Biodegradable Polyesters for Tissue Engineering

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Summary: Paper describes basic characteristics of synthesis and properties of aliphatic polyesters used for tissue engineering. Described is also synthesis of polyester containing block copolymers suitable for surface modification. Described are methods used for scaffold fabrication with required porosity. In particular, presented are methods according to which scaffolds are made from prefabricated polyester micro- and nanoparticles.

Keywords: aliphatic polyesters; polymeric scaffolds; tissue engineering

Introduction

In many instances diseases and accidents result in so severe damage of soft and hard tissues that at normal conditions they cannot regenerate and heal only by natural forces. Sometimes defects are due to genetic disorders or other developmental reasons. In such situations at least partial restoration of affected functions may be achieved by using appropriate prostheses, in particular the implanted ones. Examples are artificial blood vessels and fascia. These implants are permanent ones and preferentially should retain their properties for the patient's life. Thus, they should be made from not degradable materials. However, there are many cases when permanent implants are not appropriate. For example, bones are in a relatively slow but continuous change due to decomposition of bone material by osteoclasts and regeneration by bone building osteoclasts. Therefore, bone substituting implants must be suitable to participate in the mentioned above turnover process that desirably should lead to restoration of the natural bone elements. In principle, the implants should serve as degradable scaffolds on the basis of which the bone is being formed by bone forming cells.

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There are several requirements that such scaffolds must fulfill. They should be not toxic, not terratogenic, not inflamatory, and not cancerogenic. Moreover, they should have necessary mechanical strength. Sponge-like or fibrous structure of scaffolds should provide compartments with appropriate size for osteclasts as well as for vascularization. Surface of pores should allow adhesion of osteoclasts, their proliferation and normal functioning. Scaffold material should be degradable, either hydrolytically, under action of osteoclasts or under action of enzymes present in body fluids penetrating scaffolds after implantation. Possibility of transfer of nutrients into and undesirable metabolites outside of scaffolds is an important property.

Many materials have been investigated till now for making scaffolds for bone regenerative procedures. However, we are still far from fully satisfactory solutions. Hydrolytically or biodegradable aliphatic polyesters constitute an important group of polymers used for fabrication of scaffolds for hard tissue engineering. This chapter briefly presents current state of knowledge on synthesis of these polymers and describes methods used for fabrication of scaffolds from them.

Synthesis of Aliphatic Polyesters

Linear aliphatic polyesters can be obtained by ring-opening polymerization of cyclic esters, by condensation of hydroxyacids or

by biotechnological processes.[1–9] The most facile for synthesis of polyesters with controlled molecular weight, molecular weight distribution and microstructure is the first from abovementioned methods. Polycondensation, in spite of its apparent simplicity, is a process quite difficult to control. It requires efficient removal of water (or alcohols when ester derivatives are used as starting material) and must be carried on at elevated temperatures at which the main process is often accompanied with side reactions. Biotechnological processes in which bacteria (e.g. *Bacillus megaterium*) produce polyesters as an energy and carbon reserve material) have been quite well developed f synthesis of poly(3-hydroxybutyrate) and its derivatives. In spite that cost of biotechnological processes is still higher than the cost of polymers obtained by synthetic methods the former one may be considered as a method of choice when copolymers with special chemical structure are needed.. In subsequent part of this section only the synthesis by ring-opening polymerization of cyclic esters will be discussed.

From series of cyclic esters the four-, six- and seven-membered ones can be homo- and copolymerized. Due to thermodynamic reasons homopolymerization of the five-membered γ–butyrolactone yields only oligomeric species.[4] However, this monomer can be copolymerized with other cyclic esters.

Polymerization of four-membered cyclic esters can be initiated with alcoholates and with carboxylates. However, even in the case of polymerization of β–propiolactone initiated with alcoholates after the short initial period polymerization proceeds on carboxylate active centers.[10]

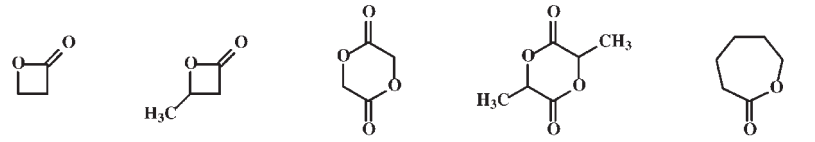
Polymerization of six- and seven-membered cyclic esters can proceed by anionic or by coordination ring-opening polymerization, depending on chemical structure of initiators. In anionic polymerization nucleophilic attack on carbonyl carbon atom and acyl-oxygen ring scission leads to addition of monomer molecule with regeneration of alcoholate active center.

Coordination ring-opening polymerization of cyclic esters can be initiated with metal alkoxides (e.g. Sn (II), Zn, Al, Sn(IV), Ti, La, Y, Zr).[2] Tin(II) 2-ethylhexanoate was often used as an initiator approved by FDA for polymers synthesized for medical applications. It has been found that initiation with tin(II) 2-ethylhexanoate proceeds efficiently in presence of alcohols (co-initiators) converting tin(II) 2-ethylhexanoate into tin alkoxide.[11] Propagation consists of coordination of monomer molecules by active centers with subsequent monomer insertion.

