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Surface-Functionalized Nanoparticles for Controlled Drug Delivery

Sung-Wook Choi, Woo-Sik Kim, and Jung-Hyun Kim

Summary

Nanoparticles have been extensively investigated in drug-delivery systems. Especially, the effectiveness of the surface-functionalized nanoparticles, which consist of copolymers with functional molecules, is well demonstrated. This chapter describes the complete technique for the preparation of surface-functionalized nanoparticles. Tetracycline with an affinity to bone was chosen as a model material for surface functionalization. There are two steps for the preparation of tetracycline-modified nanoparticles. The first step is the conjugation of poly(D,L-lactide-co-glycolic acid) with tetracycline via carbodiimide chemistry and is the most often employed. Three kinds of techniques—the emulsification-diffusion method, nanoprecipitation, and the dialysis method—are used for nanoparticle formation of the resulting copolymer. Prepared nanoparticles having a size <200 nm and a hydrophilic surface layer can be applied for bone-specific drug delivery.

Key Words

Surface-functionalized nanoparticles; carbodiimide chemistry; poly(D,L-lactide-co-glycolic acid); tetracycline; emulsification-diffusion method; nanoprecipitation method; dialysis method; bone-specific drug delivery.

1. Introduction

Well-designed functional nanoparticles are of great interest for drug-delivery systems. Functional nanoparticles suitable for biomedical applications are defined as polymeric particles with submicron size having the characteristics of protective ability of encapsulated therapeutic agents, avoidance of the reticuloendothelial system, long circulation time in the body, and site-specific delivery with the targeting moiety. The size of the nanoparticles as well as their surface properties is the crucial factor for practical in vivo study. Numerous works ***(1–4)*** have recommended the nanoparticle (less than 100 nm diameter) with

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the hydrophilic surface, which can minimize opsonization and subsequent clearance by the macrophage.

Surface-functionalized nanoparticles have been proven to be useful in controlled-release ***(5)*** and site-specific delivery. Many researchers have demonstrated site-specific delivery using surface-functionalized nanoparticles with a high affinity to target sites ***(6–8)***. In this chapter, bone-specific drug delivery using surface-functionalized nanoparticles is discussed. Poly(D,L-lactide-co-glycolic acid) (PLGA) was used as a biodegradable polymer, which is approved for in vivo use by the Food and Drug Administration. Tetracycline was chosen as a model functional molecule because it has a strong affinity for adsorption to calcium phosphate ***(9)*** and thus can serve as a targeting moiety for the bone-specific drug-delivery system. Detailed procedures for the conjugation of tetracycline to PLGA and the three kinds of methods for nanoparticle formation are described.

2. Materials

1. PLGA (lactic acid : glycolic acid ratio of 75 : 25, mol wt = 10,000; Wako, Saitama, Japan).
2. Ethyl acetate.
3. Acetone.
4. Diethyl ether.
5. Dimethylformamide (DMF).
6. Tubular dialysis membrane (mol wt cutoff = 12,000 g/mol).
7. Poloxamer 188.
8. Homogenizer (Omni, Waterbury, CT).
9. Tetracycline (store at 0°C).
10. Hydroxyapatite (HA).
11. HCl.
12. *N*-Hydroxysuccinimide (NHS).
13. Dicyclohexylcarbodiimide (DCC).
14. Phosphate-buffered saline (PBS) (pH 7.4).
15. Syringe filter (0.45-μm pore size).
16. Pyrene.

3. Methods

Complete techniques on the conjugation of tetracycline to PLGA and nanoparticle formation of conjugated polymer are described. The overall procedures for the preparation of functionalized nanoparticles are introduced in **Subheading 3.1.** (*see* **Fig. 1**) and the detailed steps follow. Tetracycline, chosen as a functional molecule, is conjugated to the PLGA via carbodiimide chemistry (**Subheading 3.2.**). Then the resulting conjugated polymer is formed into nanoparticles by three different methods (**Subheding 3.3.**). Finally, the specific affinity of tetracycline-modified nanoparticles to HA is examined (**Subheading 3.4.**).

