Particle Coating Procedures

Introduction

Currently, there are several methods of attaching biological ligands to polystyrene particles. These methods include adsorption to plain polystyrene particles, covalent attachment to surface functionalized particles, and attachment of the ligand of interest to particles that are pre-coated with a binding protein such as Streptavidin, Protein A or Protein G. Presented in this Technical Note are protocols such as adsorption, covalent coupling, and other methods used to attach ligands to polystyrene particles .

Procedures and Disscussion

The following protocols are developed byfor the convenience of microparticleusers. They are to be utilized only as initial conditions.encourages the optimization of the coating conditions by changing the buffer, pH or reagents concentration.

In general, polyclonal antibodies are coated to polystyrene particles by adsorption without using any coupling agents. The binding of polyclonal antibodies to polystyrene particles is strong. However, care should be taken not to over load the antibodies to the particles. If over loading occurs, leaching of the coated antibody will happen during storage. This is due to the weak interaction between antibodymolecules compared to the interaction of antibody molecules to the surface of polystyrene particles.

Typically, centrifugation is used for removing unbound proteins after coating for polystyrene particles larger than 0.4 µm. On the contrary, gel filtration, dialysis or diafiltration is used for particles smaller than 0.4 µm. The magnetic particles are processed easily during the coating with a commercially available magnetic separator. The Separator will provide a very convenient and cost effective way of cleaning magnetic particles after coating. A schematic presentation of covalent coupling of ligands to functionalized particles is shown in Fig. 1 below.

