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SCAFFOLDS FOR TISSUE ENGINEERING

RUSZTOWANIA KOSTNE DLA INŻYNIERII TKANKOWEJ

Abstract

The paper presents the current trends in medicine of regenerative tissue defect caused by resection of tumors or fractures. Although it is a relatively young field of science, it creates new possibilities for the reconstruction of pathologically altered tissue with the use of three-dimensional scaffolds. Tissue engineering places particular emphasis on the type of scaffold from which they are made because of a number of requirements of medical materials including biocompatibility, mechanical strength and porosity.

Keywords: scaffolds, tissue engineering, biomaterials

Streszczenie

Artykuł przedstawia trendy panujące w medycynie w regeneracji ubytków tkankowych powstałych na skutek resekcji nowotworów bądź złamań, skupione na wykorzystaniu inżynierii tkankowej. Ta stosunkowo młoda dziedzina nauki stwarza nowe możliwości odbudowy patologicznie zmienionych tkanek z wykorzystaniem trójwymiarowych rusztowań – skafoldów. Inżynieria tkankowa kładzie szczególny nacisk na rodzaj materiału, z jakiego produkowane są skafoldy, gdyż musi on spełniać szereg wymagań, m.in. biozgodność, wytrzymałość mechaniczna i porowatość.

Słowa kluczowe: skafoldy, rusztowania kostne, inżynieria tkankowa, biomateriały

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1. Introduction

The definition of tissue engineering was delineated in 1993 by Robert Langer and Joseph Vacanti. They identified it as an interdisciplinary field that combines various aspects of biology, materials science, engineering, and medicine [4, 17]. The main goal of this discipline is to develop biological substitutes that restore, maintain, or improve tissue function or a whole damaged organ [7].

Formerly, it was thought that damaged tissues should be replaced only by allogenic transplantation or artificial implants. However, these methods are not able to intercept all functions of lost tissue or organ. Concerned about the health of the patient, scientist began research on innovative biohybrid implants, which could be a temporary biological substitute for the loss of bone tissues. They are based on a combination of three-dimensional scaffolds made of a biocompatible material and cells cultures grown in the laboratory sterile conditions. The main aim was to get the information about the chemical, physical and mechanical properties of biomaterials and choose the material with specific, functional properties that favor the reconstruction of damaged tissues. It was also necessary to know the phenomena associated with the proliferation and differentiation of cells in their natural environment and under laboratory conditions [5, 19].

It is predicted that development in tissue engineering can eliminate the problems related to organ transplantations, such as rejection or lack of suitable donors. It also eliminates the use of materials with a relatively low biocompatibility, and therefore, a decrease in post-operative complications is observed [5].

2. Tissue engineering

The first achievement of tissue engineering is based on the generation of skin substitutes for burned patients. Over the past 20 years, the tissue engineering field has allowed the development and testing of artificial cartilage, bone, blood vessels, pancreas, heart valves, breast, nerves, trachea, bowel, kidney, lung and liver. Much of the research focuses on understanding and utilizing the potential of cells and improving materials to control cell behavior [13, 19].

The basic steps of tissue engineering are illustrated in Fig. 1. The first step involves the seeding of donor cells and growth factors on 3D scaffold which provides a framework and initial support for the cells to adhere proliferate and differentiate. To induce the growth of new, healthy tissue matrix is cultured in *in vitro* conditions in bioreactor. The final stage includes the implantation into the patient. It is desirable for scaffold to be a biodegradable because, after fulfilling, newly growing tissues can intercept its function [19, 25, 27].

Many different types of cells can be use in tissue engineering. The choice of cells is determined by their availability and the ability to multiply. The most attention is focused around stem cells which have the remarkable potential to differentiate into specialized cells. After division, every new cell can remain as a stem cell or become another type of cell like a muscle cell, a red blood cell, or a brain cell [15].

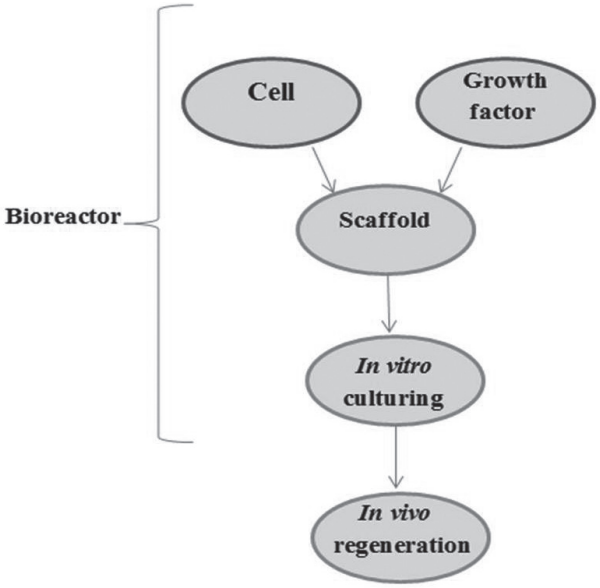

Fig. 1. Basic steps in tissue engineering [19]

Due to the potential for differentiation, there are the following types of stem cells:

- totipotential – cells that can differentiate into any cell type and give rise to whole organism,
- pluripotential – they can differentiate into any cell type, except germ cells,
- multipotential – somatic (adult) cells that are capable of replenishment new cells, often with similar properties,
- unipotential – progenitor cells, their differentiation is restricted for the manufacture of only one type of cell.

