FRESH Blended Gelatin Slurry Preparation Protocol

Freeform Reversible Embedding of Suspended Hydrogels

This protocol is for making the FRESH blended gelatin slurry for 3D bioprinting. It has been optimized for printing with Lifeink® collagen bioinks.

Protocol:

Part 1: Preparing the Gelatin (day 1)

1. Pre-heat 250 mL of 1X PBS to 40-45°C in a glass mason jar.
2. Mix in 10 g of gelatin into the 1X PBS and stir until fully dissolved.
3. Store overnight (+10 hrs) in a 4°C refrigerator.
4. Make 1L of sterile 1X PBS solution and place in refrigerator overnight (+10 hrs) for next steps.

Part 2: Blending gelatin (day 2)

5. Remove mason jar from the refrigerator and add approximately 100 mL of cold 1X PBS.
6. Take a spatula and separate the gelatin from the walls of the jar to introduce PBS between the sides of the gelatin puck and the container. Don't over-agitate the gelatin puck.
7. Fill the jar to the brim with additional cold 1X PBS.
8. Place the rubber O-ring on the jar and again fill to the brim of the O-ring.
9. Quickly put the blade on over the O-ring; some solution might spill (overfilling the container with PBS solution ensures as little air as possible is trapped within the container).
10. Screw the blender adapter tightly onto the jar. Only a small bubble of air should be seen when tipping the jar.

Video Demonstration for Steps 5-10

11. Place the sealed jar into a -20°C freezer. This will help to prevent the gelatin from melting during blending.
12. Remove the jar from the freezer when ice crystals clearly start to form between the top surface of the gelatin puck and the fluid solution (~45 minutes).
13. Blend mixture by holding down the "pulse" switch of the blender for 60 seconds. Almost immediately after starting to blend, the gelatin puck should dislodge from the roof of the jar.

Video with Additional Tips for Step 13

Part 3: Centrifugation

14. Pipette ~40 mL of the gelatin slurry into 50 mL centrifuge tubes.

Video Demonstrating Consistency of Blended Slurry

15. Centrifuge at 3,800G for 4 minutes at 4°C. After centrifugation, the tube should contain a compacted pellet of gelatin slurry with a somewhat cloudy supernatant, and a white foamy top layer. This white foam raft is the soluble gelatin (sometimes there is little to no foam after the first centrifugation).
16. Aspirate or pour off the white gelatin raft and supernatant.
17. Refill tube with cold 1X PBS solution.
18. Vortex to re-suspend the slurry.

Video Demonstrating Steps 14-18

19. Repeat steps 15-18 until no to little foam is observed at the top of the supernatant (at least 3 times). This indicates that most of the soluble gelatin has been removed.
20. After the last centrifuge, pour off the supernatant, refill the tube with 1X PBS and vortex.
21. To save for later use, store the suspended slurry at 4°C. To use right away, continue to Part 4.

Part 4: Preparing FRESH for printing

22. With your slurry in suspension, centrifuge it at 225G for 5 minutes at 4°C.
23. Pour off the supernatant.
24. Use a spatula to scoop the slurry into your print container (e.g. 35 mm dish).
25. Place 2 Kimwipes on top of the gelatin slurry to draw water out of the slurry.
26. Allow the Kimwipes to become saturated and then remove.

Video and Tips Demonstrating Steps 23-26

For additional printing suggestions, parameters, or help, contact support@advancedbiomatrix.com.

References

1. Hinton, T. J. *et al*, "Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels," *Science Advances,* vol. 1, no. 9, October 2015.