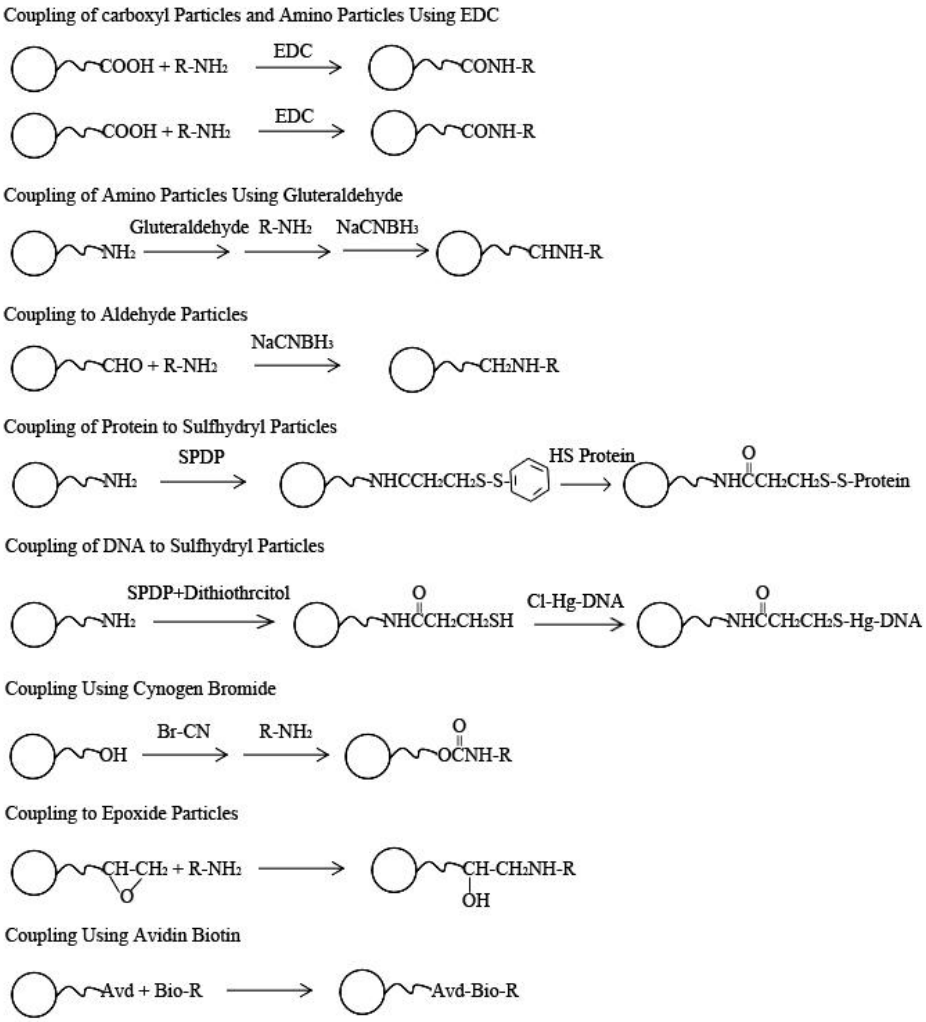

Fig.1 General Procedures For Particle Coating

General Procedures For Particle Coating

Passive adsorption:

1. Add the following to a 15 mL glass centrifuge tube:

a. phosphate buffer, 0.1 M, pH 7.4

b. 1 mg/mL protein solution

c. 5% w/v 0.8 µm polystyrene particles

2. Vortex and incubate for at least one hour at ambient temperature.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant carefully.

5. Add Isotonic Buffered Saline (IBS).

6. Mix well using a vortex mixer.

7. Centrifuge at 3000x g for several minutes.

8. Remove the supernatant carefully.

9. Add IBS and mix well to obtain 0.25% w/v suspension.

Note: 1. For 4.0~4.5 µm magnetic particles, use appropriate amount of proteins per mL of 2.5% w/v magnetic particles and Separator for the separation of particles in Steps 3 and 7.

2. This procedure is used for the passive adsorption of immunoglobulins, antigens or other ligands to polystyrene particles or polystyrene magnetic particles.

Coating of Amino Particles with Ligands or Proteins Using EDC

Covalent Coupling (one step EDC coupling):

1. Add the following to a 12x75 mL glass centrifuge tube:

a. 0.05M MES buffer, pH 5.0

b. ligands or proteins

c. 5% w/v 0.8 µm Amino particles

d. EDC

2. Vortex and incubate at ambient temperature on a rotary mixer or with occasional vortexing or shaking.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant carefully.

5. Resuspend the pellet in Isotonic Buffered Saline.

6. Repeat Steps 3 and 4 and resuspend the pellet in IBS to obtain 5% w/v suspension.

Note:

1. Use the Separat for the separation of particles in Step 3.

2. For 1.0~2.0 µm magnetic particles, use 1.0 mg of ligands or proteins per mL of 2.5% w/v magnetic particles and 10 mg of EDC.

3. EDC(1-ethyl-3(-3-dimethylaminopropyl) carbomiimide hydrochloride), Sigma Chemical

Coating of Carboxyl Particles with Avidin Using EDC

Covalent Coupling (one step EDC coupling):

1. Add the following to a 15 mL glass centrifuge tube:

a. sodium acetate buffer, 0.01 M, pH 5.0

b. Avidin or Streptavidin

c. 5% w/v 0.8 µm Carboxyl particles d. 20 mg of EDC

2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant carefully.

5. Resuspend the pellet in Isotonic Buffered Saline.

6. Repeat Steps 3 and 4 and resuspend the pellet in IBS to obtain 5% w/v suspension.

Note:

1. Use the Separato for the particle separation in Step 3.

2. This procedure is also for covalent coupling of other proteins such as monoclonal or polyclonal antibodies, antigens or other ligands. Acidic buffers such as phosphate, 0.1M or MES, 0.05 M can be used instead of acetate buffer.

Covalent Coupling (two step EDC coupling):

For two step EDC coupling, wash the particles with coupling buffer, centrifuge and remove ~80% of the supernatant. Add EDC to the pellet, mix, and incubate. Wash the particles with coupling buffer and resuspend with protein solution. Continue with Steps 2 to 6 of the Covalent Coupling (one step) procedure.

Coating of Avidin Particles with Biotinylated Proteins

Affinity Coupling:

1. Add the following to a 15 mL glass centrifuge tube:
a. biotinylated protein in sodium phosphate buffer (PB), 0.1 M, pH 5.5
b. Avidin coated polystyrene particles, 5% w/v

2. Vortex and incubate for at least one hour at ambient temperature.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant carefully.