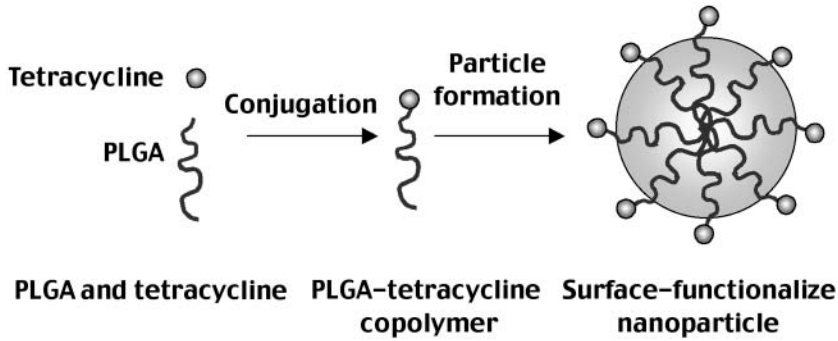

Fig. 1. Overall procedure for preparation of surface-functionalized nanoparticle.

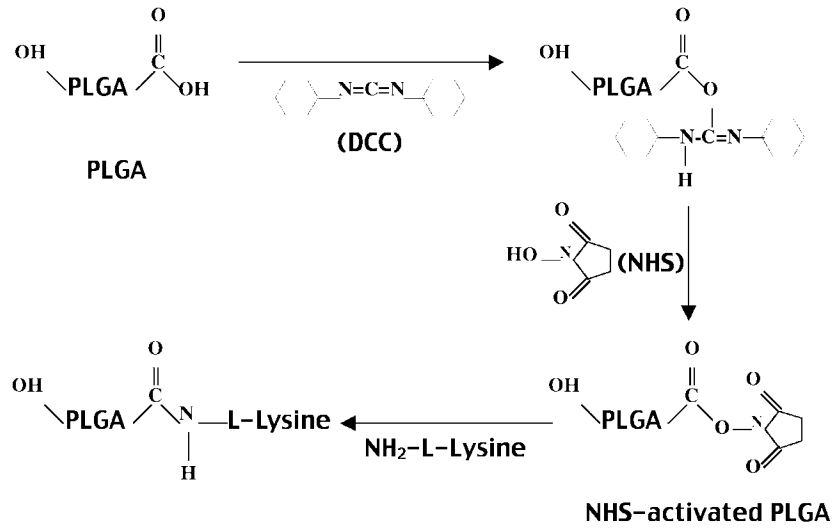

Fig. 2. Schematic diagram of conjugation of PLGA-tetracycline copolymer.

3.1. Surface-Functionalized Nanoparticles

3.1.1. Conjugation

Carbodiimide chemistry is well known and often used for the amide bond reaction between the carboxyl group and primary (or secondary) amine group ***(10,11)***. Conjugation between PLGA and tetracycline is shown schematically in **Fig. 2**. The carboxyl group of PLGA is activated with NHS in the presence of DCC. After the addition of tetracycline, NHS is replaced with tetracycline to form the PLGA-tetracycline-conjugated polymer.

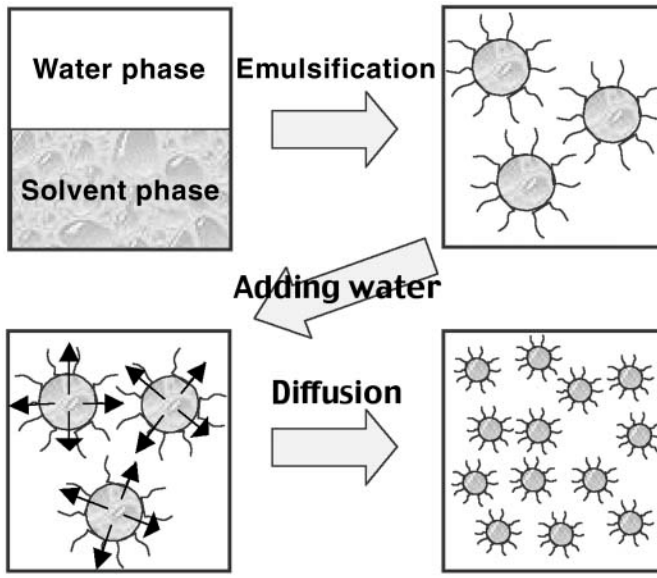

Fig. 3. Nanoparticle preparation by emulsification-diffusion method.

