Functionalized Biodegradable Nano- and Microspheres for Medical Applications

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Summary: This minireview describes strategies for preparation of biodegradable (from polylactides, and poly(ε-caprolactone) and from their derivatives) nano- and microspheres for medical applications, in particular for drug delivery. In addition to standard methods of particles' formation by emulsification of polymer solution in water-miscible organic solvent with subsequent solvent evaporation or extraction there are described methods of particles formation by self assembly of polymer macromolecules, by dialysis of polymer solutions in organic water-miscible solvents carried on against water and by dispersion ring-opening polymerization of heterocyclic monomers. Strategies for encapsulation of bioactive compounds into nano- and microspheres are presented.

Keywords: drug delivery; encapsulation; microspheres; nanoparticles; polylactide; poly(ε-caprolactone)

Introduction

Polymer nano- and microparticles are gathering increasing attention of researchers interested in materials for medical applications. Initially, the microparticles were used as constituents of simple diagnostic tests.[1–3] Later, drug delivery systems based on nano- and microparticles from polymers degradable and metabolized to not harmful products were elaborated.[4–6] Recently, polymer particles found new applications as building blocks for implantable scaffolds for tissue engineering.[7–9]

Dimensions of particles tailored for drug delivery should be close to the dimensions of such natural carriers as transport proteins (for example human serum albumin) or to the dimensions of viruses or cells. In an ideal situation the synthetic carriers should be ''invisible'' for immune-defense system of the treated organism and at the same time should allow for the targeted delivery to selected tissue. There are four main strategies for fabrication of particles for drug delivery. The simplest and the oldest one consist in emulsification of polymer or of polymer and drug solutions in water-miscible organic solvent with subsequent water extraction or evaporation from the droplets of emulsion. Dimensions of these particles are usually close to dimensions of living cells (a few micrometers). The second one is based on self-assembly of amphiphilic block copolymers and drug into nanoparticles with a core-shell structure. With respect to the size these carriers are similar to viruses (from 20 to 100 nm). Reactive groups (for example the pH and ionic strength sensitive carboxyl groups or chromophores changing conformation under irradiation) allow for the stimuli controlled release of particles' content. The third method consists in dialysis of polymer and drug solution in organic water-miscible solvent (often stabilizers are added to the solution) against water.[10] This method usually yields particles with diameters slightly exceeding 1 μm. The last one, developed in our laboratory involves dispersion polymerization of cyclic esters leading to formation of biodegradable microspheres.[11–16] These particles

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could be loaded with bioactive compounds either during polymerization or after particle synthesis.

Tissue engineering opens new ways for treatment of a variety of illnesses otherwise very difficult to be cured. Especially this applies to the cases with bone defects which should be filled or replaced with bone substituents. Such substituents could be created by bone-forming cells (osteoblasts) cultivated on temporary biodegradable scaffolds. List of requirements concerning materials for the scaffolds for tissue engineering includes, among other, morphology allowing in-growth of cells, transport of nutrients in and removal of unwanted metabolites as well as appropriate mechanical properties. Direct synthesis of poly)L,L-lactide) microspheres elaborated earlier in our laboratory, and more recently developed methods for production of polyester microparticles (poly(L,L-lactide) and poly(D,L-lactide-*co*-glycoliode) with various morphologies (microspheres, flakes, particles with irregular shape) by dialysis provided basis for simple ways for scaffold preparation.[1–9,17]

Particles by Emulsification of Polymer and Drug Solutions in Organic Solvent and by Subsequent Organic Solvent Evaporation or Extraction

This method being the simplest one is still used for preparation of particles containing bioactive compounds. Typically, 6.6 g of poly(L,L-lactide) was dissolved in 6.6 ml of methylene dichloride.[17–19] The solution was added dropwise to stirred (from 50 to 200 revolutions per minute) 150 ml of water solution containing surfactant (e.g. 4% wt/v of poly(vinyl alcohol) or 3% wt/v of sodium dodecyl sulfate. The mixture was kept at 30 °C and stirring was continued for 30 min. This was a sufficient time for solvent evaporation. Formed particles were isolated by sedimentation. Diameters of particles ranged from 2 to 35 μm, depending on stirring rate and on concentration of surfactant. Addition of bioactive compounds to the primary solution of polyester in organic solvent yielded drug or other substance loaded microparticles. It is worth noting that diameter distribution of such particles is rather high (dispersity parameter D_w/D_n – where D_w and D_n denote weight and number average diameters, respectively – was usually close or even exceeding 3).

