KATARZYNA KURZEPA[1)], KRZYSZTOF RÓŻYCKI[1)], MARTA BOCHYŃSKA[2)], MAREK KONOP[2)], ZOFIA URBANCZYK-LIPKOWSKA[3)], ANDRZEJ W. LIPKOWSKI [2), 4), *)]

Molecular scaffolds for three-dimensional cell and tissue cultures

Summary — Regenerative medicine and cell therapy are the most growing fields of medical sciences in the last decade. The successes in development of scaffolds for three-dimensional cells and tissue breeding are one of the key factors of this progress. A broad spectrum of synthetic polymers, natural biopolymers and their combinations are available for testing and applications. Growing knowledge of biological and physicochemical properties of various (bio-) polymers allow tailoring macromolecules to particular medical application.
Keywords: 3D scaffolds, cell culturing, regenerative medicine, cosmetology.

MOLEKULARNE UKŁADY SZKIELETOWE DO TRÓJWYMIAROWYCH HODOWLI KOMÓREK I TKANEK
Streszczenie — Artykuł stanowi przegląd literaturowy dotyczący szkieletowych układów molekularnych wykorzystywanych do trójwymiarowych hodowli tkankowych. Odnotowane w ostatnich latach sukcesy w opracowywaniu takich układów, przyczyniły się do obserwowanego szybkiego rozwoju medycyny regeneracyjnej i terapii komórkowej. Obecnie do testowania i aplikacji dostępna jest szeroka gama polimerów naturalnych, syntetycznych oraz ich kombinacji. Niniejszy przegląd podaje przykłady ilustrujące różnorodność zarówno właściwości, jak i zastosowań takich szkieletowych układów wielkocząsteczkowych.
Słowa kluczowe: skafoldy trójwymiarowe, hodowle komórkowe, medycyna regeneracyjna, kosmetyka.

INTRODUCTION

Tremendous progress of biomedical and material sciences observed in last decades, created a new field — regenerative medicine that by definition „replaces or regenerates human cells, tissues or organs, to restore or establish normal function" [1]. The possibilities of three-dimensional (3D) *de novo* creation of cell aggregates or/and tissues are milestones determining progress of regenerative medicine. In normal cell culture conditions thin monocellular layers are formed. To obtain 3D constructs, special 3D molecular skeletons are needed. The name „scaffolds" has been adopted for such molecular skeletons. Scaffolds are support structures used in tissue engineering to provide the 3D growth of cells in an organized way. The cell/tissue type as well as target place of implantation of scaffolds with cultured cells determine properties of optimal scaffolds. Such properties include cell attachment, proliferation and differentiation, delivery and retaining cells and growth factors, enabling diffusion of cell nutrients and oxygen, and preservation of an appropriate mechanical and biological environment for organized tissue regeneration [2—5].

The scaffolds could be divided into three categories, considering their construction method — synthetic polymers, semisynthetic polymers of biological macromolecules and preparations of natural biopolymers. This mini-review will present some examples of various scaffolds to illustrate their variability and advantages.

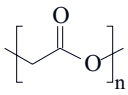
SYNTHETIC POLYMERS

The search for cell culture scaffolds for regenerative medicine started in the time when polymers for drug carriers have been quite well developed. Although their function as a carrier of drugs differs from cell-scaffold complexes, the general properties like lack of toxicity, biocompatibility to host organism, defined metabolic biodegradation after implantation [6] are desirable for both types of applications.

Lactic acid based chemical polymers are the most common both as drug carriers [7] and as scaffolds for cell culture. Lactic acid is a natural intermediate/by product of an anaerobic respiration, that during the Cori cycle is converted into glucose in the liver. Glucose is then used as an energy source in the body. Poly(lactic acid) [PLA,

[1)] Industrial Chemistry Research Institute, ul. Rydygiera 8, 01-793 Warsaw, Poland.
[2)] Mossakowski Medical Research Institute, Polish Academy of Sciences, ul. Pawińskiego 5, 02-106 Warsaw, Poland.
[3)] Institute of Organic Chemistry, Polish Academy of Sciences, ul. M. Kasprzaka 44/52, 01-224 Warsaw, Poland.
[4)] Tufts University School of Medicine, 145 Harison Avenue Boston, MA 02111, USA.
[*)] Author for correspondence; e-mail: andrzej@lipkowski.org

Formula (I)] is a biocompatible and biodegradable polymer which is broken down to monomeric units of lactic acid in the body. Therefore, the use of PLA nanoparticles is safe and devoid of any major toxicity. PLA nanoparticles could be prepared by the solvent evaporation,

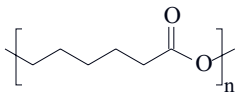


(I)

solvent displacement, salting out and solvent diffusion methods [8, 9]. In particular, this polymer has been used to test new techniques especially designated for scaffold preparation like lithographic process [10] or electro-spinning [11, 12].

PLA scaffolds have been used for hematopoietic cell differentiation [13]. Physical properties of 3D polymer may strongly influence proliferation and cells differentiation. Systematic studies of PLA with various pore sizes showed that polymer with smaller sizes of cavities and higher polymer concentration generated significantly more hematopoietic cells than polymer with larger pore sizes and smaller concentration [14]. Similarly, collagen scaffolds cultured with additional hepatocytic growth factors exhibited differentiation towards hepatic cell types [15].

