Seeding Cells on Microfiber Scaffolds
without hydrogel or 3D ECMs

Instructions For Use / Users Guide

Materials

- Ultra-low attachment (ULA) Wellplates
- 50 mL sterile conical tubes
- Aspirator
- Sterile forceps

Reagents

- Media containing minimum 10% FBS

Procedure

1. **Submerge in Media –** Add sterile media containing at least 10% FBS. Incubate scaffolds at 37 C overnight.
    - Minimum of 12 hours incubation.
    - Media may contain more than 10% serum.
    - If using bead bath, parafilm the cap to prevent evaporation and maintain sterility
        - Do not use water bath for incubation

2. **Transfer to ULA Wellplates -** Open and label ULA wellplates. Use sterile forceps to transfer scaffolds into wells, ensuring the top and bottom of scaffolds stay the same
    - Make sure the BSC is thoroughly cleaned before starting this step

3. **Prepare Cells**
    - Lifting cells from flasks - Follow protocols for lifting and counting cells.
        - Apply trypsin (or other reagent)
        - Incubate cells for 5-15 minutes
        - Add media @ 2:1 ratio
        - Transfer to falcon tubes
        - Centrifuge
        - Resuspend in media and count
    - Adding cells from cryopreservation
        - Thaw cells
        - Add to media
        - Centrifuge
        - Resuspend in media and count

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4. **Seed Cells**
    - Pipette cell solution directly on top of scaffolds
        - For 24 well plates add 100 µL of solution
    - Place plates in incubator @ 37°C for 1 hour

5. **Add Media–**add media to each well
    - For 24 wells, add 500 µL to each well
    - Carefully dispense media to the side of each well with minimal jet disturbance to the scaffolds.
    - Return plates to incubator

Troubleshooting

- Cell attachment
    - Visualizing cells attached to the scaffolds can be challenging with brightfield microscope. In order to verify cells have attached the standard is to fix scaffolds and perform immunofluorescence imaging with DAPI and phalloidin or similar stains.
    - It may take the cells 24-48 hours until they visually spread in culture.

    - If cell attachment to scaffolds is an issue, consider
        1. Extend attachment period to 1 hour (from 30 minutes)
        2. Add additional pipetting step to attachment period, where liquid excess liquid is aspirated and re-dispensed onto scaffold after 30 minutes.
        3. Pre-treat scaffolds with NaOH to induce hydrophilic surface of microfibers (see troubleshooting protocol).

References and Citations

- Bolle, E.C.L., Nicdao, D., Dalton, P.D., Dargaville, T.R. (2021). Production of Scaffolds Using Melt Electrospinning Writing and Cell Seeding. In: Rainer, A., Moroni, L. (eds) Computer-Aided Tissue Engineering. Methods in Molecular Biology, vol 2147. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0611-7_9
- Tourlomousis F, Jia C, Karydis T, Mershin A, Wang H, Kalyon DM, Chang RC. Machine learning metrology of cell confinement in melt electrowritten three-dimensional biomaterial substrates. Microsyst Nanoeng. 2019 Mar 25;5:15. doi: 10.1038/s41378-019-0055-4. PMID: 31057942; PMCID: PMC6431680.