The described above method was used for preparation of particles loaded with a number of bioactive compounds including, among others: triclosan (an antimicrobial agent),[18,19] recombinant hepatitis B surface antigen,[20] progesterone,[21] neuroleptic haloperidol,[22] anticancer drugs 10-hydroxycampothecin,[23] irinotecan (CPT-11 or camptothecin-11),[24] disodium norcantharidate,[25] paclitaxel,[26] etoposide,[27] antiviral drug ganciclovir,[28] human growth hormone,[29] antiinflamatory ibuprofen[30] and flurbiprofen,[31] immunosuppressant cyclosporin A.[32]

During formation of particles by the polymer solution emulsification solvent extraction method the diffusion of solvent from outer parts of polymer solution droplets often results in creation of a solid polymer ''skin'' on particles' surface impeding further solvent diffusion. Penetrating solvent forms channels which latter are converted into pores. The scheme illustration the described above process is shown in Figure 1.

In Figure 2 there is shown an example of the scanning electron microscope picture of the porous microsphere obtained by the polymer solution emulsification solvent extraction method.

It is worth noting, however, that proper adjustment of parameters of particle formation allows for obtaining particles with smooth surface.[18,22,28] Especially important is control of the rate of solvent evaporation.

Formation of particles by polymer solution emulsification solvent extraction method consists in preparation of an emulsion of polymer solution in another organic medium used as a continuous phase. This second liquid being a nonsolvent for polymer should be miscible with solvent used for preparation of polymer

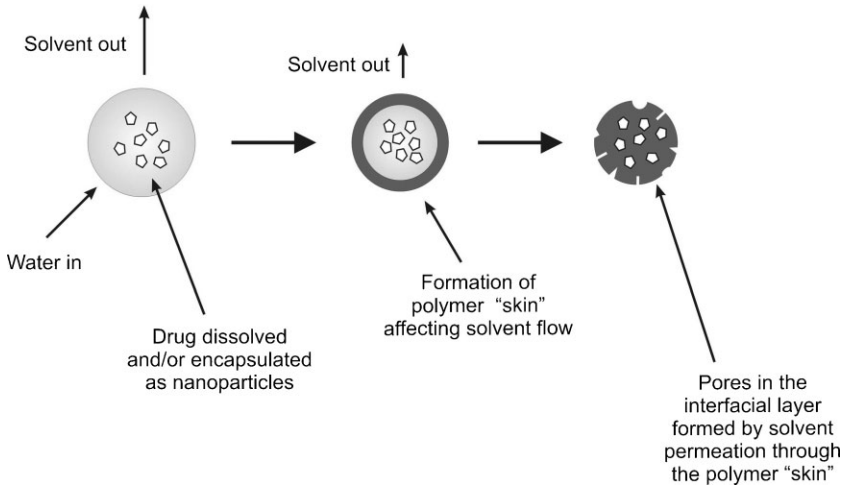


Figure 1.
Scheme illustrating formation of drug loaded polymer particles by the polymer solution emulsification solvent evaporation method.

solution. An example of microspheres preparation based on our earlier work is given below.[17]

Poly(L,L-lactide) was dissolved in ε-caprolactone (1:1 wt:v). This solution was dispersed in heptane (tenfold volume with respect to the volume of ε-caprolactone) containing surfactant (Span 85, 3% wt/v). Obtained emulsion was poured into isopropanol containing polyvinylpirrolidone (PVP, 5% wt/v). Extraction of ε-caprolactone from polymer solution droplets into the isopropanol rich medium yields solid microparticles. The final product was obtained by extensive washing of particles with water containing PVP (0.1% wt/v). The number average diameter of microspheres obtained by the method described above was $D_n = 20\,\mu m$ and the dispersity parameter was equal $D_w/D_n = 2.06$.[17]

