Copner Biotech Ltd - Spheroid and Organoid Protocol (3D PETG Cell Culture Scaffolds – 24 Well Format)

Protocol

Notes Before Starting

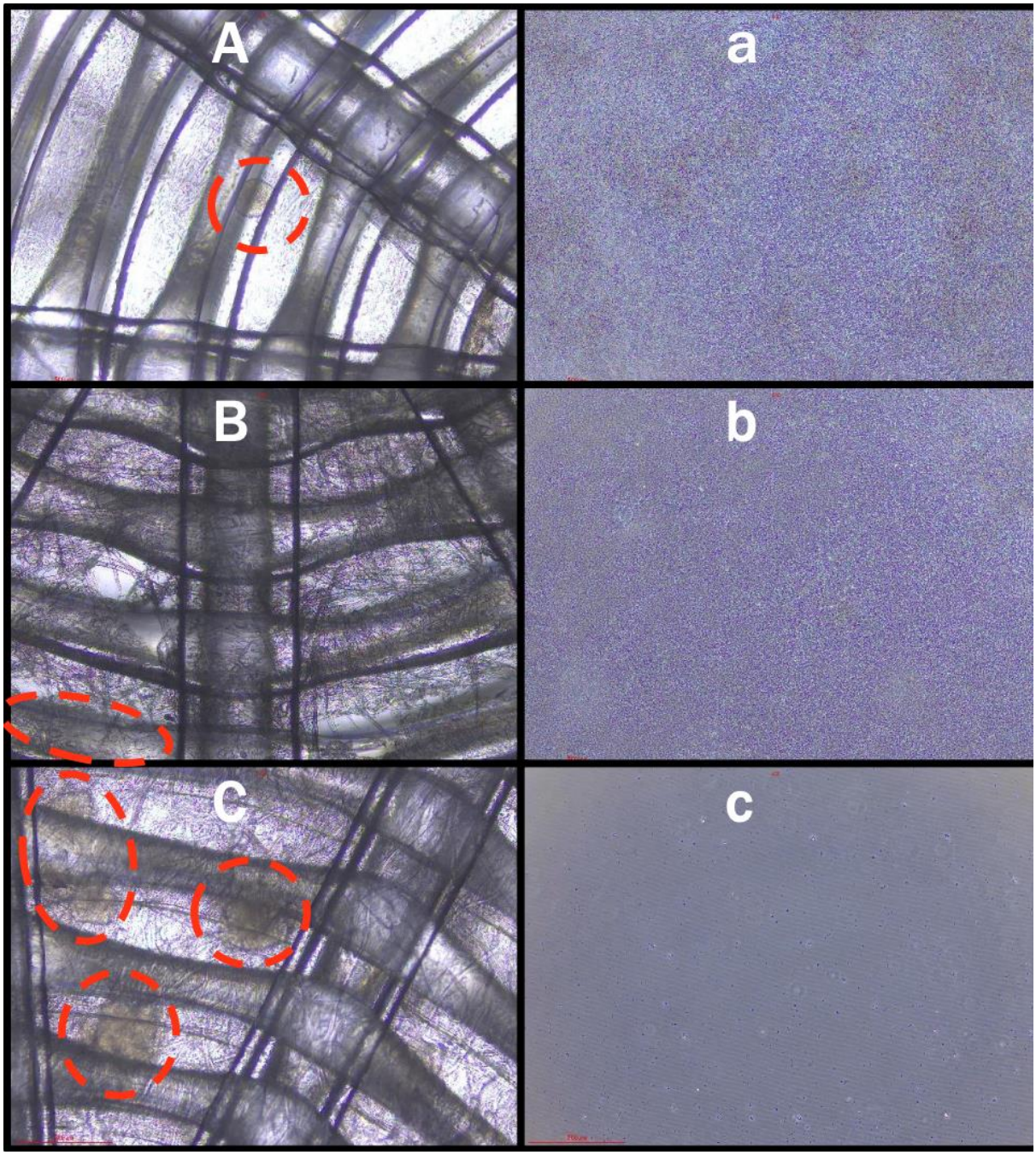
• Prior to use, scaffolds must be rendered hydrophilic with a 70 % ethanol wash for 15 minutes.
• Add enough ethanol to completely cover the scaffold, remove, and wash 2x with appropriate culture media (2ml).
• To avoid drying, leave the scaffold in the final medium (2ml) wash until required.
• We recommend leaving scaffolds in this final medium for 4h to enhance subsequent cell attachment.

