

Supplementary material

In our study, we utilized an ion gelation method to prepare chitosan microspheres. Our experiments did not involve traditional gel electrophoresis (Gels) or Western blotting (Blots), we focused on the preparation and characterization of chitosan microspheres in solution.

Mechanism of Ion Gelation Method for Preparing Chitosan Microspheres:

In an acidic solution, chitosan, which carries a positive charge, interacts with sodium tripolyphosphate (TPP), which carries a negative charge, through intermolecular electrostatic interactions. This mutual adhesion leads to the formation of chitosan nanospheres.

Preparation Steps of Chitosan Microspheres Containing Different Growth Factors

A: Preparation of Materials

Chitosan Acetic Acid Solution: Weigh an appropriate amount of chitosan powder and place it in a centrifuge tube. Mix it with 1% acetic acid solution by vortexing until a homogeneous viscous solution is obtained. Monitor the pH using a pH meter, and adjust the pH to 5 using saturated sodium hydroxide solution.

TPP Solution: Weigh TPP powder and place it in a new centrifuge tube. Prepare a 0.8 mg/mL solution using double-distilled water.

Mass Ratio of Chitosan to Sodium Tripolyphosphate: Maintain a mass ratio of 3:1 (chitosan:TPP).

Sterile Filtration: Filter both the chitosan acetic acid solution and the TPP solution using a 0.22 μm sterile filter.

Growth Factor Solution: For lyophilized BMP-2 and PDGF-BB powders, reconstitution is required before use.

- a. Centrifuge at 10,000–12,000 rpm for 30 seconds to ensure any protein adhering to the tube lid or walls is collected at the bottom.
- b. Add reconstitution buffer to the lyophilized powder and gently mix using a pipette to resuspend the protein to a concentration of at least 100 $\mu\text{g}/\text{mL}$.
- c. To ensure complete dissolution, allow the reconstituted protein to stand at room temperature for several minutes.

B: Preparation of Chitosan Microspheres Containing BMP-2 and PDGF-BB

Based on the requirement of 0.5 µg of BMP-2 and PDGF-BB per scaffold:

Add the growth factor solution dropwise to the TPP solution.

Pour the chitosan acetic acid solution into a 25 mL beaker. Stir at room temperature using a magnetic stirrer at a speed of 200 rpm. Slowly add the TPP solution containing the growth factors using a pipette while continuing to stir. Maintain stirring for 30 minutes until the solution turns milky white, indicating the formation of chitosan microspheres. If this state is not achieved, continue stirring.

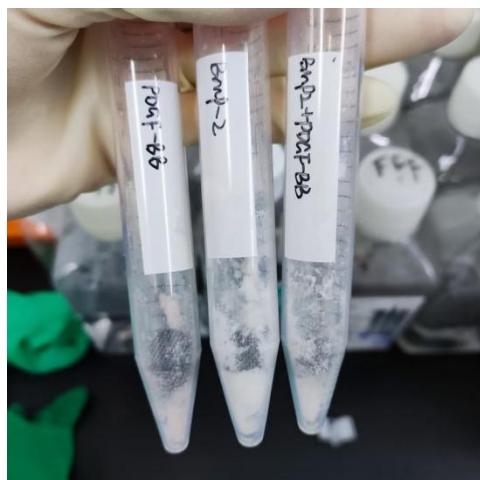
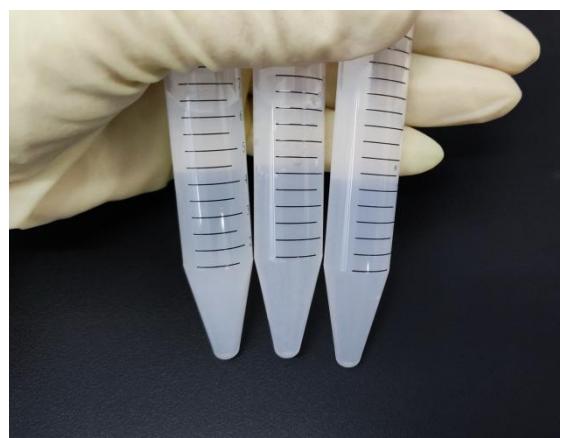


Fig.1 3 types of Lyophilized Growth Factor Powder



(a)



(b)

Fig.2 PDGF-BB Chitosan microsphere solution, BMP-2 Chitosan microsphere solution, PDGF-BB and BMP-2 Chitosan microsphere solution.(a) on the magnetic stirrer (b)in test tubes



Fig 3. PDGF-BB and BMP-2 Chitosan microsphere solution



Fig 4. High-resolution images of the chitosan microspheres (white netted part)