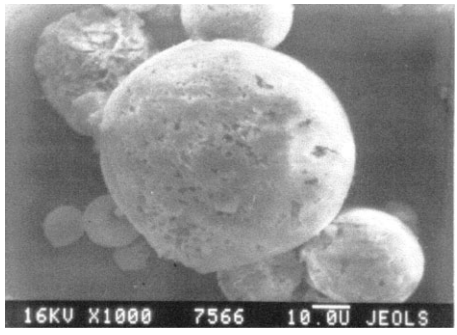


Figure 2.
SEM picture of the poly(L,L-lactide) microspheres obtained by the polymer solution emulsification solvent evaporation method.

It is needed to note that for the obvious reasons the preparation of drug-loaded microspheres by emulsion solvent extraction method is possible only in the case when solvents used for extraction (e.g. isopropanol in the described system) are nonsolvents not only for polymer but also for the drug. Otherwise it would be difficult to achieve sufficiently high degree of drug encapsulation.

Poly(L,L-lactide) and Poly(L,L-lactide-co-glycolide) Microparticles by Dialysis

Physicochemical processes occurring during dialysis of polymer solution in organic water-miscible solvent carried on against water are similar to those which do occur during solvent extraction from polymer solution droplets. In the first case the polymer solution phase is a continuous one whereas in the second case it is dispersed into microdroplets. Moreover, usually there is a significant difference in the rate of solvent extraction from droplets and transport through the membrane

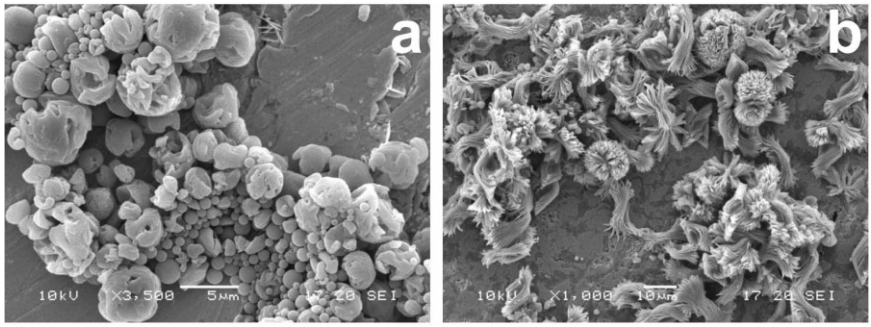


Figure 3.
SEM picture of the poly(L,L-lactide) microspheres obtained by dialysis from polymer solutions in 1,4-dioxane (a) and in acetonitrile (b) (reproduced with permission from Ref. 10).

during dialysis. Due to the very large surface to volume ratio (for particles with radius 1 μm this ratio $S/V = 4\pi R^2/(4/3)\pi R^3$ equals $3 \cdot 10^6$ 1/m) the extraction of solvent from emulsion – and in result formation of polymer microspherers – is much faster than formation of microspheres by dialysis. The former usually requires minutes, the latter often many hours. It is worth noting that slow formation of microparticles by dialysis (with continuous but slow change of the medium from solvent to nonsolvent yielded poly(L,L-lactide) microspheres with number average diameters much lower ($D_n = 1.0$ μm, dialysis from polymer solution in 1,4-dioxane)[10] than in the case of particles obtained emulsification-solvent evaporation or extraction methods (D_n larger than 2 and often even larger than 20 μm). However, diameter dispersity of poly(L,L-lactide) microspheres obtained by dialysis was also very broad ($D_w/D_n = 2.76$).[10]

It should be noted that very important is proper selection of organic solvent used for preparation of poly(L,L-lactide) microspheres by dialysis. From many water-miscible solvents: 1,4-dioxane, DMF, DMSO, THF, and acetonitrile only the first one was suitable for preparation of microspheres. In the case of all other solvents polymer precipitation was accompanied with crystallization of poly-(L,L-lactide) and various polymer crystal aggregates were formed. This difference is illustrated in Figure 3.

