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Nanoparticles for targeted drug delivery to cancer stem cells and tumor

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Summary

Due to the drug resistance of cancer stem cells (CSCs), CSC-targeted delivery of multiple drugs in nanoparticle-based drug delivery system holds great potential for the destruction of the CSCs and tumor. In this chapter, we describe the preparation of multi-layered pH-responsive polymeric nanoparticles (NPs) by multiple emulsifications to encapsulate multiple hydrophilic and hydrophobic theranostic agents for controlled and sequenced release. Hyaluronic acid (HA) is used for not only actively targeting the CSCs to reduce their drug resistance due to dormancy (i.e., slow metabolism), but also replacing the commonly used poly (vinyl alcohol) (PVA) as a stabilizing agent to synthesize the nanoparticles.

Keywords

Nanoparticles; cancer stem cells; polymer; controlled release; combination therapy; responsiveness; active targeting

1. Introduction

Cancer stem cells (CSCs) are rare subpopulations of cancer cells that can initiate and/or reinitiate the formation of tumors including metastasis after the conventional radio and chemotherapy (1, 2). Because of their resistance to chemotherapeutic drugs, the CSCs have attracted a great deal of attention in the field of oncology in the past ~10 years (3, 4). One of the major strategies for overcoming drug resistance of CSCs is combination therapy, which is the combined use of two or more chemotherapeutic agents with different anticancer mechanisms or multiple treatment modalities (5–7). However, due to the vast difference in physiochemical and pharmacokinetic properties of different chemotherapeutic drugs including solubility, biodistribution, circulation time in blood, and cell membrane transport properties, the current practice of simply using multiple free agents with no control of their delivery and release is far from being optimal in making use of the therapeutic capacity of the agents for cancer treatment. Therefore, it is important to develop a vehicle for delivering all the different chemotherapeutic drugs together in a controlled fashion (8, 9).

Amphiphilic block copolymer-based nanoparticles have attracted much attention for the delivery of chemotherapeutic drugs for several decades (10, 11). The emulsion-solvent

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evaporation method is usually used for encapsulating hydrophobic drugs in the polymeric nanoparticles by fabricating an “oil-in-water” configuration (12). The double emulsion method is the commonly used approach to form a “water-in-oil-in-water” configuration for encapsulating hydrophilic drugs (13, 14). Both methods have been extensively utilized to encapsulate chemotherapeutic drugs for cancer treatment. However, for most studies using the two methods, either hydrophobic or hydrophilic agent (but not both) has been encapsulated in the nanoparticles for delivery. In this chapter, we report a series of emulsion-based methods for assembling multi-layered core-shell polymeric nanoparticles to encapsulate one or more hydrophilic and hydrophobic agents for co-delivery.

Furthermore, in order to actively target the CSCs, we have developed the method by using hyaluronic acid (HA) instead of poly (vinyl alcohol) (PVA) during the preparation of the multi-layered core-shell polymeric nanoparticles. Since HA can bind to the variant CD44 antigen commonly overexpressed on the surface of many types of CSCs (15, 16), the resultant nanoparticles can be used to specifically co-deliver multiple drugs into the CSCs. In this protocol, curcumin (CUR, hydrophobic), doxorubicin hydrochloride (DOX, hydrophilic), irinotecan or camptothecin (CPT, hydrophobic), and indocyanine green (ICG, hydrophilic) were used as the model agents. The nanoparticles are prepared with four polymers that are approved by the US Food and Drug Administration (FDA) for medical use: Poly(d,l-lactide-co-glycolide) (PLGA), Pluronic F127 (PF127 with and without chitosan modification), chitosan, and HA. By combining PLGA and PF127 together, more stable and uniform-sized nanoparticles can be obtained than using PLGA or PF127 alone. Chitosan has also been shown to specifically bind to the variant CD44 overexpressed on the CSCs (17).

2. Materials

2.1. Nanoparticle Synthesis

1. PLGA (lactide:glycolide: 75:25, Mw: 4000–15,000)
2. Pluronic F127 (PF127)
3. Hyaluronic acid (HA, Mw: 66–90 kDa)
4. Polyvinyl alcohol (PVA, Mw: 100 kDa)
5. Chitosan oligosaccharide of pharmaceutical grade (Mw: 1.2 kDa, 95% deacetylation)
6. Irinotecan/camptothecin

2.2 Preparation of drug solutions

1. For DOX, weigh the desired amount of DOX (1–10 mg/ml) and mix it with 5 ml of deionized water for 1 h. Store the solution at 4 °C for no more than one month and keep it away from light.
2. For ICG, mix it with deionized water for 0.5 h before use.