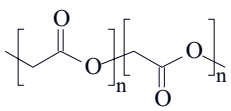
Poly ε-caprolactone polymer [PCL, Formula (II)] is another simple polymer that is prepared by catalytical ring-opening polymerization of the ε-caprolactone



(II)

monomer. PCL is degraded by hydrolysis of its ester linkages at physiological conditions and therefore, has received a great deal of recognition as an implantable biomaterial. In particular, it was especially designed for the preparation of long term implantable devices. For example, polycaprolactone (PCL) was found to promote aligned neurite orientation and supported myelination [16]. However, most often PLC is an element of multicomponent scaffolds.

Poly-D-L-lactide-co-glycolide [PLGA, Formula (III)] is the simplest example of multicomponent synthetic co-polymer that is widely used for drug delivery [17—20]. PLGA is metabolized to further biodegradable monomers — lactic and glycolic acids. Both acids are endogenous intermediates of biochemical processes. Therefore, PLGA as well as PLA expresses low systemic toxicity.



(III)

The metabolic rate and mechanical properties can be tailored by altering the composition ratio of lactic acid and glycolic acid, using polymers of different molecular weight or varying porosity and pore size [21, 22]. Stem cells growing on PLGA in the presence of various active components could differentiate to broad spectrum of cell lines. Upon addition of TGF-β, activin or retinoic acid, the human stem cells differentiated into cartilage, liver, or neural tissue respectively. These scaffolds also induced neural differentiation upon addition of NGF [23]. Further studies showed that PLGA scaffolds coated with laminin, collagen I, collagen IV or fibronectin actively interacted with pluripotent stem cells but with modified differentiation properties [24]. However, application of PLA as well as PLGA scaffolds *in vivo* is limited due to developing of local acidity due to hydrolysis products that in consequence induces inflammation [25].

MULTICOMPONENT SCAFFOLDS FOR SOFT TISSUE REGENERATION

Several novel multicomponent scaffolds have been developed with improved properties. ε-Polycaprolactone (PCL) scaffold was modified with galactosylated chitosan (GC) to achieve better bioactivity and mechanical stability. The incorporated GC was rather stable against incubation in the medium, and could significantly enhance the compression strength of the PCL scaffold in the wet state. With galactose ligands on the surface, the PCL scaffold could be better recognized by hepatocytes, and show better cell viability, spheroid formation and long-term maintenance of liver-specific functions such as albumin secretion [26]. Heterogeneous scaffolds with incorporated additional various stimulating or regulatory components can be also designed. For example, three component nanofibrous scaffolds based on blends of a hydroxyl-functionalized polyester [poly(hydroxymethylglycolide-co-ε-caprolactone), pHMGCL] and poly(ε-caprolactone) (PCL), loaded with bovine serum albumin (BSA) as a protein stabilizer and with vascular endothelial growth factor (VEGF) as a potent angiogenic factor have been developed and the effect of the released protein on the attachment and proliferation of endothelial cells was investigated. It was shown that the incorporated protein preserved its biological activity and resulted in higher initial number of adhered cells [27].

Recently, a hybrid hydrogel has been prepared that includs a synthetic polymer (Poloxamer-407) and a self-assembling octapeptide with the amino acid sequence of KFEFKFEF [28]. Poloxamers are a series of tri-block (PEO-PPO-PEO) copolymers, containing poly(propylene glycol) (PPO) and poly(ethylene oxide) (PEO). Poloxamer-407 is non-toxic, biocompatible, bioabsorbable and FDA approved copolymer for use in humans [29—31]. It forms in aqueous solution a thermo-reversible hydrogels at certain concentrations. It has also been used as a scaffold for differentiation of bone mar-

row-derived mesenchymal stem cells into adipocytes [32]. KFEFKFEF belongs to a group of self-assembling peptides (SAP) that form nanofiber scaffolds that have been used for cell culture in tissue engineering [33, 34]. A variety of cells have been encapsulated in these SAP scaffolds and demonstrated to maintain functionality [35—40]. The objective to develop this hybrid hydrogel consisting of two components was to incorporate two complementary chemical properties of synthetic polymer and peptide bioactivity. Recently, a fluorescent group was added to a similar hybrid polymer-peptide combination [of poly(N-isopropylacrylamide)-GRKPG-Dns] which allowed to follow aggregation, *in vitro* metabolism and prospectively *in vitro* distribution [41].

Specific topographical architecture of natural macromolecules in endogenous conditions regulates interactions with cells and tissues. These topographic structures form adhesion sites and signaling cues to support cellular functions in normal conditions and natural regeneration after pathological breakdown. Collagen and its derivatives are the most popular natural macromolecules applied in scaffold development. Although approximately thirty types of collagens function in the body [42], collagen IV has been implicated in mesodermal differentiation including hematopoietic, endothelial and smooth muscle formation [43].

Stem cells breaded on collagen IV differentiated into smooth muscle cells, whereas modified (through adsorption or covalent binding) with VEGF promoted differentiation into endothelial cells [44].

Fibronectin is often used as a cell adhesive layer due to the presence of the peptide sequence -Arg-Ser-Asp-(RGD) that is widely involved in integrin-mediated cell adhesion [45]. It has been found that stem cells attachment to fibronectin promote neuronal differentiation [46]. Fibronectin surface plays critical role in creation of 3D environment of fibrin gel (coagulated thrombin and fibrinogen proteins) [47, 48].