It is worth noting that particles obtained by dialysis of poly(D,L-lactide-*co*-glycolide) (Resomer® 504, product of Boehringer, Ingelheim, Germany, ratio of polylactide and polyglycolide units 51:49) were spherical, regardless of the solvent in which polymer was dissolved and their diameters and diameter dispersity parameters were significantly lower than in the case of particles obtained from poly(L,L-lactide) (see Table 1). Such behavior should be related to noncrystallinity of poly(D,L-lactide-*co*-glycolide) which in the case of poly(L,L-lactide) could interfere with formation of spherical particles.

Nanoparticles from Block Copolymers Containing Polyester and Polyether Blocks

Particles with diameters exceeding 10 μm are too large to pass capillary blood vessels and therefore they are used mainly as drug

Table 1.
Number-average diameter (D_n) and diameter dispersity (D_w/D_n) of spherical particles obtained by dialysis of Resomer® 504 from various solvents (based on data from Ref. 10, with permission of e-Polymers Foundation).

Solvent	D_n, μm	D_w/D_n
1,4-Dioxane	0.660	1.57
THF	0.545	2.02
DMF	0.380	1.37
Acetonitrile	0.580	2.05
DMSO	0.360	1.58

depot with localized action. For intravenous administration are needed particles with much smaller diameters, usually with sub-micrometer radii. Very small particles are needed for drug delivery through various barriers (e.g. through the blood-brain barrier, through endothelium or through the barriers separating tumors from normal tissue).[33–37] These nanoparticles are most often made from block copolymers which consist of polylactide or poly(ε-caprolactone) and poly(ethylene oxide) blocks. Di- and triblock copolymers (polyether-*b*-polyester-*b*-polyether or polyester-*b*-polyether-*b*-polyester) were used. Nanoparticles were produced by nanoprecipitation (nanoprecipitation consists in rapid change of continuous phase properties by pouring polymer dissolved in water-miscible solvent (e.g. in acetone) into water),[34,36,37] or by dialysis.[33,35]

Recently in our laboratory there was developed synthesis of a triblock poly(ethylene oxide)-*b*-polyglycidol-*b*-polylactide (poly(L,L-lactide) and poly(D,L-lactide)) copolymer.[38–41] The copolymer was synthesized by sequential anionic polymerization of ethylene oxide, 1-ethoxyethylglycidyl ether (glycidol with blocked hydroxyl groups) and lactide. Deblocking of hydroxyl groups in synthesized copolymer lead to the desired poly(ethylene oxide)-*b*-polyglycidol-*b*-polylactide product. The scheme showing copolymer synthesis is shown in Figure 4.

Copolymers with M_n of polylactide blocks from 2400 to 11800, of polyglycidol blocks from 500 to 9100 and poly(ethylene oxide) blocks from 1700 to 11200 were obtained. The hydroxyl groups in copolymers were used for further functionalization introducing into copolymers pH sensitive carboxyl or light sensitive azobenzene side groups.[39,41,42]

Drug loaded nanoparticles were obtained by addition of appropriate bioactive compound to solution of copolymer used for dialysis or nanoprecipitation. In this way nanoparticles loaded with pyrene (model of hydrophobic drug),[39,41,41] papaverine,[33] 10-hydroxycamptothecin,[34]