3. For hydrophobic drugs, dissolve in dichloromethane (DCM, 1–30 mg/ml) before use.

2.3 *In vitro* drug release study

1. Phosphate buffered saline (PBS) buffer of pH 7.4 or 5.0
2. Dialysis bags (MWCO: 20 kDa)

3. Methods

3.1 Preparation of chitosan-modified PF127 (chitosan-PF127)

1. Add a total of 30 ml of PF127 solution (26 mM in benzene) dropwise into 30 ml of 4-nitrophenyl chloroformate (4-NPC) (160 mM in benzene) solution.
2. Stir the mixture for 3 h in N₂ atmosphere at room temperature to activate PF127.
3. Precipitate and filter the activated polymer in excess (ice-cold) diethyl ether for three times and dried under vacuum overnight.
4. Add a total of 10 ml of chitosan solution (200 mg/ml, in DI water) dropwise into 10 ml of the activated PF127 solution (400 mg/ml, in deionized water).
5. After stirring for 12 h, dialyze (MWCO: 7 kDa) the mixture against DI water for 24 h.
6. Freeze-dry the sample for 48 h to remove water to obtain dry chitosan-modified PF127 (chitosan-PF127).

3.2 Preparation of HA solution

0.125% HA solution: Weigh 125 mg of HA and mix with 100 ml of deionized (DI) water (Resistivity > 10 Ω .m) for 24 h. Store at 4 °C.

3.3 Preparation of PVA solution

1. Slowly add PVA powder into DI water to fully disperse it in water.
2. Once the powder is fully dispersed (~2 h), heat the suspension to the temperature at which the polymer is soluble (temperature from 90 to 95 °C is suitable).
3. Stir the sample at this temperature until fully solubilizing the PVA (~5 h). Store the solution at room temperature for no more than 1 month before use.

3.4 Particle Fabrication using the Single-Emulsion Method

1. Dissolve 10 mg of PLGA, 10 mg of PF127, and the desired amount of CUR (starting from 1 mg, and the amount can be adjusted according to the drug encapsulation efficiency and the desired amount of drug in the nanoparticles) in 1 ml of DCM. Transfer this solution together with 10 ml of the aqueous solution of chitosan-PF127 (10 mg) and HA into a centrifuge tube and emulsify the two immiscible solutions by sonication for 2 min using a Ultrasonic Liquid Sonicators (see Note 1).

2. Remove the DCM by rotary evaporation of the resultant emulsion for 15 min (see Note 2).
3. Collect the nanoparticles by centrifugation at 13,800×g for 10 min at room temperature and washing twice with deionized water.
4. Suspend the nanoparticles at the desired concentration for further use.

In summary, the single-emulsion method can be used for encapsulating one or more hydrophobic agents. A schematic illustration of the procedure of single-emulsion method and the characterization of the resultant nanoparticles are summarized in Fig. 1 (see Note 3). The nanoparticles can be used immediately or store at −20 °C for ~1 month.

3.5 Particle Fabrication using the Double-Emulsion Method

1. Transfer a total of 0.4 ml of DOX solution (in deionized water) at the desired concentration, together with 2 ml of DCM containing 10 mg of PLGA, 10 mg of PF127, and the desired amount of CUR (same as that in section 3.4) into a centrifuge tube and emulsify the two immiscible solutions by sonication for 2 min using a Ultrasonic Liquid Sonicators (see Note 1).
2. Mix the first emulsion with 10 ml of aqueous solution of chitosan-PF127 and HA.
3. Emulsify the mixture by sonication for 2 min (see Note 4).
4. Remove DCM by rotary evaporation of the resultant emulsion for 15 min (see Note 2).
5. Collect nanoparticles by centrifugation at 13,800×g for 10 min at room temperature and wash twice with deionized water.
6. Suspend the nanoparticles in a desired solution (e.g., culture medium) for further use.

In summary, the double-emulsion method can be used for encapsulating both hydrophobic and hydrophilic agents. A schematic illustration of the procedure of the double-emulsion method and the characterization of the resultant nanoparticles are given in Fig. 2 (see Note 3). The nanoparticles can be used immediately or store at −20 °C for ~1 month.

3.6 Particle Fabrication using the Triple-emulsion method

1. Dissolve a total of 10 mg of PLGA, 10 mg of PF127, and the desired amount of CUR (same as that in section 3.4) in 0.5 ml of DCM. Then, transfer this solution

¹The amount of polymers and drugs can be adjusted. The volume of oil (i.e., organic solvent) and aqueous solution can be adjusted to make nanoparticles of different sizes in the core and shell(s). The power and time of the ultrasound for emulsification can be changed also to make nanoparticles of different sizes.

²Complete removal of organic solvent is crucial to obtain small-sized nanoparticles and minimize the potential toxicity of the resultant nanoparticles. Usually, no bubble formation in the mixture indicates complete removal of organic solvent.

³The polydispersity of the resulting particles are mainly dependent on the sonication process. A better polydispersity can be achieved if the emulsion is more homogeneous during sonication. The size of the nanoparticles is also associated with sonication process. Under higher power, the size of the nanoparticles is smaller compared with the ones made with lower power.

⁴To minimize overheating during the emulsification, the tube with the mixture can be placed in the ice or water bath to control the temperature.

together with 2 ml of 2% PVA solution (in deionized water) into a centrifuge tube, and emulsify the two immiscible solutions by sonication for 1 min (see Note 1).