It is worth noting that number of chains growing from one active center is equal to the number of alkoxide groups attached to the metal atom.[2,12]

Coordination polymerization of ε-caprolactone and lactide has important advantage over anionic polymerization of these monomers. Namely, strongly nucleophilic alcoholate anions – active centers of anionic polymerization – participate not only in propagation but also in intramolecular (cyclization) and intermolecular transfer reactions. For much less reactive species responsible for coordination polymerization the transesterification reactions are much slower than propagation and thus clean synthesis of linear polymers can be achieved.[13–16]

Active centers are often aggregated and reactivity of nonaggregated and aggregated species usually differs significantly. For example, in the case of coordination polymerization of ε–caprolactone initiated with dialkylaluminum alkoxides the propagating not aggregated species are in equilibrium with not propagating (dormant) dimers or trimers.[17,18] Aluminum triisopropoxide, often used as an initiator in polymerization of cyclic esters, can be present as trimer or tetramer. In polymerization of ε–caprolactone trimer was found to be an effective initiator whereas tetramer was not reactive towards monomer. At room temperature the rate of tetramer conversion to reactive trimer is much slower than initiation. Thus, in a particular experiment the rate of polymerization depends on fraction



Scheme 1.
Structures of homopolymerizing cyclic esters.

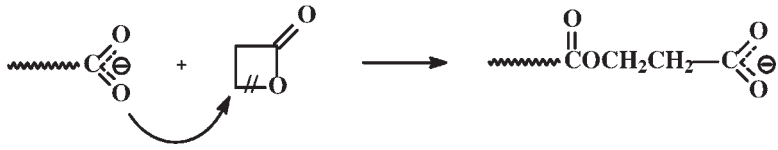
of trimer in a mixture used for initiation. Initiation with pure isolated trimer leads to controlled polymerization yielding polymer with molecular weight predicted by the monomer/initiator ratio.[19,20]

Aggregation of active species in coordination polymerization of cyclic esters can be reduced by using initiators with bulky substituents. Moreover, Spassky found that some bulky initiators with chiral centers allow for enantioselective polymerization of racemic lactide (due to the presence of two chiral centers in each molecule there are lactides with two *R* ((*RR*)-lactide), two *S* ((R*S*)-lactide) and with *RS* (*meso*-lactide) centers).[21–23] Example of chiral initiators (1,1′-binaphthyl-2,2′-diamine complex of aluminum alkoxide – SBO_2Al–OR) used for polymerization of lactides is given in Scheme 5. Chirality in these initiators comes from restricted rotation of 2- and 2′- substituted naphthyl groups.

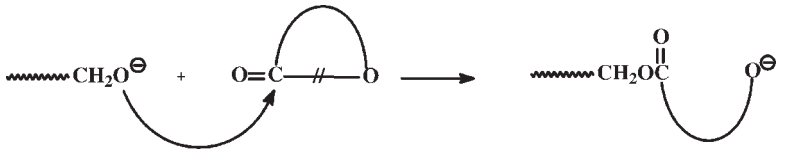
Polymerization of racemic lactides initiated with racemic SBO_2Al–OR or achiral initiators in which binaphthyl moiety was replaced with $–(CH_2)_n–$ linkage yields polylactides with multiblock copolymers composed from poly[(*SS*)-lactide] and poly[(*RR*)-lactide] blocks.[24–27] Recently Duda reported on synthesis of poly[(*S*)-lactide]-grad-poly[(*RR*)-lactide] obtained in polymerization in which chirality of active centers was changed by addition (when (SS)-enantiomer was already consumed) of a ligand with opposite chirality to the one in initiator.[28] The process wascarried on as follows. First a chiral initiator was produced in THF solution in reaction of (*S*)-2,2′- [1,1′-binaphthyl-2,2′-diylbis (nitrylomethylidyne)]diphenol and aluminum-*tris*-isopropoxide. In each initiator molecule was present Al-(O^iPr) reactive group and a chiral ligand. Thereafter, racemic lactide was added to this mixture and polymerization was carried on to the almost complete conversion of (*SS*)-lactide enantiomer. At this stage (*R*)-2,2′-[1,1′-binaphthyl-2,2′-diylbis(nitrylomethylidyne)]-diphenol was added to the polymerizing mixture what in result led to polymerization of (*RR*)-lactide.

Synthesis of poly(*SS*)- and poly(*RR*)-lactides attracted attention of many researchers because macromolecules of these stereoisomers in solid state form stereocomplexes. Properties of stereocomplexes in solid state significantly differ from those of pure stereoisomers alone. For example, melting point of poly[(*SS*)-lactide]/poly[(*RR*)-lactide] stereocomplexes from high molecular weight stereoisomers are about 50 °C higher than for pure stereoisomers alone.[22,26–36]

Typically, ring-opening polymerizations of cyclic esters are carried on either in bulk or in solution. However, when product is



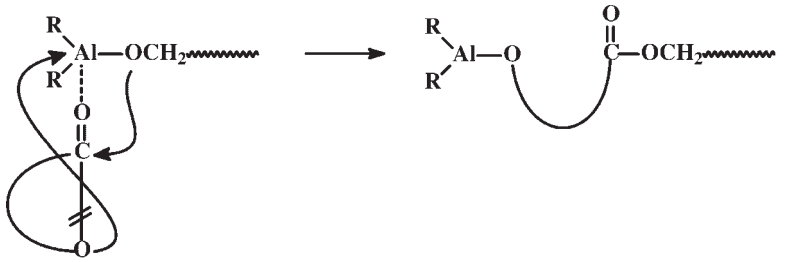
Scheme 2.
Anionic polymerization of β-propiolactone (counterion omitted).