Hyaluronic acid (HA), a non-sulfated, linear polysaccharide composed of alternating monomers of D-glucuronic acid and D-*N*-acetylglucosamine, linked *via* alternating β-1,4 and β-1,3 glycosidic bonds, is known to regulate gene expression, adhesion, proliferation and morphogenesis [49]. This indicates potential role of HA for use as a biomaterial for stem cell differentiation [50]. HA scaffolds were prepared mainly by covalent crosslinking [51]. Stem cells growing on HA hydrogel with proangiogenic growth factor VEGF initiated stem cells differentiation to vascular lineages [52]. Dextran is another polysaccharide with molecular composition similar to HA. Its chemical structure has been modified towards enhancement of cell adhesiveness. Moreover, modifying dextran-based hydrogels with regulatory factors like peptides containing a RGD fragment and VEGF initiated vascular differentiation [53]. Combination of two scaffolds naturally coexisting in the nature provides advantages over the use of either material alone. HA and collagen naturally coexist in extracellular matrix. Covalent crosslinking results with scaffolds with well-defined proportions of both components that in turn modify physicochemical properties [54] as well as biocompatibility to cells and tissues [55, 56].

Hair, wool, horns, hooves and nails are natural constructs of insoluble proteins with different molecular weights and amino acid compositions [57]. These proteins contain various but high content of cystines. These cystine residues form specific poly-disulfide bridges that determine very rigid unique structures [58]. Proteins in these natural materials are simply divided into keratins that contain in sequence relatively low percentage of cystines and keratin associated proteins (KAPs) [59] that are much more rigid as a result of exceptionally high content of cystines (up to 25—30 %). Both native keratins and KAPs are not soluble in water and in any organic solvent. These technical problems are the main reason why these proteins have not been extensively studied as a biomaterial system, when compared to other structural proteins. To solubilize them, intra-molecular disulfide crosslinks must first be broken down. Applications of mercaptans seem to be the most efficient method. Unfortunately, reversible oxidation of cysteine residues resulted in formation of native structures. Nevertheless, recent successful application of keratin derivatives in development of scaffolds for regenerative medicine and drug delivery biomaterials has been reported [60]. The possibility to develop scaffolds containing natural structures of KAPs has been proposed as well. Chemical activation followed by enzymatic digestion allowed to remove fragments called „soluble keratins", leaving 3D scaffolds of KAPs intact [61] (Fig. 1). These scaffolds have been successfully

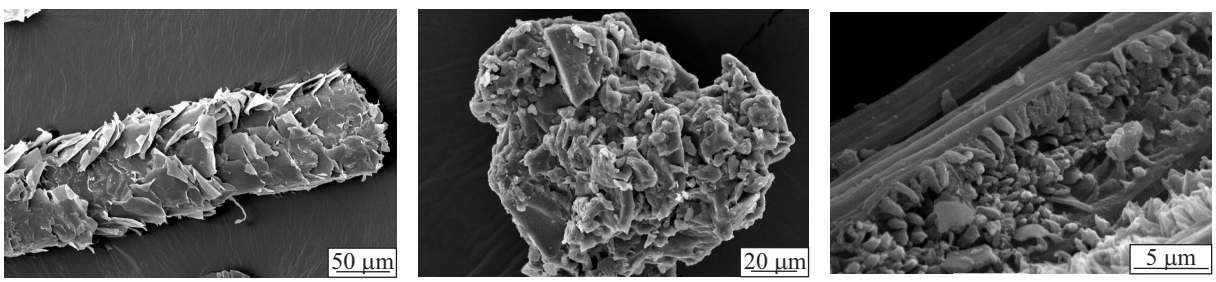


Fig. 1. Different types of KAPs scaffolds obtained by modified methods [author's unpublished results]

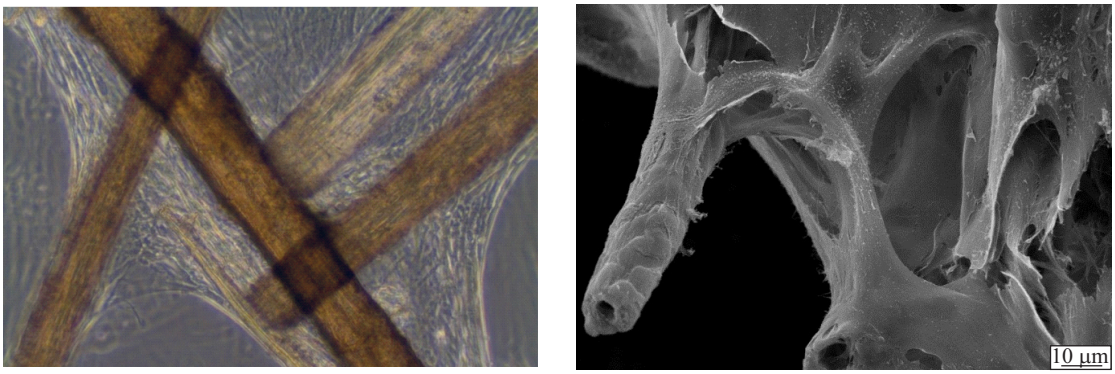


Fig. 2. Example of interaction of KAPs scaffolds with cells (optical microscope and ESM) [author's unpublished results]

applied for differentiation of stem cells into neural cells that form active network [62] as well as for other cell type cultures (Fig. 2).

