Supporting Informaiton

Incorporation of Gentamicin-Encapsulated Poly (lactic-co-glycolic acid) Nanoparticles into Polyurethane/Poly (ethylene oxide) Nanofiber Scaffolds for Biomedical Applications

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Methods:

S1. PLGA Nanoparticles Synthesis

First, 200mg PLGA powder was dissolved in 4ml dichloromethane, resulting in an oil phase, while 45mg gentamicin was dissolved in 0.5ml DI water, resulting in an aqueous phase 1. An 8ml of 3% PVA solution was an aqueous phase 1 where 3% PVA solution was made of 3g PVA dissolved in 100ml DI water. The oil phase solution was mixed with the aqueous phase 1 solution. Then, the mixture was combined with an aqueous phase 2. Thus, a water-oil-water solution was formed that contained three phases where nanodroplets were floating. The nanodroplet dispersion was sonicated for 10min at 40% amplitude and 10s pause after each round of 30s, resulting in a uniform emulsion solution. The emulsion solution was titrated into a beaker that contained a second 25ml 0.1% PVA solution. The solution mixture was placed on a hot plate and stirring shear force was applied, which allowed the solution to evaporate for 8 hours. An opaque solution containing PLGA nanoparticles was obtained after the evaporation. The obtained PLGA nanoparticles were examined using a scanning electron microscope (SEM, JEOL JSM-6500).