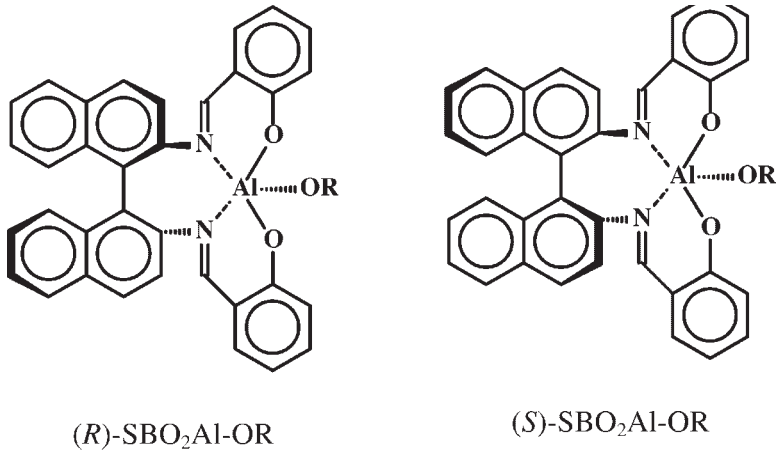
Scheme 3.
Anionic polymerization of cyclic esters with larger than four-membered rings (counterion omitted).

needed in form of micron-size particles dispersion polymerization of cyclic esters may be the method of choice. First reports on synthesis of polyesters by dispersion polymerization of cyclic esters are dated to 1972 when Union Carbide patented synthesis of polyester microparticles by dispersion polymerization of ε–caprolactone, methyl-ε–caprolactone and δ–valerolactone.[37] Systematic studies of this type of polymerization begun, however, only about twenty years later.[38] In the mentioned above work there were investigated polymerizations of ε–caprolactone and (*R,S*)–lactide initiated with $(CH_3CH_2)_2$-$AlOCH_2CH_3$ and carried on in presence of poly(dodecyl acrylate)-*g*-poly(ε–caprolactone) used as a suspension stabilizer. 1,4–Dioxane-heptane mixtures (1:4 and 1:9 v/v for polymerizations of lactide and ε–caprolactone) were used as reaction medium. Polymerizations of ε–caprolactone and of lactides were carried on at room temperature and at 80 °C, respectively. At these conditions at initial stage monomer, initiator and stabilizer were dissolved in reaction medium. However, shortly after onset of polymerization polymer precipitated into spherical particles. The mentioned above polymerizations of ε–caprolactone and (*R,S*)–lactide yielded particles with number average diameters (D_n) equal 0.63 and 2.50 μm, for poly(ε–caprolactone) and poly[(R,S)-lactide] respectively. Diameter distributions of synthesized particles were narrow. In case of poly(ε–caprolactone) D_w/D_n (ratio of weight to number average diameter) was equal 1.038. For poly[(R,S)- lactide] particles D_w/D_n was equal 1.15. Subsequent studies allowed optimization of polymerization process (by using poly(dodecyl acrylate)-*g*-poly(ε– caprolactone) stabilizer with ratio of M_n of poly(dodecyl acrylate) main chain and of poly(ε–caprolactone) grafts equal 4). At these conditions particles with $D_w/D_n = 1.03$ were obtained.[39] It has been found also that addition of new monomer portions does not lead to formation of new particles but to an increase of size of the already existing ones. Increase of particle volume was proportional to amount of added monomer.[40]

It is worth noting that proper post-synthesis thermal treatment allows production of polylactide microspheres with controlled degree of crystallinity (in a range



Scheme 4.
Coordination polymerization of cyclic esters initiated with dialkylaluminumalkoxides.



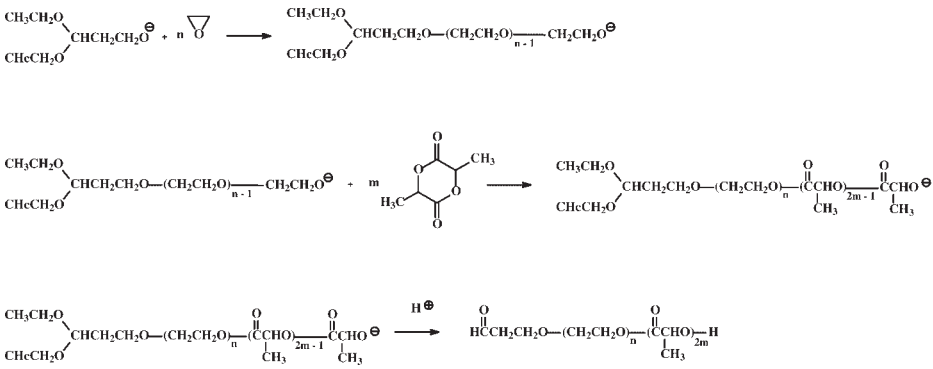
Scheme 5.
Chiral aluminum alkoxides, initiators of stereospecific polymerization of lactides.

from 0 to 60%).[41] When poly[(SS)-lactide] microspheres suspended in dodecane are heated to 175 °C the polymer melts converting each solid partuicle into a droplet. Subsequent rapid cooling to – 30 °C yields fully amorphous miscrospheres. Annealing of poly[(SS)-lactide] microspheres in heptane at 80 °C for controlled periods of time gives particles with required degree of crystallinity.