2. Mix the first emulsion with 100 μ l of DOX solution (in deionized water).
3. Add a total of 4 ml of DCM with PLGA and PF127 (10 mg for both PLGA and PF127) and emulsified by sonication for 2 min.
4. Mix the second emulsion with 100 μ l of CPT solution (in DCM).
5. Add a total of 10 ml of aqueous solution of chitosan-PF127 and HA and emulsify the mixture by sonication for 2 min (see Note 5).
6. Remove DCM by rotary evaporation of the resultant emulsion for 15 min (see Note 2).
7. Collect nanoparticles by centrifugation at 13,800 $\times$ g for 10 min at room temperature and wash twice with deionized water.
8. Suspend the nanoparticles in a desired solution (e.g., culture medium) for further use.

In summary, both hydrophobic and hydrophilic agents can be encapsulated into the multi-layered nanoparticles produced by the triple-emulsion method. The hydrophobic agents can be encapsulated in the core or the outer hydrophobic layer. The hydrophilic agents can be encapsulated in the middle hydrophilic layer. A schematic illustration of the procedure of the triple-emulsion method and the characterization of the resultant nanoparticles are given in Fig. 3 (see Note 3). The nanoparticles can be used immediately or store at -20°C for ~ 1 month.

3.7 Particle fabrication using the quadruple-emulsion method

1. Dissolve 10 mg of PLGA and 10 mg of PF127 in 1 ml of DCM (see Note 1).
2. After adding 0.2 ml of DOX solution (in deionized water), emulsify the immiscible solutions by sonication for 1 min.
3. Mix this first emulsion with 100 μ l of CUR solution (in DCM).
4. Add a total of 2 ml of 2% PVA solution (in deionized water) and the mixture emulsified by sonication for 2 min.
5. Mix the second emulsion with 100 μ l of ICG solution (in deionized water).
6. Add a total of 4 ml of DCM with PLGA and PF127 and emulsify the mixture by sonication for 2 min.
7. Mix the third emulsion with 100 μ l of CPT solution (in DCM).
8. Add a total of 15 ml of chitosan-PF127 and HA solution (in deionized water) and emulsify the mixture by sonication for 2 min.

⁵At the last emulsion, the total volume of the sample is large compared with previous emulsions. Therefore, in order to achieve homogenous emulsion, the solution can be divided and emulsified at a higher power.

9. Remove DCM by rotary evaporation of the resultant emulsion for 15 min (see Note 2).
10. Collect the nanoparticles by centrifugation at 13,800×g for 10 min at room temperature and wash twice with deionized water.
11. Suspend the nanoparticles in a desired solution (e.g., culture medium) for further use.

In summary, both hydrophilic and hydrophobic agents can be encapsulated into the multi-layered nanoparticles produced by quadruple-emulsion method. The hydrophilic agents can be encapsulated in the core or the middle hydrophilic layer, while hydrophobic agents can be encapsulated in the middle or outer hydrophobic layer. A schematic illustration of the procedure of the quadruple-emulsion method and the characterization of the resultant nanoparticles are given in Fig. 4 (see Note 3). The nanoparticles can be used immediately or store at −20 °C for ~1 month.

3.8 *In vitro* drug release study

1. Drug-laden nanoparticles (20–30 mg) are dissolved in PBS (5 ml, pH 5 or 7.4).
2. Transfer the nanoparticles solution into dialysis bags (MWCO: 20 kDa).
3. Put the dialysis bags in 30 ml of the same PBS at 37 °C and stir at 110 rpm using a mini-stir bar.
4. At appropriate time points, 100 µl of the dialysate is collected, and the sample is replenished with the same amount of fresh PBS.
5. The concentration of the released agents/drugs in the removed dialysate is determined using UV-Vis

spectrophotometry based on the absorbance of drug (see Note 6).

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Fig. 1. (A) A schematic illustration of the single-emulsion method. (B) Scanning electron microscopy (SEM) and (C) transmission electron microscopy (TEM) images of the resultant nanoparticles. The inset in (C) is a zoom-in view of the nanoparticle showing its core-shell morphology (reproduced from (9) with permission from Royal Society of Chemistry).

Fig. 2. (A) A schematic illustration of the double-emulsion method. (B) SEM and (C) TEM images of the resultant nanoparticles. The inset in (C) is a zoom-in view of the resultant nanoparticle showing its core-shell morphology (reproduced from (9) with permission from Royal Society of Chemistry).

Fig. 3. (A) A schematic illustration of the triple-emulsion method. (B) SEM and (C) TEM images of the resultant nanoparticles. The inset in (C) is a zoom-in view of the resultant nanoparticle showing its core and two-layered morphology (reproduced from (9) with permission from Royal Society of Chemistry).

Fig. 4.

(**A**) A schematic illustration of the quadruple-emulsion method. (**B**) SEM and (**C**) TEM images of the nanoparticles. The inset in (**C**) is a zoom-in view of the nanoparticle showing its core and three-layered morphology (reproduced from (9) with permission from Royal Society of Chemistry).