
APPENDIX A

Equipment of experiment

Scanning electron microscopy (JSM--6610LV Scanning Electron Microscope from JEOL USA)

Atomic Force Microscopy (Park XE7 atomic force microscopy)

Freezer (-20°C)

Cell culture plates

Micro-plate Reader

Glass molds

Lyophilizer (Freeze-Drying Machine)

Micropipette



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APPENDIX B

Chemical reagents

Squid chitosan polymers (FLAKE, TMECO)

Gelatin powder from bovine skin (Type B; HiMedia)

PVA powder (Loba Chemie)

50% glutaraldehyde solution (Merck)

Methyl thiazolyl tetrazolium (MTT)

Ethanol aqueous solution

Physiological saline

Trypsin-EDTA (Gibco)

Dulbecco's modified Eagle Medium (DMEM) powder (Gibco)

Fetalbovine serum (Gibco)

Pen Strep (Gibco)



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APPENDIX C

Preparation of solutions

Preparation of 10wt% gelatin solution

1. The following was dissolved in 80 ml distilled water.
10 g of Gelatin powder from bovine skin (Type B; HiMedia)
2. Distilled water was added to adjust the total volume to 100 ml.
3. Solution was kept at 50 °C in the water bath and was stirred with glass rod until the solution became homogeneous.

Preparation of 5wt% PVA solution

1. The following was dissolved in 80 ml distilled water.
5 g of PVA powder (Loba Chemie)
2. Distilled water was added to adjust the total volume to 100 ml.
3. Solution was kept at 80 °C in the water bath and was stirred with glass rod until the solution became homogeneous.

Preparation of 0.5wt% chitosan solution

1. The following was dissolved in 80 ml 1% acetic acid solution.
0.5 g of Squid chitosan polymers (FLAKE, TMECO)
2. 1% acetic acid solution was added to adjust the total volume to 100 ml
3. Solution was kept at room temperature for one week until solution was homogeneous.

Preparation of 10×PBS

1. The following were dissolved in 800 ml distilled water.

80g of NaCl,

2g of KCl,

14.4g of Na₂HPO₄

2.4g of KH₂PO₄

2. pH was adjusted to 7.4

3. Distilled water was added to adjust the volume to 1L

4. Sterilized by autoclaving.

Preparation of complete D. M. E. M media

1. The following were dissolved in distilled water

13.5 g of Dulbecco's modified Eagle Medium (DMEM) powder (Gibco)

3.7 g of NaHCO₃

2. 10 ml of Pen Strep (Gibco) was add to the resulted solution from last step and the total volume was adjusted to 1 L

3. 50 ml of Fetalbovine serum (Gibco) was mixed with the resulted solution from last step and the total volume was adjusted to 500 ml