Ofter molecular weight of polyesters should be taiolored keeping particular applications in mid. Since, in coordination polymerization of cyclic esters side transesterification reactions are essentially eliminated the molecular weight of synthesized polymer can be planned taking into account the following simple equation:

$$M_n = \frac{MW([M]_0 - [M]_e)}{[I]_0 f} \quad (1)$$

In the equation MW denotes molecular weight of monomer, $[M]_0$ and $[M]_e$ initial and equilibrium monomer concentrations, $[I]_0$ and f initial concentration of



Scheme 6.
Synthesis of poly(ethylene oxide)-*b*-polylactide block copolymer with aldehyde end-groups (as proposed in Ref.[49]).

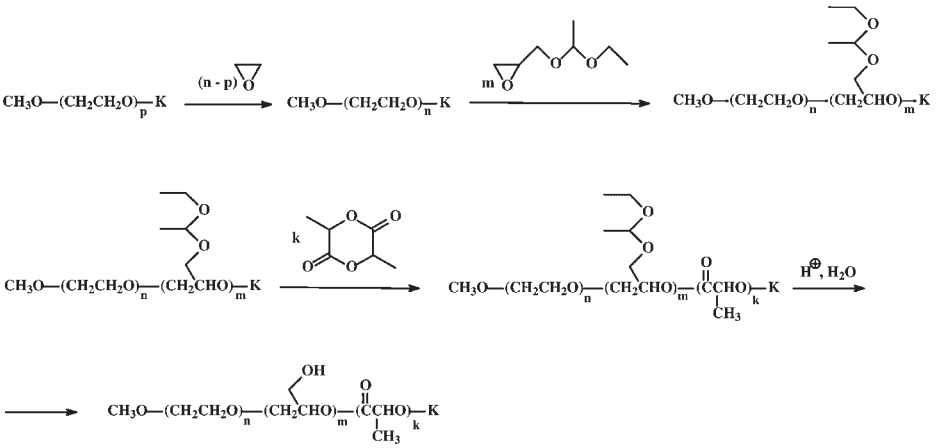
initiator and initiator functionality (number of propagating chains initiated by one initiator molecule). Obviously, presence of any impurities decreasing number of propagation species strongly molecular weight of synthesized polymer. Technical difficulties with preparation of sufficiently pure systems put certain limits on maximal attainable molecular weight of polyesters synthesized by ring-opening polymerization. In polymerization of ε–caprolactone and lactides initiated with $Al(O^iPr)_3$ polymers with molecular weight up to 300 000 were obtained.[42,43] In polymerization of lactide initiated with $Sn(OC_4H_9)_2$ polymer with M_n close to 1 000 000 was synthesized.[44]

As it has been already mentioned anionic polymerization of cyclic esters is accompanied with side reactions that decrease molecular weight of synthesized polymer (intramolecular transesterification – cyclization) and increasing molecular weight dispersity (cyclization and intermolecular chain transfer). Thus it is worth noting that in the anionic dispersion polymerization of ε–caprolactone with sodium counterion polymer with $M_n = 106\,600$ and with narrow molecular weight dispersity ($M_w/M_n = 1.15$) was obtained.[45] Reasons of such low dispertipy of poly(ε–caprolactone) is not clear. There is a possibility that low chain mobility inside of polymer particles, lower than in solution, decreases rates of transesterification.

Synthesis of Hydrophilic-Hydrophobic Copolymers with Polyester Blocks

For fabrication of polyester scaffolds are sometimes needed biocompatible additives suitable for modification of scaffold surfaces. These modifications include controlled tuning of scaffolds hydrophilicity and introduction of reactive groups suitable for immobilization of bioactive compounds (e.g. growth factors). Especially suitable for the mentioned above purpose are amphiphilic copolymers with polyester and polyether blocks. There are several reports on synthesis of poly(ethylene oxide)-*b*-polylactide block copolymers.[46–49] Usually the synthesis is a one-pot two-step process. At the beginning ethylene oxide is polymerized into the poly(ethylene oxide) block. This block is used as a macroinitiator for polymerization of lactide.

Copolymers with relatively short poly (ethylene oxide) and long polylactide blocks (e.g. for DP of poly(ethylene oxide) equal 30 and polylactide containing in average over 80 –C(O)OCH(CH_3)- units) are water insoluble. Those with shorter polylactide chains have limited water solubility and



Scheme 7.
Synthesis of poly(L,L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) block copolymer.

above critical concentration form polymeric micelles that are considered as promising carriers for drug delivery.

Block copolymers shown in Scheme 6 may be used not only for modification of hydrophilicity of the surface of polyester scaffolds but also for covalent immobilization of bioactive compounds (e.g. proteins or oligopeptides). Unfortunately they have only one functional (aldehyde) group per polymer chain.

Recently, in our laboratory there was developed a method for synthesis of polylactide-*b*-polyether block copolymers with polyglycidol block that contains one functional (hydroxyl group) per one monomeric unit.[50] Scheme 7 illustrates synthesis of these copolymers.