Mesenchymal stem cells – MSC isolated from the bone marrow are promising material used for the regeneration of damaged tissues. These multipotential cells have unique properties which make able to differentiate into specialized cells to form other tissues and organs and release substances that stimulate the surrounding cells to regeneration. MSC have also potential to *in vitro* into osteoblasts and chondrocytes, therefore, they can be used to reconstruction of damaged cartilage, bones, ligaments and tendons. Cells are seeded into artificial structure which provides supporting 3D tissue formation. Scaffolds should make cell attachment and migration possible, allow diffusion of vital cell and deliver and retain cells and biochemical factors [29].

3. Scaffolds

The key role of scaffolds is to provide temporary mechanical integrity at the defect site until the damaged tissue is repaired or regenerated, and normal biomechanical function is restored. Therefore, to fulfil the function, scaffolds should meet some specific requirements [8, 21].

At first, scaffolds must provide appropriate conditions to promote cell viability, proliferation and differentiation. A sufficiently high porous architecture of scaffold and an adequate pore size are necessary to facilitate cells seeding and diffusion throughout the whole structure of both dividing cells and nutrients. A large surface area is desirable for cell adhesion, therefore, the volume of the pores should be relatively high (50–90%) [20, 21].

Scaffold should be characterized by a high open porosity in which the pores are connected with each other and with the surface of the material. Interconnected porous structure of scaffold is required for cell penetration, tissue ingrowth, nutrient and waste transport. The presence of isolated pores prevents the diffusion of gases and fluids between cells. Most research shows that the best bone tissue ingrowth occurs in materials with a pore size of 100–500 μm, however, the high porosity and pore size affects the strength scaffold parameters. Therefore, it is necessary to evaluate the optimal value of the mentioned properties [21, 30].

Biodegradability and biocompatibility are important features for tissue engineering. The ideal scaffold made from a suitable biomaterial should be absorbed by the surrounding tissues without the necessity of a surgical removal and minimal degree of immune and inflammatory. It is important that the rate of degradation must be coincided as much as possible with the rate of tissue formation. That means that while cells fabricates their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually break down leaving the neotissue, newly formed tissue which will take over the mechanical load [22, 23].

To provide tight integration with surrounding tissue, scaffold should be characterized by high bioactivity which is responsible for chemical bond formation. Osteoconductivity is another desirable scaffold property which support the regeneration process of damaged bones by assisting cells to adhere to the graft surface and to proliferate [28].

Scaffold should have mechanical properties compatible with the anatomical location and it must be strong enough to allow surgical handling during implantation. Production of scaffolds with appropriate mechanical and physical properties is one of the biggest challenges in tissue engineering [20].

4. Biocomposites applied for tissue engineering

Many types of biomaterials can be used to generation of 3D scaffolds for tissue engineering. Requirements for these materials are non-antigenic, non-carcinogenic, non-toxic, non-mutagenic actions and high cell biocompatibility. Such properties significantly affect to the quality of scaffolds like cell survival, growth, propagation and reorganization. Fig. 2. presents general groups and examples of biomaterials such bioceramics, polymeric materials and composites. Typically, ceramics, synthetic and natural polymers are used in the fabrication of scaffolds for tissue engineering. Each of these materials has specific advantages and disadvantages, consequently, the use of composite scaffolds is becoming increasingly common [18, 24].

In tissue engineering, composite materials are increasingly being used, about 30% of currently applicable biomaterials are composites. Interest in composites results from the biomimetic approach, which tries to understand and mimic the structure and mechanisms of natural tissue [1, 6, 31]. In tissue engineering, the most commonly used type of composite is