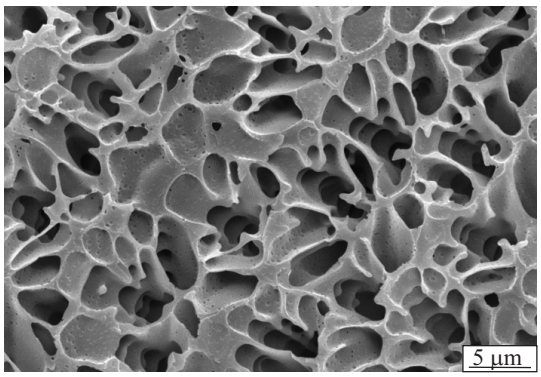


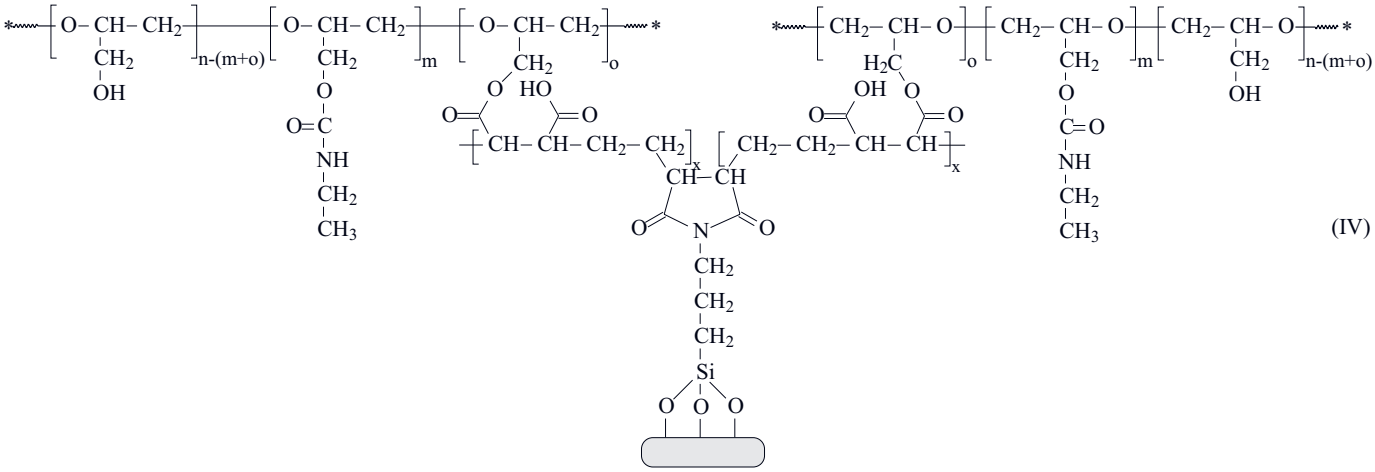
Fig. 3. Structure of temperature-responsive polymer at 37 °C [author's unpublished data]

The application of thermo-sensitive block copolymers (Fig. 3) is one of the most advanced and promising approaches, primarily in drug delivery and presently in regenerative medicine [63]. *Ex vivo* cells into tissues breading with simple isolation of final materials and/or low temperature injectable mixtures that adopt proper structure in the body temperature, are the two prospective approaches. An excellent example is the application of poly(glycidol-co-ethyl glycidyl carbamate) [Formula (IV)] for harvesting skin cells adhesion at temperatures above the phase separation and separation of final tissue layer by simple temperature change [64].

In contrast to natural biopolymers, most of the synthetic polymers applied as scaffolds are linear. The development of branched polymers [65, 66] seems to be one of the most promising approaches. The branched structures give the opportunity to construct on relatively small molecules multifunctional surface topographically related to big biomacromolecules. Preliminary studies of dendrimer polymers confirmed their prospective applicability as a component of new scaffolds [67, 68].

SCAFFOLDS FOR BONE REGENERATION

Most of the scaffolds for tissue regeneration were developed for soft tissues. However, since the discovery of the human adipose-derived mesenchymal stem cells osteogenesis [69], regeneration of bone tissues is one of the most important targets in regenerative medicine. For this purpose, a large number of specifically designed scaffolds are under studies [70]. The main direction of these studies is focused on development of porous rigid materials itself or hybridized with typical 3D polymers



(IV)

used for soft tissue regeneration. Apatites are the most commonly studied for osteogenesis due to their chemical and structural similarities to the native bone [71]. The most common apatite used in bone tissue engineering application is hydroxyapatite (HA) [72, 73]. It could be simply prepared by co-precipitation with ammonia from aqueous solutions of calcium chloride and bi-substituted ammonium phosphate according to the following reaction [74]:

$$10\ CaCl_2 + 6\ (NH_4)_2HPO_4 + 14\ NH_4OH \rightarrow$$
$$\rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20\ NH_4Cl + 6\ H_2O$$

Akermanite ($Ca_2MgSi_2O_7$) and β-tricalcium phosphate (β-TCP) [75] are another ceramic materials synthesized in a porous form that could be used itself or in combination with other, structural polymers, *e.g*. PCL [76]. A self-setting composite consisting of chitosan/tricalcium phosphate microparticles and sodium alginate has been proposed as an alloplastic bone substitute material [77]. There are promising data documenting the biocompatibility of selected biodegradable polyurethanes (PURs) *in vivo* and the tolerance of certain cells toward PURs *in vitro* — potentially to be used as scaffolds for tissue-engineered products (TEPs) [78]. It appeared, that rigidity of the surface materials influence differentiation of stem cells [79].