5. Resuspend the pellet in 0.1M PB.

6. Repeat Steps 3 and 4 and resuspend the pellet in PB to obtain 0.25% w/v suspension.

Note: This procedure is also used for coating biotinpolystyrene or magnetic particles with various avidin-protein conjugates or other avidin-ligand conjugates.

Coating of Protein to Hydroxyl Particles

Covalent Coupling using Cyanogen Bromide (CNBr):

1. Add 1.25% w/v 0.8 µm hydroxyl polystyrene particles to a centrifuge tube:

2. Adjust the pH with 1N NaOH.

3. Add CNBr in a fume hood.

4. Readjust the pH with 1N NaOH.

5. Incubate for several minutes.

6. Add cold borate buffer (0.1M, pH 8.5).

7. Cool to 4℃ and add protein at a concentration of 1 mg/mL.

8. Incubate at 4℃ for at least four hours.

9. Add glycine buffer (0.1M, pH 8.5).

10. Centrifuge for several minutes.

11. Remove the supernatant and resuspend the pellet in 0.1M phosphate buffer, pH 7.2.

12. Repeat Steps 10 and 11 twice to give particles at 0.25%.

Coating of Dimethylamino Particles with DNA

Ionic Interaction Coupling:

1. Add the following to a 15 mL glass centrifuge tube:

a. 0.25% w/v 0.8 µm dimethylamino particles in carbonate buffer, 0.1 M, pH 9.0

b. DNA in 0.1M carbonate buffer, pH 9.0

2. Vortex and incubate for three hours at ambient temperature.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant and resuspend the pellet in 0.1M carbonate buffer, pH 9.0.

5. Centrifuge at 3000x g for 15 several minutes.

6. Remove the supernatant and resuspend the pellet in 0.1M tris buffer, pH 7.5. Store refrigerated.

Coating of Epoxy Particles with Proteins

1. Add 5% w/v epoxy polystyrene particles to a microfuge tube.

2. Microfuge for several seconds.

3. Aspirate the supernatant and resuspend the pellet in 0.1M carbonate buffer, pH 9.0.

4. Add proteins in carbonate buffer.

5. Rotate at 60℃ for dozens of hours.

6. Microfuge at 15000 rpm for several seconds and wash two times with PBS (0.1M, pH 7.4). Resuspend with PBS to get 1% particles.

Note: For 3.0 µm epoxy polystyrene particles use proteins in carbonate buffer.

Coating of Ligands to Modified Amino Proteins

Covalent Coupling Using SPDP[2] :

2-Pyridyldisulfide particles:

1. Add the following to a 15 mL glass centrifuge tube:

a. sodium phosphate buffer (PB), 0.1 M, pH 7.0

b. amino polystyrene particles, 0.8 µm, 5% w/v

c. DMSO containing of SPDP [3-(2- pyridyldithio) propionic acid N-hydroxy succinimide ester)]

2. Incubate for at least one hour at ambient temperature on a rotary mixer.

3. Centrifuge at 3000x g for several minutes.

4. Remove the supernatant carefully.

5. Resuspend the pellet in 0.1M PB, pH 7.0.

6. Centrifuge at 3000x g for several minutes.

7. Repeat Steps 3 and 4 and resuspend the pellet in deionized water. Store at 4℃.

Thio Ester Particles:

1. Repeat Steps 1-5 from the above procedure, or use the suspension obtained in Step 6 and centrifuge at 3000x g for several minutes.

2. Remove the supernatant carefully.

3. Resuspend the pellet in 0.1M acetate buffer, pH 5.0.

4. Add DTT (1,4-dithiothreitol).

5. Incubate for several minutes at ambient temperature on a rotary mixer.

6. Centrifuge at 3000x g for several minutes.

7. Aspirate and save the supernatant. If cloudy, filter through Acrodisk. Save the filtrate for optical density (OD) measurment at 343 nm. Multiply the absorbance by $8.08x10^3$ to obtain the molar concentration of the thio ester on the particles.