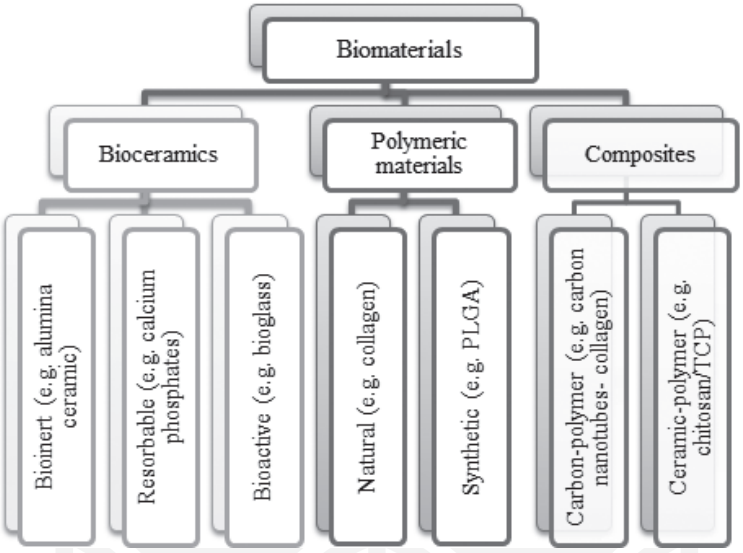

Fig. 2. The general group of biomaterials

combination of polymers with ceramics. Scaffolds made only from polymers have insufficient mechanical properties, mainly mechanical strength, Young's modulus and toughness. Another problem is low bioactivity of the polymer scaffolds, however, it can be eliminated by the addition of ceramic material, like hydroxyapatite or tricalcium phosphate. The connection of flexible polymer with active ceramic supports the regeneration of tissues [2, 6, 14, 32].

An exemplary material for scaffold production is a composite consisting of biodegradable polyurethane matrix and calcium carbonate (calcite). Preparation of three-dimensional scaffold is based on a combination of polymer coagulation technique with eluting of particles. In this method, the polymer solutions in 20% of 1-methyl-2-pyrrolidone and calcium chloride as porogenic particles are used in experiment. The obtained composite was cooled in liquid nitrogen, ground, dissolved and mixed with NaCl particles size of 300–420 microns (ratio PUR/NaCl – 1:1). Next, the material was poured into forms immersed in distilled water and left to maturate for the next 24 h. During the process, the polymer precipitation and eluting of porogen particle were observed. The final stage of scaffold preparation was dried at 37°C under vacuum for 24 h. Fig. 3. presents obtained scaffolds [10–12].

The resultant three-dimensional matrices exhibits improved properties compared with scaffolds made of pure PUR allowing the transport of gases, proliferation and cell differentiation. The research on PUR/calcite composites demonstrate that they fulfill the requirements for tissue engineering applications in bone defects repair [10–12].

It is also worth mentioning about research into the production of scaffolds based on chitosan and calcium – phosphate ceramics. They mainly focus on several preparation methods, i.e. rapid prototyping, freeze-drying, microgranules agglomeration and foaming. Using these methods, it is possible to construct spatial scaffolds with diversified structure, properties and thus different application. Fig. 4. shows chitosan/TCP composites prepared by varied techniques.

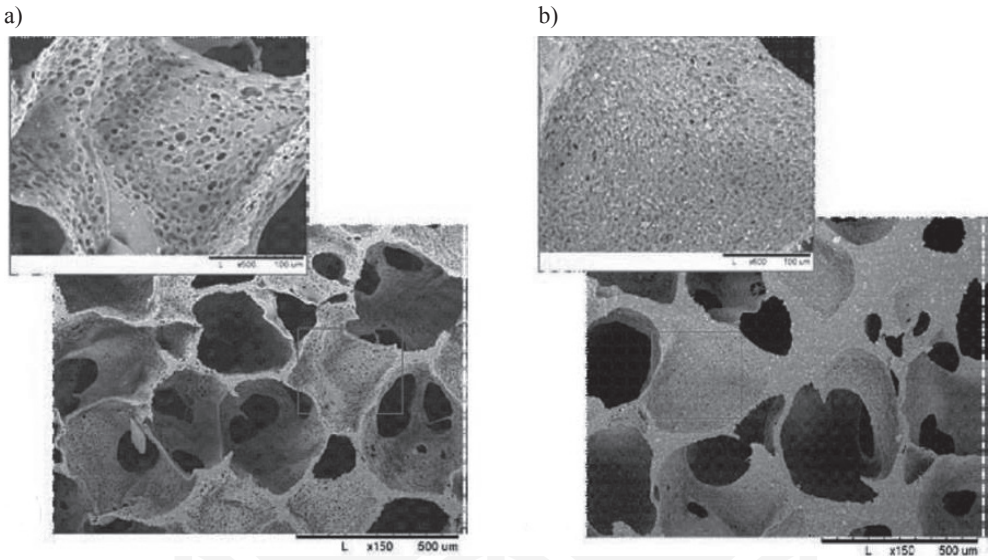

Fig. 3. The structure of scaffolds: (a): PUR + 20% calcite; (b): PUR + 40% calcite [11]