Cell behavior (proliferation and differentiation) is modulated by substrate rigidity to a degree dependent on the substrate stiffness in relations to the stiffness of the native tissue [80]. Bone tissue is rigid, so it could be assumed that a substrate of greater stiffness would favor osteogenic differentiation more than a substrate of lower stiffness. The dependence of osteogenic response on rigidity of the surface has been presented also on the synthetic polyacrylamide hydrogels (PAAMs) [81].

The synthetic scaffolds, polyester of D,L-lactid, glycolid or ε-caprolactone and their copolymers were studied intensely for biomedical applications in bone and cartilage repair, as drug delivery systems, and as surgical sutures [82—86]. Porosity and pore size of scaffolds play an important role in bone formation. Whereas lower porosities can enhance osteogenesis by suppressing cell proliferation and forcing cell aggregation, higher porosities and pore size result in greater and faster bone growth [87, 88]. Another important factor is hydrophilicity of material that can be achieved for example by treatment the polycaprolactone-co-lactide surface with NaOH [89]. Cell adhesion to artificial materials is mediated by molecules of the extracellular matrix, like fibronectin, vitronectin, collagen or fibroin which are normally adsorbed spontaneously to the material from different body fluids. Most of these natural proteins contain hydrophilic niches that are responsible for interactions with cells. Silk, example of fibroin proteins, is composed mainly of stacked antiparallel β-sheets. In antiparallel sheets, hydrophobic side chains will typically be on just one face of the sheet, while hydrophilic side chains will reside on the other. The fully extended form of antiparallel β-sheets of silk makes the resulting protein resistant to stretching. Therefore, silk proteins themselves are under development as scaffolds for bone tissue regeneration [90—96].

Bioactive protein molecules adsorb on hydrophobic polymers are in a denatured and rigid state and their conformation often is inappropriate for cells to bind [97]. Enhancement of hydrophilicity of porous synthetic polymer scaffolds has been considered as an effective approach to obtain more cell/tissue compatible scaffolds in tissue regeneration [98]. Indeed, treatment of polymer with NaOH, results in the exposure of carboxylate and hydroxyl groups on the surface [99], allowing more specific ionic interactions with the positively charged amino groups of proteins [100]. Several bone tissue engineering studies have co-delivered recombinant human bone morphogenetic protein-2 (BMP-2) and bone marrow mesenchymal stem cells (MSCs) [101]. Such approach has been applied for poly(lactic-co-glycolic acid) [102, 103]. The other scaffolds for MSCs and/or BMPs delivery have been also successfully applied including different scaffolds, like silk proteins [104, 105], collagen [106], and poly(fumarate) [107], as well as synthetic injectable pH/thermo-sensitive biodegradable block copolymer, consisting of sulfamethazine oligomer-poly(ε-caprolactone-co-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-co-lactide)-sulfamethazine oligomers (SMOPCLA-PEG-PCLA-SMO) [108].

CONCLUSIONS

A booming progress of the biomedical research area called regenerative medicine and/or cell therapy has been observed in the last decade. The availability of new 3D scaffolds for cell and tissue engineering is one of the major reasons of this spectacular achievement. The years of experience in development of polymers and biopolymers for drug delivery resulted in methods to produce (bio-) polymers of quality required by medicine. Fortunately, the basic principles concerning application of polymer scaffolds like biocompatibility, metabolism, toxicology, *etc*. were similar for drug delivery and cell breading. A large number of these (bio-) polymers received approval of governmental drug regulating agencies (*e.g*. FDA). It is easy to predict that the current focus of chemical and biomedical sciences on the cell therapy would shortly become „a billion dollar global business with unlimited potential" [109].

This work has been partially supported by NCN grant UMO-2011/01/B/ST5/07818.

REFERENCES

[1] Mason Ch., Dunnill P.: *Regen. Med*. 2008, **3**, 1. [2] Bártolo P. J., Almeida H., Rezende R., Laoui T., Bidanda B.: „Advanced processes to fabricate scaffolds for tissue

engineering" in „Virtual Prototyping & Bio Manufacturing in Medical Applications", (Eds. Bidanda B., Bártolo P. J.) Springer, New York, NY, USA, 2008. [3] Hoque M. E., Feng W., Wong Y. S., *et al*.: *Tissue Eng. Part. A* 2008, **14**, 907. [4] Rath S. N., Cohn D., Hutmacher D. W.: *Virt. Phys. Prototyp*. 2008, **3**, 209. [5] Hutmacher D. W.: *J. Biomater. Sci., Polymer Ed*. 2001, **12**, 107. [6] Lazzari S., Moscatelli D., Codari F., Salmona M., Morbidelli M., Diomede L.: *J. Nanopart. Res*. 2012, **14**, 920. [7] Mahapatro A., Singh D. K.: *J. Nanobiotech.* 2011, 9, 55. [8] Pinto Reis C., Neufeld R. J., Ribeiro A. J., Veiga F.: *Nanomed: Nanotech. BiolMed*. 2006, **2**, 8. [9] Tu C., Cai Q., Yang J., Wan Y., Bei J., Wang S.: *Polym. Adv. Technol*. 2003, **14**, 565. [10] Wang G-J., Ho K-H., Hsueh C. C.: DTIP 2007.