3.1.2. Emulsification-Diffusion Method

Many researchers ***(13–16)*** have studied the emulsification-diffusion method developed by Leroux et al. ***(12)***. The use of partially water-miscible solvent, which allows the additional diffusion of solvent, is the unique characteristic of the emulsification-diffusion method. After homogenization, oil and water phases are in a state of mutual saturation. However, the addition of water leads to interfacial turbulence at the interface of the oil and water phases, which allows the diffusion of solvent into continuous water phase ***(13)***. In this stage, PLGA is aggregated in the form of nanoparticles (*see* **Fig. 3**).

3.1.3. Nanoprecipitation Method

The nanoprecipitation method is very simple for the preparation of nanoparticles ***(17–19)***. Briefly, polymer is dissolved in volatile water-miscible solvent, and then the polymer solution is injected into the water phase through a syringe under stirring (*see* **Fig. 4**, left). In some cases, polymer solution is added dropwise into an aqueous phase ***(18,19)***. The particle formation is dominated by the solvent diffusion. The rapid diffusion of solvent is assumed to result in the reduction of nanoparticle size.

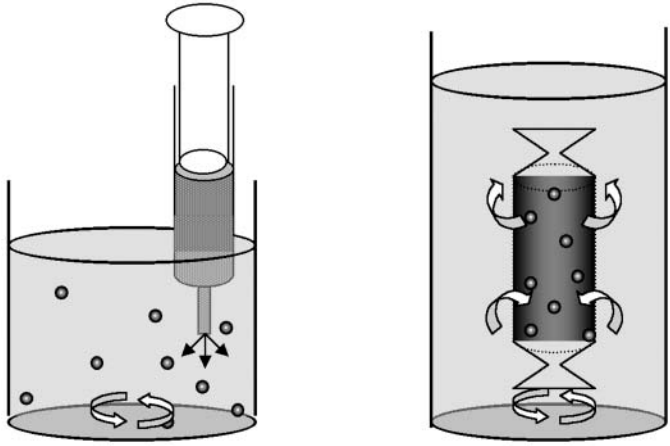

Fig. 4. Schematic illustration of nanoprecipitation (**left**) and dialysis method (**right**).

3.1.4. Dialysis Method

Micellar nanoparticles can be prepared by the dialysis method without any other surfactants ***(20–22)***. The polymer is first dissolved in water-miscible solvents, and then the polymer solution is dialyzed against the water. During the dialysis process, micellar nanoparticles are induced and the organic solvent is removed by water exchange (*see* **Fig. 4**, right). The employed solvent significantly affects the size and size distribution of the micellar nanoparticles. The mutual miscibilities of a polymer, solvent, and water are assumed to be the key factors ***(22)***.

3.2. Conjugation Between PLGA and Tetracycline

The PLGA used in this procedure has a hydroxyl group and a carboxyl group at its terminal ends (*see* **Note 1**).

1. Dissolve 1 g (0.1 mmol) of PLGA in 20 mL of acetone.
2. For activation of the carboxyl group of PLGA, add 0.1 g (0.5 mmol) of DCC and 0.06 g (0.5 mmol) of NHS to the polymer solution.
3. Stir the solution overnight at room temperature.
4. Filter the PLGA solution through a syringe filter to remove the precipitated dicyclohexylurea (byproduct of the reaction).
5. After the evaporation of 5 mL of acetone, precipitate the PLGA solution in cold diethyl ether to remove the unreactants and dry under a vacuum oven (*see* **Note 2**).
6. Dissolve 0.088 g (0.2 mmol) of tetracycline in 10 mL of acetone/0.1 mL of HCl.
7. Dissolve 1 g of the NHS-activated PLGA in 20 mL of acetone.

8. Add the 10 mL of tetracycline solution (**step 6**) to the NHS-activated PLGA solution (**step 7**) under stirring.
9. To remove the insoluble particulates, filter the mixed solution through a syringe filter and stir the mixed solution for 5 h.
10. Precipitate the resulting polymer solution in cold diethyl ether and dry under a vacuum oven.

3.3. Preparation of Functional Nanoparticles

After the conjugation of tetracycline to PLGA, the next step is the nanoparticle formation of the resulting copolymer. The three preparative methods are detailed next. Pyrene is used as a hydrophobic dye for the assay of the concentration of nanoparticles (*see* **Notes 3–5**).