Formation of Scaffolds for Hard Tissue from Polyesters

Scaffolds for hard tissue support and development not only should be made from materials degradable to non toxic compounds during time needed for new bone formation but also must have appropriate morphology. Interconnected pores should provide space sufficient for accommodation of bone forming cells. The optimal pore size is in a range from about 150 to 500 μm. Several methods were used for formation of objects with the mentioned above morphology. One of the simplest and very often used is called solution casting – salt leaching method.[51–54] The method consists on preparation of polymer solution in low boiling solvent (e.g. chloroform). To this solution sufficient amount of salt (NaCl) particles with needed size (matching required size of pores) are added. The mixture prepared in the above mentioned way is cast into the mould. After solvent evaporation the sample is placed into water into which salt is washed out. Water is changed several times for time period needed for complete salt removal (usually from 72 to 96 hours). Instead of NaCl also sugar has been used as porogen.[55]

Porous scaffolds were prepared also by using paraffin as a porogen and hydrocarbon as a solvent for paraffin removal. Polymers (polylactide or poly(lactide-*co*-glycolide)) were dissolved in chlorophorm and compounded with paraffin into a pulp. Thereafter solvent was evaporated. Eventually, paraffin was washed out from this mixture using hydrocarbon (e.g. hexane) that did not dissolve polyester.[56]

Some researchers prepared the highly porous scaffolds by thermally induced phase separation process.[57,58] This process consists on preparation of polyester solution in dioxane, dioxane/water mixture or in dimethyl carbonate. Solutions are poured into molds and frozen (depending on particular procedure to temperatures from −5 to −196 °C). Then, temperature is raised to the one in a range from −5 to −10 °C and solvents are sublimed at high vacuum. Scaffolds with a very high, up to 98%, porosity were obtained by this method. The size of the pores was in a range from about 100 to 500 μm, depending on temperature of sample freezing.

A very interesting method for scaffold preparation was reported recently by Li et al.[59] This method leading to the bone replica did consist on preparation of calcined bovine cancellous bone. This bone has been used as a mold soaked with poly[(R,S)-lactide] solution in chloroform during several times repeated steps including sample evacuation and immersion in polymer solution. In this way the solution could penetrate inside of the bone. Thereafter the sample was dried and afterwards treated with HCl (concentration 1 mol/l). During this step bone elements were removed and scaffolds with porosity from 86 to 93% were obtained.

An important problem in all processes based on preparation of scaffolds from polymer solutions is related to removal of traces of organic solvents. This difficulty may be overcome either by fabrication of scaffolds in processes in which pores are created by foaming with gases or in processes when starting material is a solid polymer (micro or nanofibres, powders or

granules). In a gas foaming method the solid compression molded polyester (polylactide, poly(lactide-*co*-glycolide)) samples are exposed to CO_2 at high pressure (e.g. 5.5 MPa) for time sufficiently long for equilibrium of CO_2 partition between the gas and solid phases (e.g. for three days).[60,61] After that time, when pressure is rapidly decreased to the atmospheric one the pores are formed.

Recently, there is a growing interest in scaffold formation from electrospun fibers.[62–64] Formation of fibers consists on flow of polyester solution (e.g., in N,N-dimethylformamide, polymer concentration in a range from 30 to 50%) through the small capillary kept at the high electric potential (often in a range from 20 to 30 kV). The polymer solution droplet pending from the capillary is strongly deformed by electric field. Above the threshold potential the droplet breaks and polymer solutions are broken into several highly charged polymer solution streams. During movement towards the receiving metal plate (counter-electrode) solvent evaporates and polyester fibers with diameters in a range from about 100 nm to 1 μm are formed. The fibers assemble into a nonwoven web of interconnected fibers that can be used as a scaffold cut to required dimensions.

Scaffolds made without using any organic solvent were obtained also by a method based on room temperature injection molding of a polyester – NaCl particles mixture and subsequent salt washing out with water.[65] The mentioned above method was used for fabrication of scaffolds from poly(ε–caprolactone) and poly(lactide-*co*-glycolide). Porosity of these scaffolds ranged from 76 to 90% and size of pores was varied from 90 to 450 μm.

Recently, C.T. Laurencin and co-workers developed a very simple and elegant method for preparation of scaffolds from poly(lactide-co-glycolide) microspheres.[66,67] First, polyester microspheres were obtained by emulsification of polyester dissolved in methylene dichloride in water containing 1% (wt/v) of poly(vinyl alcohol). Methylene dichloride was removed by stirring water suspension of microspheres for 24 hours in water. The solidified microspheres were isolated by filtration, washed with water, dried in air and under vacuum. Eventually, the particles were sieved into fractions with narrow diameter distribution. Required amounts of dried particles were placed into molds and heated to 62 °C; it is above the glass transition temperature of polymer in microspheres. Heating was carried on for 24 hours. During this time particles were sintered due to polymer chain migration between microspheres. Porosity of scaffolds prepared by sintering of microspheres was in a range from 32 to 39%. Diameters of pores were controlled by size of microspheres. For particles with diameters ranging from 300 to 350 μm the average pore size was equal 100 μm. When diameters of particles

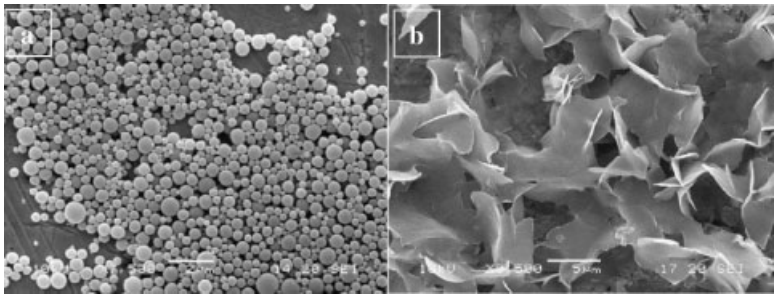


Figure 1.
Poly[(RS)-lactide-co-glycidol] microspheres obtaine by dialysis from dimethylformamide and poly(SS)-lactide particles obtained by dialysis from dimethylsulfoxide (reproduced with permission from Ref.[69]).