[11] Yang F., Murugan R., Wang S., Ramakrishna S.: *Biomaterials* 2005, **26**, 2603. [12] Kim K., Yu M., Zong X., Chiu J., Fang D., Seo Y. S., *et al*.: *Biomaterials* 2003, **24**, 4977. [13] Liu H., Roy K.: *Biomaterials* 2005, **11**, 319. [14] Taqvi S., Roy K., *Biomaterials* 2006, **27**, 6024. [15] Beharvand H., Hashemi S. M., Ashtiani S. K., Farrokhi A.: *Int. J. Dev. Biol*. 2006, **50**, 645. [16] Donoghue P. S., Lamond R., Boomkamp S. D., Sun T., Gadegaard N., Riehle M. O., Barnett.S. C.: *Tissue Eng. Part. A*. 2013, **19**, 497. [17] Scholes P. D., Coombes A. G. A., Illum L., Daviz S. S., Vert M., Davies M. C.: *J. Controlled Release* 1993, **25**, 145. [18] Niwa T., Takeuchi H., Hino T., Kunou N., Kawashima Y.: *J. Controlled Release* 1993, **25**, 89. [19] Govender T., Stolnik S., Garnett M. C., Illum L., Davis S. S.: *J. Controlled Release* 1999, **57**, 171. [20] Sendil D., Maszczynska-Bonney I., Carr D. B., Lipkowski A. W., Wise D. L., Hasirci V.: *Biomaterials* 2003, **24**, 1969.

[21] Wu L., Ding J.: *Biomaterials* 2004, **25**, 5821. [22] Linbo W., Jiandong D.: *J. Biomed. Mater. Res. Part A*. 2005, **75A**, 767. [23] Levenberg S., Burdic J. A., Kraehenbuehl T., Langer R.: *Tissue Eng.* 2005, **11**, 506. [24] Gao S. Y., Lees J. G., Wong J. C. Y., Croll T. I., George P., Cooper-White J. J., Tuch B. E.: *J. Biomed. Mater. Res.* 2010, **92A**, 683. [25] Meinel L., Hofmann S., Karageorgiou V., Kirken-Head C., McCool J., Gronowicz G., *et al*.: *Biomaterials* 2005, **26**, 147. [26] Qiu Y., Mao Z., Zhao Y., Zhang J., Guo O., Gou Z., Gao C.: *Macromol. Res*. 2012, **20**, 283. [27] Seyednejad H., Ji W., Yang F., van Nostrum C. F., Vermonden T., van den Beucken J. J., Dhert W. J., Hennink W. E., Jansen J. A.: *Biomacromolecules* 2012, **13**, 3650. [28] Huang J., Wang S., Wei C., Xu Y., Wang Y., Jin J., Teng G.: *Tissue Cell.* 2011, **43**, 344. [29] Khattak S. F., Bhatia S. R., Roberts S. C.: *Tissue Eng*. 2005, **11**, 974. [30] Matthew J. E., Nazario Y. L., Roberts S. C., *et al*.: *Biomaterials* 2002, **23**, 4615.

[31] Ruszymah B. H., Chua K., Latif M. A., *et al*.: *Int. J. Pediatr. Otorhinolaryngol*. 2005, **69**, 1489. [32] Vashi A.V., Keramidaris E., Abberton K. M., *et al*.: *Biomaterials* 2008, **29**, 573. [33] Zhang S., Holmes T., DiPersio M., *et al*.: *Biomaterials* 1995, **16**, 1385. [34] Zhang S., Holmes T., Lockshin C., *et al*.: *Proc. Natl. Acad. Sci. USA* 1993, **90**, 3334. [35] Holmes T., Lacalle S., Su X., Liu G., Rich A., Zhang S.: *Proc. Natl. Acad. Sci. USA* 2000, **97**, 6728. [36] Ellis-Behnke R. G., Liang Y. X., You S. W., Tay D. K., Zhang S., So K. F., Schneider G. E.: *Proc. Natl. Acad. Sci. USA* 2006, **103**, 5054. [37] Kisiday J., Jin M., Kurz B., Hung H., Semino C., Zhang S., Grodzinsky A. J.: *Proc. Natl. Acad. Sci. USA* 2002, **99**, 9996. [38] Narmoneva D. A., Oni O., Sieminski A. L., Zhang S., Gertler J. P., Kamm R. D., Lee R. T.: *Biomolecules* 2005, **26**, 4837. [39] Semino C. E., Merok J. R., Crane G. G., Panagiotakos G., Zhang S.: *Differentiation* 2003, **71**, 262. [40] Genové E., Shen C., Zhang S., Semino C. E.: *Biomaterials* 2005, **26**, 3341.

[41] Trzebicka B., Robak B., Trzcinska R., Szweda D. D., Suder P., Silbering J., Dworak A.: *Eur. Polymer J*. 2013, **49**, 499. [42] Gordon M. K., Hahn R. A.: *Cell Tissue Res*. 2010, **339**, 247. [43] Schenke-Layland K., Rhodes K. E., Angelis E., Butylkova Y., HeydarkhanHagvall S., Gekas C., Zhang R., Goldhaber J. I., Mikkola H. K., Plath K., MacLellan W. R.: *Stem Cells* 2008, **26**, 1537. [44] Chiang C. K., Chowdhury M. F., Iyer R. K., Stanford W. L., Radisic M.: *Acta Biomater*. 2010, **6**, 1904. [45] Maheshwari G., Brown G., Lauffenburger D. A., Wells A., Griffith L. G.: *J. Cell Sci*. 2000, **113**, 1677. [46] Buzanska L., Ruiz A., Zychowicz M., Rauscher H., Ceriotti L., Rossi F., Colpo P., Domańska-Janik K., Coecke S.: *Acta Neurobiol. Exp. (Wars).* 2009, **69**, 24. [47] Willerth S. M., Faxel T. E., Gottlieb D. I., Sakiyama-Elbert S. E.: *Stem Cells* 2007, **25**, 2235. [48] Liu H., Collins S. F., Suggs L. J.: *Biomaterials* 2006, **27**, 6004. [49] Toole B. P.: *Sem. Cell Dev. Biol*. 2001, **12**, 79. [50] Gerecht S., Burdick J. A., Ferreira L. S., Townsend S. A., Langer R., Vunjak-Novakovic G.: *Proc. Natl. Acad. Sci. USA* 2007, **104**, 11298.

