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| **Poly (D-lactic acid) Fused Deposition Modelling (FDM) 3D Printed Scaffolds for Cell Culture: Printing reproducibility and influence of internal geometry on cell viability and growth** |
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| **Background:** 3D printed scaffolds allow recapitulation of the extracellular environment by providing attachment sites, allowing cells to grow in 3D shape, providing a rigid environment for cell growth and delivery of soluble factors to facilitate cell growth. Development of 3D printed scaffolds which enable optimal cell growth conditions remains a constant issue in the field of tissue engineering. Scaffold internal geometry has previously been shown to influence the rate of cell growth, with previous studies indicating scaffolds with hexagonal internal structure to offer greatest cell growth, whilst other studies show scaffolds with triangular geometry to be optimal for cell growth. Disparity in results found in the literature highlight the need to further investigate the influence of scaffold internal geometry on cell growth. In addition, the reproducibility of printing scaffolds using fused deposition modelling (FDM) 3D printers and the impact this has on cell growth is unknown and has not previously been investigated.The aims of this study were two-fold. Firstly, to identify the optimal internal geometry of FDM 3D printed scaffolds for the growth of human colorectal adenocarcinoma (Caco-2) cells using Poly (D-lactic acid) (PLA) to inform scaffold design when using novel and bespoke 3D printing filaments. Secondly, to delineate a possible link between FDM 3D printer reproducibility and Caco-2 cell growth in PLA scaffolds. |
| **Methods:** In this work, FDM 3D printing was used to print PLA scaffolds with varying internal geometry (i.e.no internal shape, triangular, square, circular, and hexagonal) and infill density (20% and 99%). Scaffolds were characterised and then seeded with Caco-2 cells at a density of 0.3 x 106 cells/mL. Caco-2 cell viability in the scaffolds was measured over a period of 6 days using the Alamar Blue assay.  |
| **Results:** Results indicated that hexagonal internal shaped scaffolds performed best over 6 days compared to all other scaffolds giving cell viabilities of more than double compared to the control for both 20% and 99% infill scaffolds. Furthermore, scaffolds with hexagonal internal geometry at 20% infill performed better over 6 days compared to their 99% infill counterparts and gave a 43% greater cell viability. When investigating the reproducibility, it was evident that as internal geometry became more complex (no internal shape being the most simple and hexagonal internal shape being the most complex), a greater difference was observed between the printed height of the scaffold and the computer automated design (CAD) height. For example, scaffolds with hexagonal internal shape showed an increase in height by up to 18%. PLA scaffolds with 20% infill density were also shown to give lower values of standard deviation compared 99% infill scaffolds when measuring scaffold weight, height, and surface roughness.  |
| **Conclusions:** To conclude, these results indicate PLA scaffolds with a hexagonal internal geometry and 20% infill density to be the best design for optimal Caco-2 cell growth over 6 days. In addition, scaffold internal geometry and infill density should be carefully considered as a higher infill density and more complex internal geometry can give increased values of standard deviation and greater dimensional inaccuracy.  |