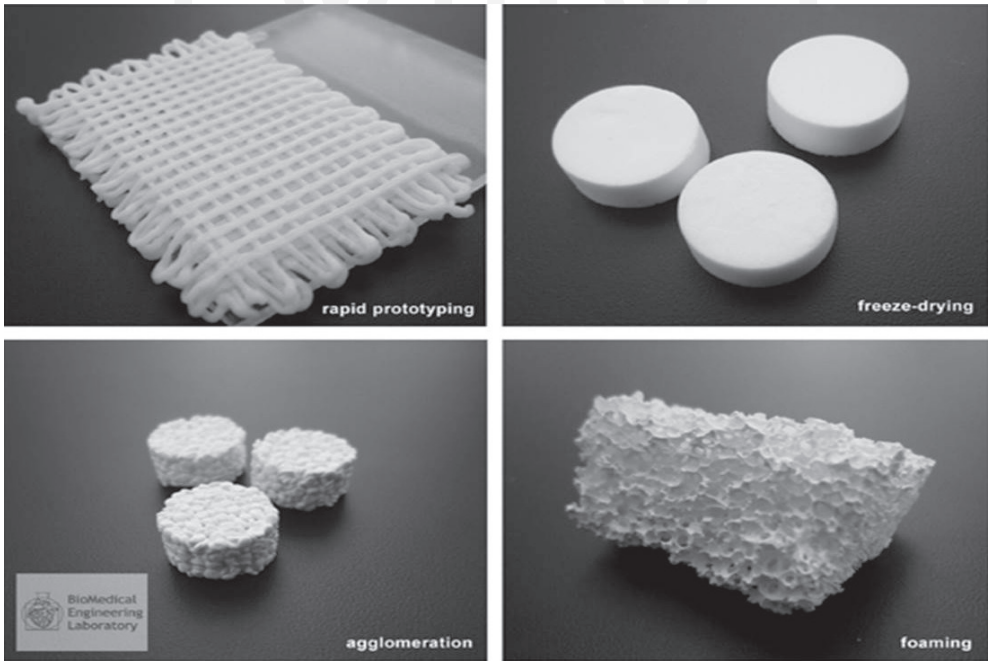

Fig. 4. Chitosan/TCP scaffolds prepared using varied techniques [33]

For example, production of scaffold by foaming method involves adding an acidic solution of chitosan to the TCP powder with particle size ranging between 3–5 μm. The quantity of introduced TCP determines the type of the results matrix structure. The formulation is mixed until a homogenous phase and then a foaming agent – $NaHCO_3$ is added. The resulting porous material was lyophilized, and after drying, washed with distilled water. The tests carried out on the obtained sponges exhibit the porosity at nearly 90%. This is possible due to gas production according to following reaction:

$$CH_3COOH + NaHCO_3 \rightarrow CH_3COONa + CO_2 + H_2O \quad (1)$$

In addition, the concentration of TCP added to the polymer affects the size of pores produced and degradation time. A low content of TCP makes distribution and pore size is irregular, but with increasing concentration of the ceramic phase, architecture of material is better organized. Moreover, the weight loss of composites with lower content of TCP (15%) is much greater than for the material with 50% of TCP [16].

An interesting group of composites, increasingly used in tissue engineering, are hydrogels. Due to its hydrophilicity, they have the ability to retain a large amount of water in its structure, without dissolution of the polymer. More important advantages of these materials are high biocompatibility, low friction coefficient, softness and plasticity, which imitate them to the natural structure of the soft tissue. In addition, hydrogels allow for the free flow of oxygen and nutrients, and therefore can be used as a scaffold [3, 9, 16]. For the preparation of hydrogels, researchers applied natural and synthetic compounds, i.e. poly (ethylene oxide), polyacrylic acid, poly (vinyl alcohol), chitosan, collagen or hydroxyapatite. One of many examples may be a hydrogel composite based on polyacrylic acid (PAA), polyethylene glycol (PEG), and HAp obtained in a few minutes [9, 26]. These type of materials are also used successfully for the production of hydrogel wound dressings or matrix for drug delivery [9].

5. Conclusions

Tissue engineering based on biology, materials engineering and medical knowledge strives to create an alternative method for the regeneration of damaged body tissues. By modifying the chemical composition of implant materials, engineers try to change and improve the functional properties of implants. Due to the fact that the architecture of scaffold plays an important role in its physicochemical characteristic, researchers develop innovative methods for the production of them. These methods ensure the obtaining matrices with desired mechanical or structural properties.

Composite materials appear to be promising biomaterial used for the production of three-dimensional matrices. A combination of at least two different properly selected phases improves properties of resulting material compared with the characteristics of the each component. Scaffolds made of polymer-ceramic materials simulated bone tissue ensure an adequate immune response, good strength, and the active bioceramic ingredient preferably affects a connection of matrix with the surrounding tissue. A major problem

faced by engineers is the time of scaffold resorption - modification of the implanted material when the material is located in the body can be changed, which may differently affect the human body.

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