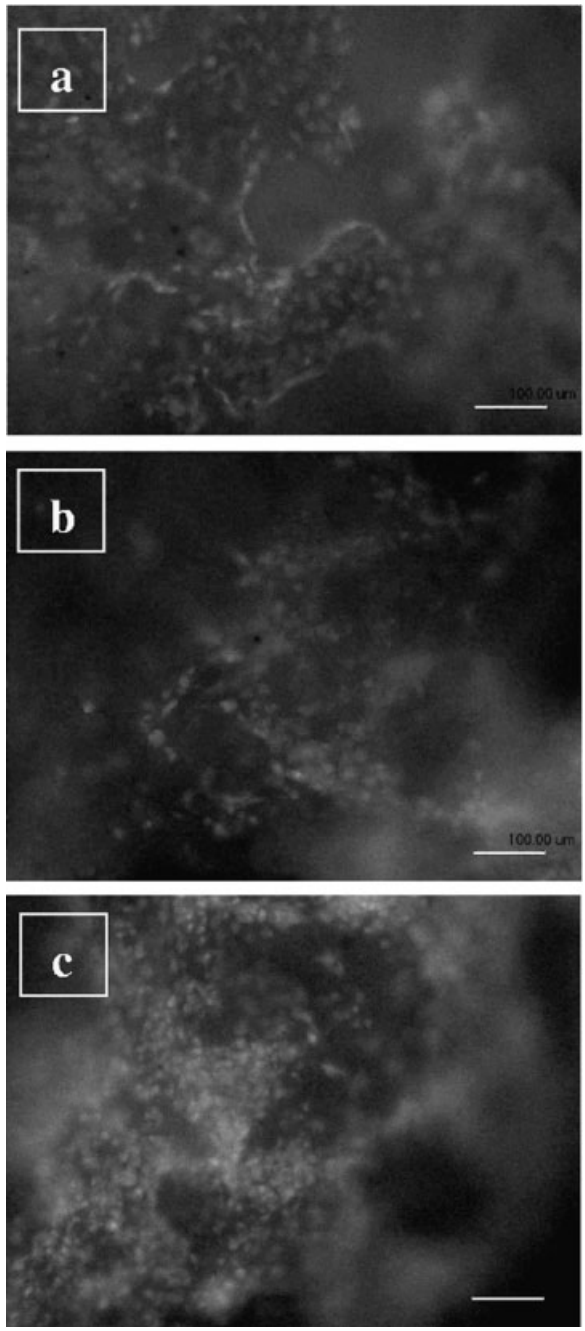


Figure 2.
Photos of MG-63 cells cultured for 34 days on macro-and mesoporous poly(SS)-lactide/poly[(RS)-lactide-co-glycidol] scaffold containing 50 wt.-% of poly[(RS)-lactide-co-glycidol] microspheres: (A) top surface; (B) a middle plane; (C) bottom surface. Bar = 100 μm (reproduced from Ref.[68]).

were from 425 to 590 μm the average diameter of pores was 150 μm.

In our laboratory there was developed a method allowing for formation of scaffolds with a bimodal pore distribution. Large pores were suitable for accommodation of the bone-forming cells, nanoporous walls were designed with purpose to facilitate transfer of nutrients into and unwanted metabolites out of the scaffolds.[68] The scaffolds were made by using a method combining of sintering a mixture of polyester (poly(S,S)-lactide, and poly[(S,R)-lactide)-*co*-glycolide]) particles) and NaCl particles, with subsequent salt leaching in water. Spherical particles and particles with irregular shape were obtained by dialysis against water of polymer solutions in water miscible organic solvents (acetonitrile, 1,4-dioxane, dimethylformamide, dimethylsulfoxide or tetrahydrofuran).[69] Shape of particles did depend on chemical structure of polyesters and on solvent from which the dialysis was carried on. Dialysis from 1,4-dioxane yielded spherical particles regardless of polyester that was used. Dialysis from other solvent yielded spherical particles only in the case of poly[(S,R)-lactide)-*co*-glycolide]; for all other mentioned above polymers particles with irregular shape were obtained.

Predetermined amounts of polyester particles and particles of NaCl (size from 50 to 400 μm were) mixed and sintered at 70 °C (slightly above glass temperature of poly[(RS)-lactide-co-glycidol]) under relatively low pressure 10 MPa. Salt leaching yielded scaffolds with macropores with size corresponding to the size of salt particles and with mesoporous walls. Dimensions of mesopores were in a range from about 10 to 65 nm, depending on proportion between homo and co-polyesters in the mixture. The largest mesopores were obtained for 50% wt content of poly[(RS)- lactide-co-glycidol].

It is worth noting that bone forming cells placed onto surface of scaffolds prepared in the mentioned above way migrated into scaffolds interior. This is clearly seen in Figure 2 shows fluorescence microsope photos of top, middle plane cut and bottom of the scaffolds. Cells and cell aggregates are visible as light spots.

In principle all the discussed above methods were used also dor preparation of scaffolds with hydroxyapatite, calcium carbonate and silica fillers. In many systems presence of fillers in the scaffolds increased theur mechanical strength.