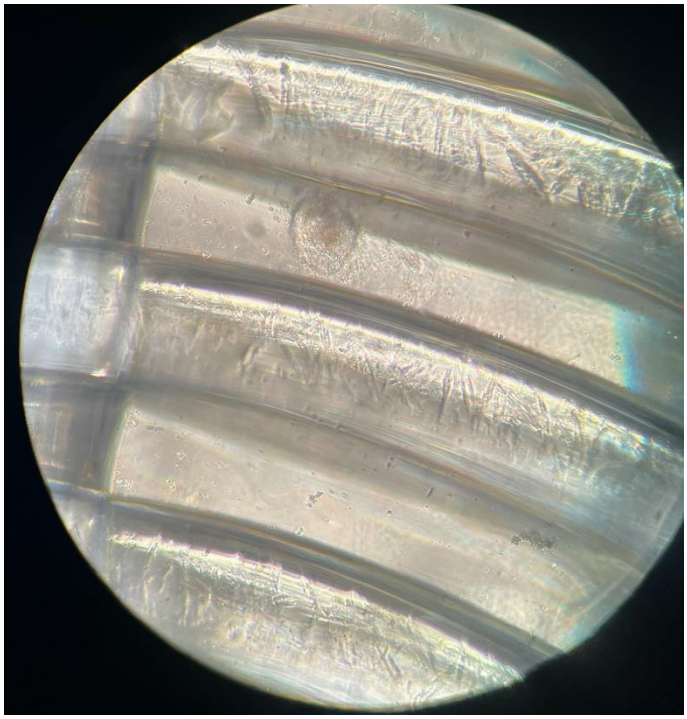
Seeding Cells on Scaffold

• Remove all cell culture media from the well prior to seeding, via pipette.
• Slowly pipette your desired cell density via a 100µl cell media solution onto the top of the scaffold, taking care to ensure the solution permeates the interconnected pores as much as possible. We recommend seeding 150,000 cells per scaffold.
• Place seeded scaffolds back into the incubator for 3h to allow cell attachment.
• Fill the remaining well with 1.4ml of complete medium.
• Change culture medium every 2 days until the end of culture. When changing media, remove original media from around the scaffold and replace with fresh media. Do not pipette directly from the scaffold.

- Spheroids can be removed from day 7. Slowly pipette from the central scaffold pore and use spheroid-rich solution in further experimentation.

Results





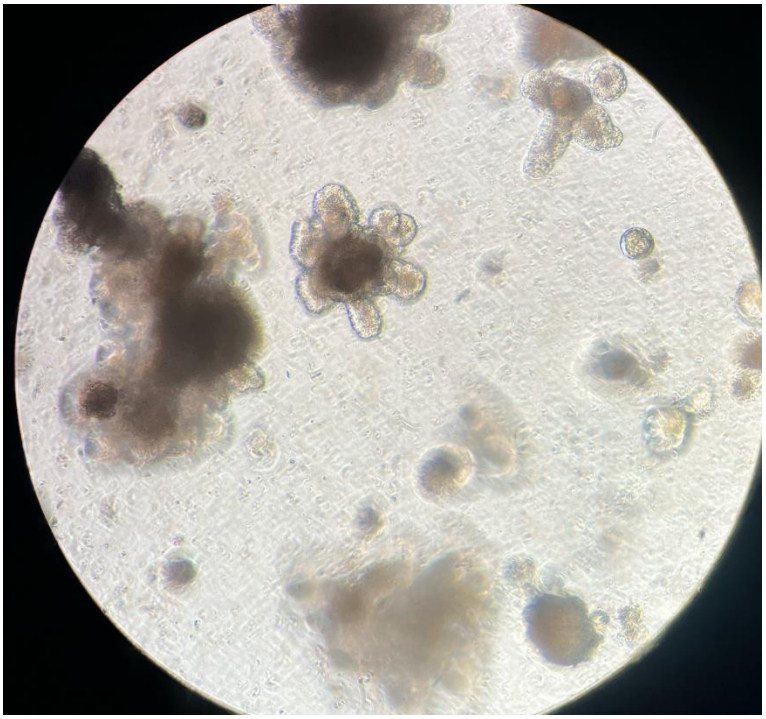


Figure 1 - iPSC cultures in Copner Biotech 3D PETG Scaffolds 7 days post seeding form spheroid structures within the interconnected pore space. The natural capillarity effect present forms a super non-attachment environment ideal for spheroid formation. Scaffolds were coated in Matrigel (A), Laminin (B) and non-coated (C), with non-coated scaffold leading to superior spheroid formation. Comparisons made to coated plates as control (a, b and c).

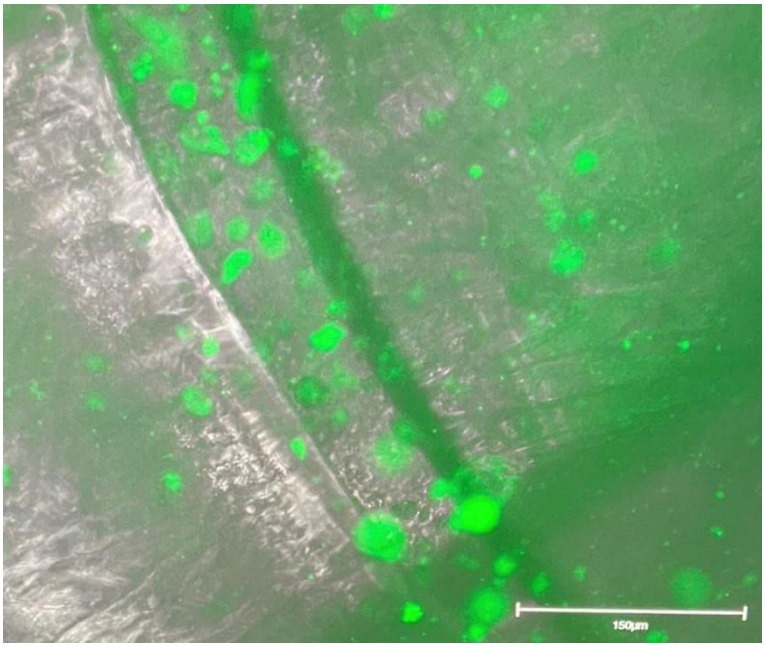


Figure 2 - Early embryoid body structures (top left image) can be pipetted from the scaffold structure and further cultured to form larger, complex organoid structures (top right image) in low attachment plates. Cellular structures can be stained and visualised via microscopy during and after culture (bottom right image).

Distribution Partners

- **UK and Ireland** – Appleton Woods
- https://www.appletonwoods.co.uk/product/3d-cell-culture-scaffolds/

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- **Benelux and Spain** – Isogen Life Sciences
- https://isogen-lifescience.com/products/reagents-and-kits/cell-culture-1/3d-scaffolds

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- **Switzerland** – Lucerna Chem AG
- https://shop.lucerna-chem.ch/7002167/24-well-3d-petg-cell-culture-scaffolds-12-scaffolds