8. Resuspend the pellet in deionized water.

9. Centrifuge at 3000x g for several minutes.

10. Remove the supernatant and resuspend the particles in deionized water to give a 5% w/v suspension of thio particles. Use the thio particles as soon as possible for coupling to thiolated ligands. The thio groups can be oxidized to disulfide groups upon prolonged storage.

Modification of Ligands with SPDP and Coupling to Thio Particles:

1. Add a solution containing of SPDP in methanol to a solution containing of ligand in phosphate buffer (PB), 0.1M, pH 7.0.

2. Incubate for at least one hour at ambient temperature on a rotary mixer.

3. Dialyze the mixture using a dialysis tubing of appropriate molecular weight cut off against three changes of PB in 24 hours.

4. Add the resulting thio ligand to 5% w/v thio particles.

5. Incubate overnight at ambient temperature on a rotary mixer.

6. Centrifuge at 3000x g for several minutes.

7. Remove the supernatant and resuspend the particles in PB.

8. Repeat Steps 6 and 7 and centrifuge at 3000x g for several minutes.

9. Remove the supernatant and resuspend the particles in PB to give a 0.25% w/v suspension.

Periodate Oxidation of Polysaccharide and Coupling to Amino Particles

1. Add a solution containing of sodium m-periodate (Sigma S-1878) in deionized water dropwise with stiring to a solution containing of polysaccharide in deionized water.

2. Stir the mixture at room temperature for several minutes and add 1 M ethylene glycol to the mixture. After several minutes, add the mixture to the packed amino polystyrene particles obtained from 5% w/v suspension by centrifugation at 4000x g for for several minutes.

3. Adjust the pH of the mixture with 10% K2CO3 and stir the mixture at room temperature for several minutes.

4. Add sodium cyanoborohydride to the mixture and stir the mixture at room temperature overnight.

5. Wash the particles twice with deionized water and resuspend the particles in 0.1 M PBS containing 100 mg of BSA.

6. Stir the mixture at room temperature for two hours and wash the particles twice as before.

7. Resuspend the particles in 0.1M PBS to give 5% w/v suspension.

Coating of Carboxyl Polystyrene Particles with Amino Modified Oligonucleotides

1. Add carboxyl polystyrene particles to 0.1M MES (2-[N-morpholino]ethanesulfonic acid)

2. Add amino modified oligonucleotide in 0.1M MES

3. Add EDC(1-ethyl-3(-3-dimethylaminopropyl) carbomiimide hydrochloride)

4. Vortex and incubate for several minutes at ambient temperature.

5. Add EDC.

6. Repeat Steps 4 and 5.

7. Incubate for another 80 minutes on a rotary mixer.

8. Centrifuge and remove the supernatant carefully.

9. Resuspend the pellet in 0.1M PBS containing 0.02% Tween-20.

10. Repeat Step 8 and resuspend the pellet in 10mM Tris [hydroxymethyl] aminomethane hydrochloride / EDTA (ethylenediamine-tetraacetic acid) pH 8.0 (TE)

15. Centrifuge and remove the supernatant carefully.

16. Resuspend the pellet in TE or IBS. Store at 4℃.

Important Notes:

1. Since the quality of the coated particles depends on the quality of reagents and on the coating procedures, high quality reagents should be used while optimizing the coating conditions. As a result of lack of control over the reagents and coating condition,can not guarantee the quality or performance of the coated particles even if the provided procedures are followed.

2. Isotonic Buffered Saline (IBS) is prepared using the following formula:

NaCl

KCl

NaHPO4

Na2HPO4

Sodium Azide

Deionized Water

References

1. M. E. Jolley, C. J. Wang, S. J. Ekenberg, M. S. Zuelke and D. M. Kelso, J. of Immunol. Methods, Vol. 67, 21-35 (1984)

2. J. Carlson et al, Biochem. J. Vol. 173, 723-737 (1878)