Conclusions

Discussion on syntheses of polyesters and methods of scaffold formation that was presented in this chapter show that it is relatively easy to control chemical structure, molecular weight and molecular weight distribution of polyesters as well as scaffold morphology. However, there are still some important questions that should be answered before the whole process of scaffold fabrication and application could be fully controlled and understood. To problems that still require explanation belong relations between bone-forming cells proliferation and cell-forming activity and chemical structure of scaffolds interfaces (including interface inside of pores) and topography on submicron level. There is also a need for much better understanding of dependence of rates of scaffold degradation due to interactions with cells present in it.

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[1] S. Slomkowski, A. Duda, in: "*Ring-Opening Polymerization*", D. J. Brunelle, Ed., Carl Hanser Verlag, Munchen **1993**, p. 87.
[2] A. Duda, S. Penczek, in: "*Biopolymers; Polyesters II – Preparation and Chemical Synthesis*", A. , Steinbüchel, Y. Doi, Eds., Wiley-VCH, Weinheim **2001**, p. 371.
[3] U. Edlund, A.-Ch. Albertsson, *Adv. Polym. Sci.* **2002**, *157*, 62.
[4] S. Penczek, M. Cypryk, A. Duda, P. Kubisa, S. Slomkowski, *Adv. Polym, Sci.* **2007**, *32*, 247.
[5] W. H. Carothers, G. A. Arvin, *J. Am. Chem. Soc.* **1929**, *51*, 2560.