[51] Dickinson L. E., Ho C. C., Wang G. M., Stebe K. J., Gerecht S.: *Biomaterials* 2010, **31**, 5472. [52] Gerecht S., Burdick J. A., Ferreira L. S., Townsend S. A., Langer R., Vunjak-Novakovic G.: *Proc. Natl. Acad. Sci. USA* 2007, **104**, 11298. [53] Ferreira L. S., Gerecht S., Fuller J., Shieh H. F., Vunjak-Novakovic G., Langer R.: *Biomaterials* 2007, **28**, 2706. [54] Pietrucha K.: *Int. J. Biol. Macromol*. 2005, **36**, 299. [55] Park S. N., Lee H. J., Lee K. H., Suh H.: *Biomaterials* 2003, **24**, 1631. [56] Davidenk N., Campbell J. J., Thian E. S., Watson C. J., Cameron R. E.: *Acta Biomater*. 2010, **6**, 3957. [57] Skopinska-Wisniewska J.: *Polimery* 2013, **58**, 100. [58] Moll R., Divo M., Langbein L.: *Histochem. Cell Biol*. 2008, **129**, 705. [59] Shimomura Y., Aoki N., Schweizer J., Langbein L., Rogers M. A., Winter H., Ito M.: *JBC* 2002, **277**, 45493. [60] Burnett L. R., Rahmany M. B., Richter J. R., Aboushwareb T. A., Eberli D., Ward C. L., Orlando G., Hantgan R. R., Van Dyke M. E.: *Biomaterials* 2013, **34**, 2632.

[61] Lipkowski A. W., Gajkowska B., Grabowska A., Kurzepa K.: Polimery 2009, **54**, 386. [62] Jurga M., Lipkowski A.W., Lukomska B., Buzanska L., Kurzepa K., Sobanski T., Habich A., Coecke S., Gajkowska B., Domanska-Janik K.: *Tissue Eng.* 2009, **15**, 365. [63] Utrata-Wesołek A., Trzebicka B., Dworak A.: *Polimery* 2009, **54**, 334. [64] Utrata-Wesołek A., Oleszko N., Trzebicka B., Aniol J., Zagdanska M., Lesiak M., Sieron A., Dworak A.: *Eur. Polymer J*. 2013, **49**, 106. [65] Polcyn P., Janiszewska J., Lipkowski A., Urbanczyk-Lipkowska Z.: *Polimery* 2009, **54**,

407. [66] Dworak A., Trzebicka B., Kowalczuk A., Utrata-Wesołek A., Wałach W., Libera M., Kronek J.: *Polimery* 2012, **57**, 441. [67] Oliveira J. M., Salgado A. J., Sousa N., Mano J. F., Reis R. L.: *Prog Polym. Sci.* 2010, **35**, 1163. [68] Shakhbazau A., Shcharbin D., Seviaryn I., Goncharova N., Ionov M., Bryszewska M.: *Mol. Biol. Rep*. 2010, **37**, 2003. [69] Zuk P. A., Zhu M., Mizuno H., Huang J., Futrell J. W., Katz A. J.: *Tissue Eng*. 2001, **7**, 21. [70] Lewandowska-Szumiel M., Wójtowicz J.: *Curr. Pharm. Biotechnol.* 2011, **12**, 1850.

[71] Low K. L., Tan S. H., Zein S. H., Roether J. A., Mourino V., Boccaccini A. R.: *J. Biomed. Mater. Res. B Appl. Biomater.* 2010, **94**, 273. [72] Guven S., Mehrkens A., Saxer F., Schaefer D. J., Martinetti R., Martin I., Scherberich A.: *Biomaterials* 2011, **32**, 5801. [73] Muller A. M., Mehrkens A.: *Eur. Cell Mater*. 2010, **19**, 127. [74] Orlovskij V. P., Ezhova J. A., Rodicheva G. V., Koval E. M., Sukhanova G. E., Tesikova L. A.: *J. Inorg. Chem*. 1992, **37**, 881. [75] Liu Q., Cen L., Yin S., Chen L., Liu G., Chang J., Cui L.: *Biomaterials* 2008, **29**, 4792. [76] Zanetti A. S., McCandless G. T., Chan J. Y., Gimble J. M., Hayes D. J.: *J. Tissue. Eng. Regen. Med*. 2012, in press. [77] Bojar W., Kucharska M., Bubak G., Ciach T., Koperski Ł., Jastrzębski Z., Gruber B. M., Krzysztoń-Russjan J., Marczewska J., Anuszewska E. L., Drozd E., Brynk T.: *Acta Bioeng. Biomech.* 2012, **14**, 39. [78] Woźniak P., Bil M., Ryszkowska J., Wychowański P., Wróbel E., Ratajska A., Hoser G., Przybylski J., Kurzydłowski K. J., Lewandowska-Szumieł M.: *Acta Biomater.* 2010, **6**, 2484. [79] Evans N. D., Minelli C., Gentleman E., LaPointe V., Patankar S. N., Kallivretaki M., Chen X., Roberts C. J., Stevens M. M.: *Eur. Cell Mater*. 2009, **18**, 1. [80] Pelham R. J., Wang Y.: *Proc. Natl. Acad. Sci. USA* 1997, **94**, 13661.