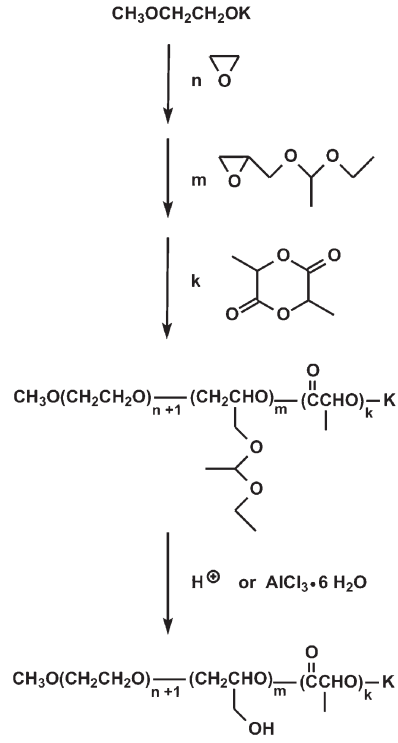


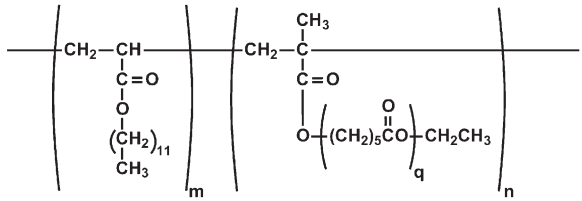
Figure 4.
Scheme illustrating synthesis of poly(ethylene oxide)-*b*-polyglycidol-*b*-polylactide triblock copolymer.

all-*trans*-retinoic acid, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide,[36] 4′-demethylepipodophyllotoxin[37] were obtained.

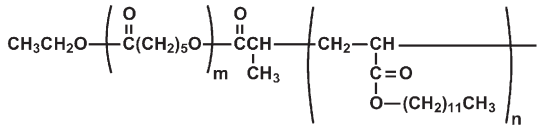
Diameters of nanoparticles composed of block copolymers were in the range from about 20 nm to 180 nm, depending on the copolymer microstructure and length of particular blocks.

Polyester Microspheres by Dispersion Polymerization of Lactides and ε-caprolactone

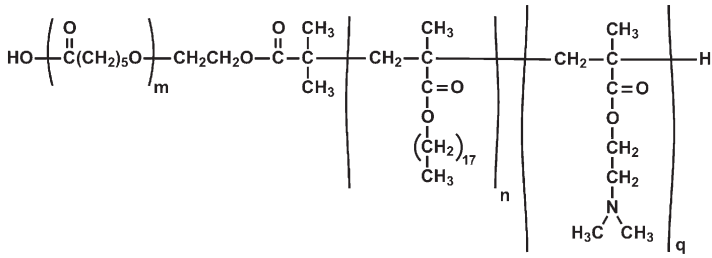
Polymerization of title monomers was carried on in mixed solvents (1,4-dioxane:-heptane 1:8 v/v for ε-caprolactone and 1:4 v/v for D,L- and L,L-lactones). In this medium monomers, initiators (tin(II) 2-ethylhexanoate or 2,2-dibutylstanna-1,3-dioxepane for lactides[11,13,14] and diethylaluminumethoxide or trimethylsilanolate for ε-caprolactone[11,12,43]) as well as dispersion



Poly(docecyl acrylate)-*g*-poly(ε-caprolactone)



Poly(ε-caprolactone)-*b*-poly(dodecyl acrylate)



Poly(ε-caprolactone)-*b*-poly(octadecyl methacrylate)-
-*b*-poly(dimethylaminoethyl methacrylate)

Figure 5.
Chemical structure of suspension stabilizers used for synthesis of polylactide and poly(ε-caprolactone) microspheres by dispersion polymerization.

stabilizers (poly(dodecyl acrylate)-*g*-poly(ε-caprolactone),[11] poly(ε-caprolactone)-*b*-poly(octadecyl methacrylate)-*b*-poly(dimethyl-aminoethyl methacrylate), and poly(ε–caprolactone)-*b*-poly(octadecyl methacrylate-*grad*-dimethylaminoethyl methacrylate)[44]) were soluble and thus, before the polymerization started the whole mixture was homogeneous. Syntheses of poly(ε-caprolactone) microspheres were carried on at room temperature whereas syntheses of polylactide microspheres required temperature elevated to 95 °C.[11] Diameters of poly(ε-caprolactone) microspheres ranged from 0.6 to 1.0 μm. In the case of poly(L,L-lactide) microspheres D_n was in a range from 2.7 to about 6.5 μm, depending on the initial monomer concentration.[17,40] Dispersity parameter of synthesized microspheres did depend on chemical structure of copolymer stabilizer. The best results ($D_w/D_n = 1.03$) were obtained for poly(dodecyl acrylate)-*g*-poly(ε-caprolactone) in which M_n of poly(ε-caprolactone) blocks was close to 25% of M_n of the whole copolymer.[40]