3.3.1. Emulsification-Diffusion Method

1. Dissolve 0.2 g of the prepared PLGA-tetracycline polymer and 0.001 g of pyrene in 10 mL of ethyl acetate, and dissolve 1 g of Poloxamer 188 in 20 mL of pH 7.4 PBS.
2. Add 10 mL of the organic solution to 20 mL of PBS containing Poloxamer 188.
3. After 1 min, emulsify the mixed solution using a high-speed homogenizer at 12,000 rpm for 7 min.
4. Add 80 mL of PBS to the resulting oil/water emulsion under moderate stirring. The rapid diffusion of solvent into water occurs and the nanoparticles are formed in 5 min.
5. Stir the nanoparticle suspension for 5 h to evaporate the solvent (*see* **Fig. 5**).

3.3.2. Nanoprecipitation

1. Dissolve 0.1 g of PLGA-tetracycline copolymer and 0.001 g of pyrene in 10 mL of acetone.
2. Dissolve 0.5 g of Poloxamer 188 in 50 mL of PBS.
3. Inject the polymer solution (**step 1**) through the needle of a syringe into stirred PBS containing poloxamer with the needle dipped in the PBS. Nanoparticle forms immediately.
4. Stir the nanoparticle suspension for 5 h to evaporate the acetone.
5. Filter the nanoparticle suspension through a syringe filter to remove some large aggregates.

3.3.3. Dialysis Method

1. Dissolve 0.02 g of PLGA-tetracycline copolymer and 0.001 g of pyrene in 10 mL of DMF.
2. Fill the tubular dialysis membrane with the polymer solution, and dialyze against PBS for 3 h using a dialysis membrane.
3. Exchange the PBS at intervals of 2–4 h for 24 h.

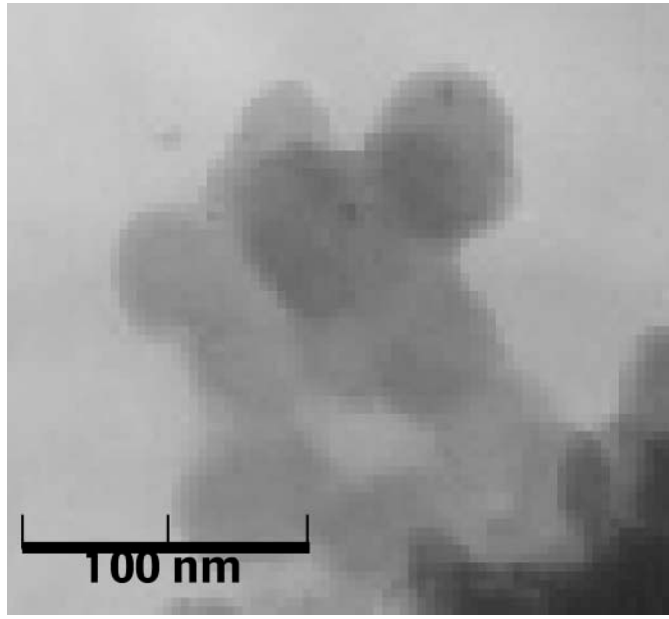

Fig. 5. Transmission electron microscopy image of PLGA nanoparticles prepared by emulsification-diffusion method.

3.4. Characterization of Tetracycline-Modified Nanoparticles

HA is used as a substitute for in vitro bone study because it is inorganic material existing in only hard tissues (bone and teeth), not in soft tissues ***(9)***. In this section, the amount of adsorption of tetracycline-modified nanoparticle to HA is examined to verify the specific affinity to bone (*see* **Fig. 6**). Pyrene is incorporated into nanoparticles as a hydrophobic dye (*see* **Notes 6–8**).

1. Prepare various concentrations of HA dispersions (0.01, 0.02, 0.03, and 0.04 g/mL).
2. Add 1 mL of the tetracycline-modified nanoparticle suspension (prepared by the emulsification-diffusion method in **Subheading 3.3.1.**) to each of the HA dispersions (**step 1**).
3. Agitate the mixed dispersions for 2 h.
4. Filter each of the mixed dispersions through a syringe filter.
5. Measure the concentration of tetracycline-modified nanoparticles using an ultraviolet spectrophotometer at 335 nm.