[81] Witkowska-Zimny M., Walenko K., Walkiewicz A. E., Pojda Z., Przybylski J., Lewandowska-Szurmiel M.: *Acta Biochim. Pol*. 2012, **59**, 261. [82] Rentsch C., Rentsch B., Breier A., Spekl K., Jung R., Manthey S., *et al*.: *J. Biomed. Mater. Res. A* 2010, **95**, 964. [83] Williams J. M., Adewunmi A., Schek R. M., Flanagan C. L., Krebsbach P. H., Feinberg S. E., *et al*.: *Biomaterials* 2005, **26**, 4817. [84] Vergroesen P. P., Kroeze R. J., Helder M. N., Smit T. H.: *Macromol. Biosci*. 2011, **11**, 722. [85] Bramfeldt H., Sarazin P., Vermette P.: *J. Biomed. Mater. Res.* A 2007, **83**, 503. [86] Tomihata K., Suzuki M., Tomita N.: *Biomed. Mater. Eng*. 2005, **15**, 381. [87] Karageorgiou V., Kaplan D.: *Biomaterials* 2005, **26**, 5474. [88] Hutmacher D. W., Schantz J. T., Lam C. X., Tan K. C., Lim T. C.: *J. Tissue Eng. Regen. Med*. 2007, **1**, 245. [89] Rentsch B., Bernhardt R., Scharnweber D., Schneiders W., Rammelt S., Rentsch C.: *Biomatter* 2012, **2**, 158. [90] Mandal B. B., Kundu S. C.: *Acta Biomater*. 2010, **6**, 360.

[91] Ak F., Oztoprak Z., Karakutuk I., Okay O.: *Biomacromolecules* 2013, in press. [92] Miyamoto S., Koyanagi R., Nakazawa Y., Nagano A., Abiko Y., Inada M., Miyaura C., Asakura T.: *J. Biosci. Bioeng*. 2012, in press. [93] Jiang J., Hao W., Li Y., Yao J., Shao Z., Li H., Yang J., Chen S.: *Biotechnol. Lett*. 2012, in press. [94] Sun L., Parker S. T., Syoji D., Wang X., Lewis J. A., Kaplan D. L.: *Adv. Healthcare Mater*. 2012, **1**, 729. [95] Kasoju N., Bora U.: *Adv. Healthcare Mater*. 2012, **1**, 393. [96] Kuboyama N., Kiba H., Arai K., Uchida R., Tanimoto Y., Bhawal U. K., Abiko Y., Miyamoto S., Knight D., Asakura T., Nishiyama N.*: J. Biomed. Mater. Res. B Appl. Biomater*. 2013, **101**, 295. [97] Bacakova L., Filova E., Parizek M., Ruml T., Svorcik V.: *Biotechnol. Adv*. 2011, **29**, 739. [98] Oh S. H., Lee J. H.: *Biomed. Mater*. 2013, **8**, 1. [99] Rentsch B., Hofmann A., Breier A., Rentsch C., Scharnweber D.: *Ann. Biomed. Eng*. 2009, **37**, 2118. [100] Atthoff B., Hilborn J.: *J. Biomed. Mater. Res. B Appl. Biomater*. 2007, **80**, 121.

[101] Luu H. H., Song W. X., Luo X., Manning D., Luo J., Deng Z. L., Sharff K. A., Montag A. G., Haydon R. C., He T. C.: *J. Orthop. Res*. 2007, **25**, 665. [102] Choi Y. S., Park S. N., Suh H.: *Biomaterials* 2005, **26**, 5855. [103] Jeon O., Song S. J., Kang S. W., Putnam A. J., Kim B. S.: *Biomaterials* 2007, **28**, 2763. [104] Meinel L., Karageorgiou V., Hofmann S., Fajardo R., Snyder B., Li C., Zichner L., Langer R., Vunjak-Novakovic G., Kaplan D. L.: *J. Biomed. Mater. Res*. A 2004, **71**, 25. [105] Haider M., Cappello J., Ghandehari H., Leong K. W.: *Pharm. Res*. 2008, **25**, 692. [106] Ikeuchi M., Dohi Y., Horiuchi K., Ohgushi H., Noshi T., Yoshikawa T., Yamamoto K., Sugimura M.: *J. Biomed. Mater. Res*. 2002, **60**, 61. [107] Hedberg E. L., Shih C. K., Lemoine J. J., Timmer M. D., Liebschner M. A., Jansen J. A., Mikos A. G.: *Biomaterials* 2005, **26**, 3215. [108] Kim H. K., Shim W. S., Kim S. E., Lee K. H., Kang E., Kim J. H., Kim K., Kwon I. C., Lee D. S.: *Tissue Eng. Part A* 2009, **15**, 923. [109] Mason C., Brindley D. A., Culme-Seymour E. J., Davie N. L: *Regen. Med*. 2011, **6**, 265.

Received 18 II 2013.