing reduced biological activity due to steric hindrance or changed conformation [54]. Therefore, it is important to perform detailed surface characterisation of new biomaterials prior to their biological evaluation.

FUTURE DIRECTIONS

Tissue engineering is a major focus of biotechnological research today, with the expectation that this type of technique strategy will ultimately transform medical practice [57]. The most ambitious tissue-engineering schemes assume that specific tissues and organs will be restored, in a multistage fabrication procedure. For example, cells derived from the patient may be processed to increase the total number available, seeded into a suitable three-dimensional (3D) resorbable scaffold and further processed in vitro to induce the elaboration of neo-tissue prior to implantation [58].

The synthesis and processing of synthetic biomaterials and the incorporation of improved biomedical implementation strategies have led to significant achievements in the complexity of today's tissue engineering research. Development of novel approaches in fabrication of polymeric scaffolds and study of interaction between biological systems and such biomaterials is bringing the field closer to world-wide clinical practice. Despite this achievement, there are still existing challenges which include the lack of suitable materials with the desired physico-chemical properties, surface characteristics, and suitable degradation rates for the specific material-guided tissue engineering. Another challenge that must be addressed is the optimization of scaffold architecture, including pore size and distribution, surface morphology, and possibility for controlled immobilisation/release of biologically active substances (proteins/drugs) mediated through scaffold properties [59].

Following the results from the stem cell research, strategies for processing of biomaterials have been redesigned to mimic the stem cell niche as a way to drive stem/ progenitor cell differentiation to the desired cell type [22]. Understanding the cellular microenvironment and then incorporating this knowledge into biomaterials design strategies is an important focus. Simultaneously, biomaterials design strategy can be used as a way to better understand the cellular microenvironment. In tissue engineering, scientists and engineers work together to better define the cellular microenvironment and then use generated knowledge to engineer better scaffolds and functional tissues. As a conclusion, the complexity of tissue engineering demands multidisciplinary approach involving materials science, biotechnology and medicine.

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Apstrakt

Ovaj pregled prikazuje ključna pitanja u sadašnjim istraživanjima u oblasti tkivnog inženjeringa, sa akcentom na dizajnu i obradi sintetičkih matričnih biomaterijala. Osnovna strategija u tkivnom inženjeringu podrazumeva regeneraciju tkiva pomoću osnovnih, tkivno specifičnih ćelija koje se seju na specijalno dizajnirane polimerne matrice. Osnovni princip u razvoju ovih matrica je da matrični materijal treba da bude sličan prirodnom vanćelijskom matriksu ciljnog tkiva. Matrica bi trebala da se biološki razgradi tokom vremena, ne ostavljajući toksične efekte prozvoda razgradnje na formirano prirodno funkcionalno tkivo. Sadašnji nivo znanja o polimernom inženjeringu pruža mogućnosti dizajna makromolekulskih mreža sa kontrolom polimerne strukture na molekulskom nivou, sa osobinama sličnim prirodno-biološkim, kao što su hidrofilnost, mehanička čvrstina, površinska hemija/morfologija i biološki profil razgradnje. Prikazani su i neki važni budući pravci razvoja tkivnog inženjeringa.

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