Recently, Muranaka et al. described synthesis of poly(L,L-lactide) microspheres by dispersion polymerization using xylene/heptanes mixture as a reaction medium and poly(dodecylmethacrylate)-*co*-poly[α-methacryloxyethoxy-poly(L,L-lactide)] as a dispersion stabilizer.[45] The most important difference between the mentioned above system and system developed in

our laboratory (see Ref. 11) did consist in using stabilizer with poly(L,L-lactide) instead of poly(ε-caprolactone grafts). This change and the change of reaction medium from 1,4-dioxane/heptanes to xylene/heptanes mixture yielded microspheres with D_n in the range from 180 to 800 nm. Very elegant refinement of particles synthesis made by Muranaka and Ono was based on using poly[(dodecyl methacrylate)-co-(2-hydroxyethyl methacrylate)] as a precursor of dispersion stabilizer produced *in-situ* during dispersion polymerization of L,L-lactide. In this process hydroxyl groups of poly(2-hydroxyethyl methacrylate) units did function as centers from which poly(L,L-lactide) chains were grafted. In this way the true stabilizer (poly(dodecyl-methacrylate)-*co*-poly[α-methacryloxyethoxy-poly(L,L-lactide)]) was produced parallel to formation of poly(L,L-lactide) microspheres.[46]

Critical analysis of experimental data allowed formulation of the following mechanism of particle formation.[16] Initiation of the polymerization proceeds in solution. When growing chains approach the critical length (M_n ranging from 500 to 800) they undergo the random coil-globule transition. Aggregation of polymer globules leads to formation of the primary particles. In this way, at the early period of polymerization almost all growing macromolecules are located in particles. Polymerization is possible due to efficient swelling with monomer of the microspheres containing propagation active centers. Thus, the main part of polymer formation occurs within particles which function as nano or microcontainers.

Four strategies were developed for formulation of drug loaded microspheres obtained by dispersion polymerization. The first one consists in adsorption of hydrophobic molecules onto the polyester particles. In this way the poly(D,L-lactide) microspheres with adsorbed proteins (human serum albumin and gamma globulin) were obtained.[11] The second one was based on swelling polyester particles with liquid bioactive compound.[47] Ethyl salicylate was used as a model drug. Swelling was carried on in the ethanol:water mixture (7:3 v/v). When partition of drug between continuous poly(ε-caprolactone) microspheres and continuous medium achieved equilibrium the particles were transferred into water in which the drug is insoluble. The microspheres with ethyl salicylate content up to 37 wt% were produced. The third strategy is appropriate for drugs with hydroxyl groups from which the polyester chains might be grown. The method was tested using N,N-bis-(hydroxyethyl)isonicotinamide as a model compound. The dispersion polymerization of ε-caprolactone initiated in the presence of N,N-bis-(hydroxyethyl)isonicotinamide yielded microparticles containing up to 6.4 wt% of covalently immobilized (via ester linkage) drug.[40] Drug release requires hydrolysis of this ester bond. The last developed method did consist in physical incorporation of bioactive compound during particle formation in the polymerization process. This method was used for synthesis of poly(L,L-lactide) microspheres loaded with omeprasol (an inhibitor of $(H^+\text{-}K^+)$ATPase).[48] The initial stage of polymerization was carried on in a standard way, without the bioactive compound. Solution containing omeprasol was added only when the reaction mixture was turbid indicating formation of microspheres. There were obtained particles containing up to 11%wt of the drug. It is important to stress that that this method could be taken into consideration only for drugs which do not contain any functional groups (e.g. hydroxyl or carboxyl) which might interfere with polymerization of L, L-lactide.

Instead of Conclusions

Until now practical applications as drug carriers found only particles from polylactide (D,L or L,L)-*co*-glycolide copolymers which most often were prepared by the polymer solution emulsification-solvent evaporation (or extraction) methods. This

minireview presents some new methods which might be considered as interesting candidates for elaboration of the pharmacologically useful drug formulations.

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