[6] M. Vert, *Angew. Makromol. Chem.* **1989**, *166/167*, 155.
[7] H. R. Kricheldorf, I. Kreiser-Sauders, *Macromol. Symp.* **1996**, *103*, 85.
[8] Y. Doi, M. Kunioka, Y. Nakamura, K. Soga, *Macromolecules* **1986**, *19*, 1274.
[9] A. Steinbüchel, T. Lütke-Eversloh, *Biochem, Eng. J.* **2003**, *16*, 81.
[10] A. Hofman, S. Slomkowski, S. Penczek, *Makromol. Chem.* **1984**, *185*, 91.
[11] A. Kowalski, A. Duda, S. Penczek, *Macromolecules* **2000**, *33*, 689.
[12] S. Penczek, T. Biela, A. Duda, *Macromol. Rapid Commun.* **2000**, *21*, 941.
[13] A. Hofman, S. Slomkowski, S. Penczek, *Makromol. Chem. Rapid Commun.* **1987**, *8*, 387.
[14] S. Penczek, A. Duda, S. Slomkowski, *Macromol. Symp.* **1992**, *54/55*, 31.
[15] J. Baran, A. Duda, A. Kowalski, R. Szymanski, S. Penczek, *Macromol. Symp.* **1998**, *128*, 241.
[16] S. Penczek, A. Duda, R. Szymanski, *Macromol. Symp.* **1998**, *132*, 441.
[17] A. Duda, S. Penczek, *Makromol. Chem., Macromol. Symp.* **1991**, *47*, 127.
[18] A. Duda, S. Penczek, *Macromol. Rapid Commun.* **1994**, *15*, 559.
[19] A. Duda, S. Penczek, *Macromol. Rapid Commun.* **1995**, *16*, 67.
[20] A. Duda, *Macromolecules* **1994**, *29*, 1399.
[21] A. Le Borgne, V. Vincens, M. Jouglard, N. Spassky, *Makromol. Chem., Macromol. Symp.* **1993**, *73*, 37.
[22] N. Spassky, M. Wisnewski, Ch. Pluta, A. Le Borgne, *Macromol. Chem. Phys.* **1996**, *197*, 2627.
[23] M. Wisniewski, A. Le Borgne, N. Spassky, *Macromol. Chem. Phys.* **1997**, *198*, 1227.
[24] Ch. P. Radano, G. L. Baker, M. R. Smith, III, *J. Am. Chem. Soc.* **2000**, *122*, 1552.
[25] T. M. Ovitt, G. W. Coates, *J. Am. Chem. Soc.* **2002**, *124*, 1316.
[26] Z. Zhong, P. J. Dijkstra, J. Feijen, *J. Am. Chem. Soc.* **2003**, *125*, 11291.
[27] N. Nomura, R. Ishi, M. Akakura, K. Aoi, *J. Am. Chem. Soc.* **2002**, *124*, 5938.
[28] K. Majerska, A. Duda, *J. Am. Chem. Soc.* **2004**, *126*, 1026.
[29] H. Tsuji, F. Hori, S.-H. Hyon, Y. Ikada, *Macromolecules* **1991**, *24*, 2719.
[30] H. Tsuji, S.-H. Hyon, Y. Ikada, *Macromolecules* **1991**, *24*, 5657.
[31] L. Cartier, T. Okihara, B. Lotz, *Macromolecules* **1997**, *30*, 6313.
[32] H. Tsuji, Y. Ikada, *Polymer* **1999**, *40*, 6699.
[33] D. Brizzolara, H.-J. Cantow, K. Diederichs, E. Keller, A. J. Domb, *Macromolecules* **1996**, *29*, 191.
[34] J. Slager, M. Gladnikoff, A. J. Domb, *Macromol. Symp.* **2001**, *175*, 105.
[35] J.-R. Sarasua, N. L. Rodriguez, A. L. Arraiza, E. Meaurio, *Macromolecules* **2005**, *38*, 8362.
[36] L. Li, Z. Zhong, W. H. de Jeu, P. J. Dijkstra, J. Feijen, *Macromolecules* **2004**, *37*, 8641.
[37] US Patent 3 632 669(1972),(Union Carbide)), R. D. Ludberg, F. P. D. Gludice.
[38] S. Sosnowski, M. Gadzinowski, S. Slomkowski, S. Penczek, *J. Bioact. Compat. Polym.* **1994**, *9*, 345.
[39] S. Slomkowski, S. Sosnowski, M. Gadzinowski, *Polym. Prep. Am. Chem. Soc., Div. Polym. Chem.* **1996**, *37*, 135.
[40] S. Slomkowski, S. Sosnowski, in: "*Polymeric Drug and Drug delivery Systems*", R. M. , Ottenbrite, S. W. Kim, Eds., CRC, Boca Raton, FL **2001**, p. 261.
[41] S. Sosnowski, *Polymer* **2001**, *42*, 637.
[42] A. Duda, S. Penczek, *Macromolecules* **1995**, *28*, 5981.
[43] Ph. Degee, Ph. Dubois, R. Jerome, *Macromol. Chem. Phys.* **1997**, *198*, 1973.
[44] A. Kowalski, J. Libiszowski, A. Duda, S. Penczek, *Macromolecules* **2000**, *33*, 1964.
[45] S. Slomkowski, S. Sosnowski, m. Gadzinowski, *Colloids Surf. A. Physicochem. Eng. Asp.* **1999**, *153*, 111.
[46] C. Scholz, M. Iijima, Y. Nagasaki, K. Kataoka, *Polym. Adv. Technol.* **1998**, *9*, 768.
[47] K. Yasugi, T. Nakamura, Y. Nagasaki, M. Kato, K. Katanka, *Macromolecules* **1999**, *32*, 8024.
[48] Y. Yamamoto, Y. Nagasaki, Y. Kato, Y. Sugiyama, K. Kataoka, *J. Control. Release* **2001**, *77*, 27.
[49] H. Otsuka, Y. Nagasaki, K. Kataoka, *Adv. Drug Delivery Rev.* **2003**, *55*, 403.
[50] M. Gadzinowski, S. Sosnowski, *J.Polym.Sci.: Part A: Polym.Chem.* **2003**, *41*, 3750.
[51] A. G. Mikos, G. Sarakinos, S. M. Leite, J. P. Vacanti, R. Tanger, *Biomaterials* **1993**, *14*, 323.
[52] A. G. Mikos, A. J. Thorsen, L. A. Czerwonka, Y. Bao, R. Langer, D. N. Winslow, J. P. Vacanti, *Polymer* **1994**, *35*, 1068.
[53] G. Shi, Q. Cai, Ch. Wang, N. Lu, S. Wang, J. Beim, *Polym. Adv. Technol.* **2002**, *13*, 227.
[54] K. Filipczak, I. Janik, M. Kozicki, P. Ulanski, J. M. Rosiak, L. A. Padewski, R. Olkowski, P. Wozniak, A. Chroscicka, M. Lewandowska-Szumiel, *e-Polymers* **2005**, no. 011; http://www.e-polymers.org.
[55] Q. Hou, D. W. Grijpma, J. Feijen, *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2003**, *67*, 732.
[56] V. P. Shastri, I. Martin, R. Langer, *Proc. Nation. Acad. Sci. USA* **2000**, *97*, 1970.
[57] Y. Hu, D. W. Grainger, S. R. Winn, O. Hollinger, *J. Biomed. Mater. Res.* **2002**, *59*, 563.
[58] L. Safinia, J. J. Blaker, V. Maquet, A. R. Boccaccini, A. Mantalaris, A. R. Bismarck, *e-Polymers* **2005**, no. 010; http://www.e-polymers.org.
[59] H. Li, K. Lin, *J. Chang, J. Biomater. Sci. Polymer Edn* **2005**, *16*, 575.
[60] D. J. Mooney, D. F. Baldwin, N. P. Suh, J. P. Vacanti, R. Langer, *Biomaterials* **1996**, *17*, 1417.

[61] X. Wang, W. Li, V. Kumar, *Biomaterials* **2006**, *27*, 1924.
[62] K. Kim, M. Yua, X. Zong, J. Chiu, D. Fang, Y.-S. Seo, B. S. Hsiao, B. Chu, M. Hadjiargyrou, *Biomaterials* **2003**, *24*, 4977.
[63] K. Fujihara, M. Kotaki, S. Ramakrishna, *Biomaterials* **2005**, *26*, 4139.
[64] A. S. Badami, M. R. Kreke, M. S. Thompson, J. S. Riffle, A. S. Goldstein, *Biomaterials* **2006**, *27*, 596.
[65] L. Wu, D. Jing, J. Ding, *Biomaterials* **2006**, *27*, 185.
[66] M. Borden, M. Attawia, Y. Khan, C. T. Laurencin, *Biomaterials* **2002**, *23*, 551.
[67] M. Borden, S. F. El-Amin, M. Attawia, C. T. Laurencin, *Biomaterials* **2003**, *24*, 597.
[68] S. Sosnowski, P. Wozniak, M. Lewandowska-Szumiel, *Macromol. Biosc.* **2006**, *6*, 425.
[69] M. Gadzinowski, S. Sosnowski, S. Slomkowski, *e-Polymers* **2005**, no. 084; http://www.e-polymers.org.