4. Notes

1. The used PLGA polymer has two functional groups such as a hydroxyl (—OH) group and a carboxyl (—COOH) group ***(23)***. In this chapter, carbodiimide

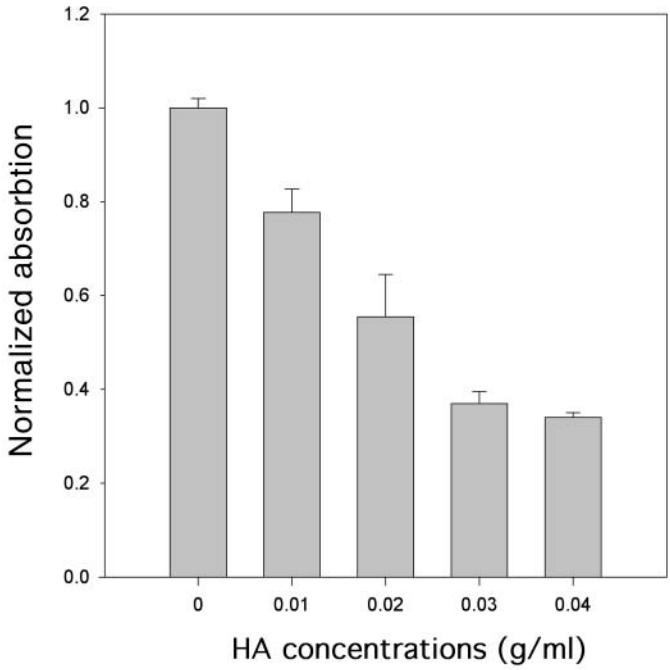

Fig. 6. Affinity of tetracycline-modified nanoparticles on HA.

chemistry is employed for the conjugation between PLGA and tetracycline, but many other methods are available to bind the functional molecule to polymer. Bromo-*tris*(pyrrolidino)-phosphonium hexafluorophosphate (PyBrop) ***(23)***, thionyl chloride ***(24)***, *p*-nitrophenyl chloroformate ***(25)***, and carbonyldiimidazole ***(26)*** are the representative coupling agents to produce amide or ester bond. The solubilities of polymer, functional molecule, and coupling agent should be taken into account for successful reaction.

2. For precipitation of the polymer solution in cold ethyl ether, the polymer solution should be concentrated by the evaporation of solvent. Otherwise, the precipitated polymer will disperse in ethyl ether and form into small particulate. In such a case, the suspension should be centrifuged to obtain the resulting polymer.
3. In the emulsification-diffusion method, the effects of process variables and the thermodynamic parameters (diffusion coefficients and solvent-polymer interaction parameter) on the particle size are investigated ***(13,16)***. The rapid diffusion of solvent is very important for the reduction of particle size. Ultrasonication in the emulsification step and the addition of heated water (about 50°C) in the diffusion step are effective at reducing the particle size, because the diffusion coefficient is proportional to temperature in Kelvin ***(13)***.
4. Volatile water-miscible solvents such as acetone and tetrahydrofuran are mainly used in the nanoprecipitation method to facilitate the removal of solvents. How-

ever, in the dialysis method all of the water-miscible solvents can be employed, because particle formation and removal of solvents occur simultaneously. The lower concentration of polymer in solvent leads to a smaller nanoparticle.

5. Generally, the size range of nanoparticles formed by the emulsification-diffusion method is 50–150 nm. Nanoparticles formed by the nanoprecipitation method are assumed to be more aggregates than micelles, having a particle size >100 nm. The dialysis method can produce micellar nanoparticles ranging from 25 to 200 nm owing to the formation of micelles.
6. Poloxamers used as surfactants are not biodegradable because they have ether, not ester, linkage although they are well known as biocompatible materials. Consequently, they may remain in the body after administration. Therefore, the nanoparticle without surfactant is thought to be suitable for clinical applications.
7. For incorporation of hydrophobic drug into nanoparticles ***(27–31)***, target hydrophobic molecules are dissolved in the organic phase containing polymer and then the nanoparticles are prepared. Incorporation efficiency is determined by the process variables and the solubility of target molecules in the used organic solvent.
8. Tetracycline-modified nanoparticles have been demonstrated to have a great affinity with HA (*see* **Fig. 6**) and thus can be employed for a potential bone-specific drug carrier. The functional molecule with the specific affinity to some organs, cells, or diseases can be utilized as the guide material for targeted drug-delivery systems ***(8,32–34)***.

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