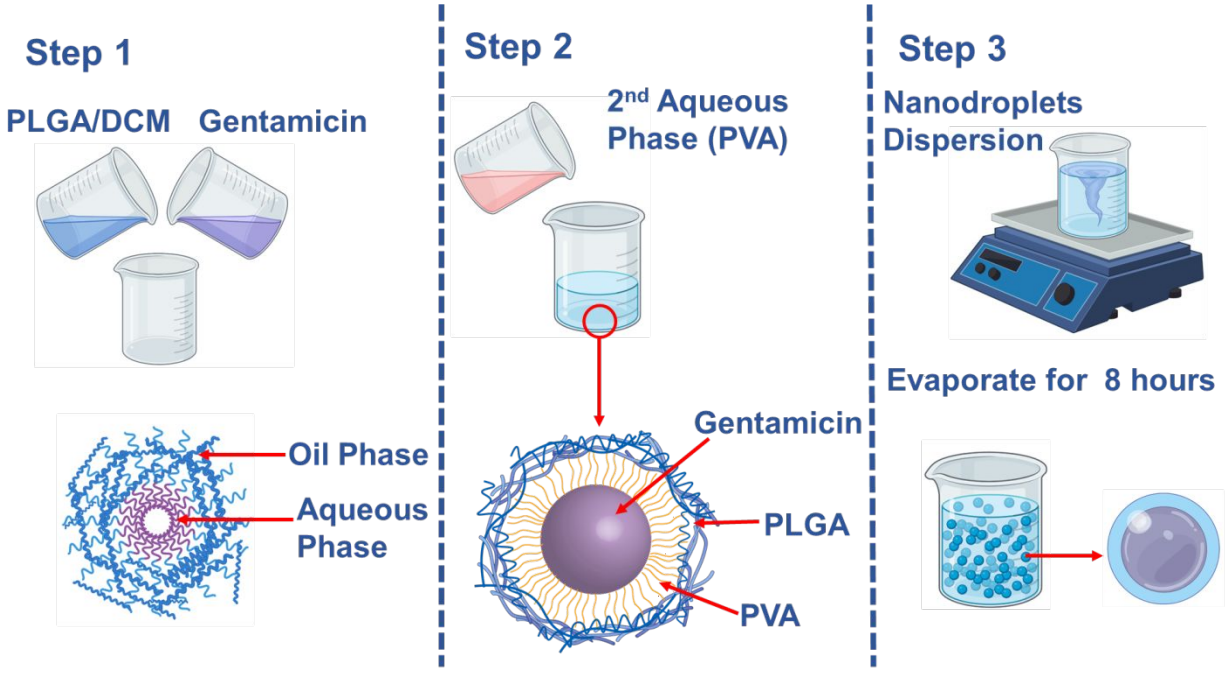

Figure S1. PLGA nanoparticles were synthesized via a double emulsion solvent evaporation method. Step 1: An oil phase of PLGA and DCM was mixed with an aqueous phase 1 of a gentamicin solution; Step 2: An aqueous phase 2 of a PVA solution was added, resulting in a double emulsion with PLGA nanodroplets; Step 3: The double emulsion was kept allowing water to evaporate. Gentamicin was encapsulated inside the PLGA nanoparticles.

S2. PLGA Nanoparticle Purification

The four steps for PLGA Nanoparticles purification were explained below.

(1) Solution condensation: the resulting solution after the double emulsion evaporation method was kept in fume hood where the temperature was controlled around 2~3^{o}C to allow evaporation and hence to consolidate the solution.

(2) Dialysis: the condensed PLGA solution was poured into a dialysis tube to further condense the solution at 2~3^{o}C. The tube was fully immersed in a beaker that contained a 25% NaCl solution and kept in refrigerator.

(3) Purification: 10ml DCM was added into the condensed PLGA nanoparticle solution before the combined solution was well mixed using appropriate agitation, resulting in an opaque solution.

(4) Centrifugation: the mixed solution was centrifuged at 6000rpm/min for 15 minutes resulting in a phase separated solution. After that, nanoparticles in water solution can be transferred by using pipettes.

In addition, several comparison experiments were conducted, which were DCM mixed with water, 3% PVA solution mixed with water. 3% PVA solution mixed with DCM. The comparison experiments were designed to prove the PLGA nanoparticles can be purified via this procedure. And the gel in the solution was caused by the combination of PVA and DCM.

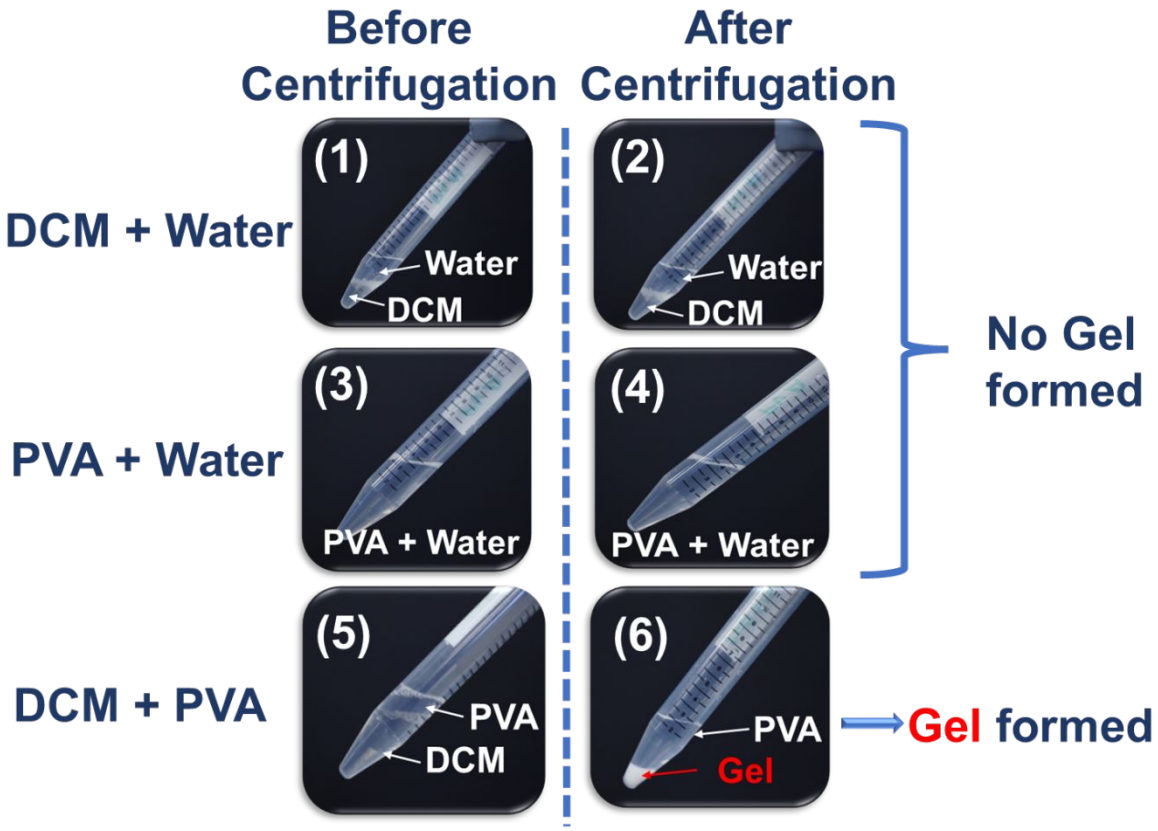

Figure S2. Purification controlled experiments. (1)-(2): DCM was mixed with water, and no gel was formed after centrifugation; (3)-(4): 3% PVA was mixed with water, and no gel was formed after centrifugation; (5)-(6): DCM mixed with 3% PVA, and gel was formed at the bottom of the test tube.

In order to further confirm the conclusion, comparison experiments were conducted to determine if the PLGA nanoparticles were purified. As illustrated by Fig S2, several sample solutions were prepared first, which were DCM mixed with water (Fig S2 (1)), 3% PVA solution

mixed with water (Fig S2 (3)), and 3% PVA solution mixed with DCM (Fig S2 (5)). Before centrifugation, all three solutions were clear and phase layers were easy to see. After centrifugation, there was no change in water DCM mixed with water (Fig S2 (2)) or 3% PVA solution mixed with (Fig S2 (4)). The separated phase layer was still in the solution of DCM mixed with water and no gel was formed. PVA solution mixed with water can only dilute the solution due to PVA solubility in water. Neither "PVA and water" nor "DCM and water" could form into gel. However, there was gel precipitation at the bottom of the tube after centrifuging PVA and DCM mixture (Fig S2 (6)). Therefore, the gel was derived from PVA and DCM, or rather DCM could drag PVA from the solution. In conclusion, adding DCM to the condensed PLGA nanoparticles can surely purify PLGA nanoparticles. The corresponding experiments can be found in the Supporting Information Figure S2.

S3. Electrospinning

In each recipe, the total mass of PLGA nanoparticles was fixed at 200mg with an average diameter of 120 nm. Then, every solution was sonicated for 10 minutes to let the nanoparticles fully disperse in the mixture. The emulsion solutions were spined under parameters of an injection rate of 0.45ml/hour, a voltage of 20kV, and a spinning distance of 20cm. Fig 2 (A) showed PLGA nanoparticles incorporated fibers that were synthesized via electrospinning. And the diagram presented emulsion spinning solution in syringe was gradually squeezed out of a needle, and polymers in the solution were stretched into fibers with PLGA nanoparticles incorporated.

S4. *E.coli* Growth

An *E.coli* ATCC25922 pellet (7.1×10^3 CFU/pellet) was mixed with 50ml LB Broth medium. The solution was incubated at 37^o C for 24 hours. An inoculation loop was used to transfer the bacteria to an LB-Agar streak plates, and the bacteria culture was placed in an

incubator for 24 hours at 37^{o} C to allow bacteria to grow. BBL Muller Hinton (MH) II Agar was used to prepare MH agar plates. 12.5 g LB powder and 7.5 g Agar powder were dissolved in 500ml distilled water. The MH agar solution was autoclaved at 131 ^{o}C for 20 minutes at 15psi. The solution was distributed to a number of petri dishes with 25ml solution for each dish. The plates were left to set in the laminar flow hood for 10-30 minutes before they were stored in the fridge for the following antibacterial textile testing.