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Editorial

Active 3D environment: A key to stable, reproducible, mimetic tissues

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Cells grown as active 3D spheroid/organoid cultures have physiological performances that mimic that seen in human tissues better than cells grown in 2D culture. We have previously proposed two extremes of cellular programming (the cultural divide). At one extreme is exponential growth with diminished functionality (as seen in wound healing or cancer, and experimentally as cells grown in traditional 2D cultures) and at the other extreme is a dynamic equilibrium with very slowly proliferating cells with a highly specialized functionality (as seen in tissues and experimentally as cells grown as active 3D spheroids). We have shown that the hepatocellular carcinoma cells HepG2/C3A grown as active microgravity 3D spheroid cultures for periods longer than 18 days have physiological performances that mimic that seen in human tissues better than cells grown in 2D culture. We have analyzed the proteome and cellular architecture at these two extremes and found that they are dramatically different. Ultra structurally, actin organization is changed, microtubules are increased and keratins 8 and 18 decreased. Metabolically, glycolysis, fatty acid metabolism and the pentose phosphate cycle are increased while Krebs cycle and oxidative phosphorylation is unchanged. Enzymes involved in cholesterol and urea synthesis are increased underpinning the attainment of cholesterol and urea production rates seen in vivo. DNA repair enzymes are increased even though cells are predominantly in G0. Transport around the cell along the microtubules, through the nuclear pore and in various types of vesicle has been prioritized. There are numerous coherent changes in transcription, splicing, translation, protein folding and degradation. The amount of individual proteins within complexes is shown to be highly coordinated. Typically, subunits which initiate a particular function are present in increased amounts compared to other subunits of the same complex. We thus conclude that 3D spheroids offer a window into in vivo physiology.

Culturing cells in 3D is often considered to be significantly more difficult than culturing them in 2D. In practice, this is not the case: the situation is that equipment needed for 3D cell culture has not been optimized as much as equipment for 2D. Here we present a few key features which must be considered when designing 3D cell culture equipment. These include diffusion gradients, shear stress and time. Diffusion gradients are unavoidably introduced when cells are cultured as clusters. Perhaps the most important consequence of this is that the resulting hypoxia is a major driving force in the metabolic reprogramming. Most cells in tissues do not experience liquid shear stress and it should therefore be minimized. Time is the factor that is most often overlooked. Cells, irrespective of their origin, are damaged when cultures are initiated: they need time to recover. All of these features can be readily combined into a clinostat incubator and bioreactor. Surprisingly, growing cells in a clinostat system do not require specialized media, scaffolds, ECM substitutes or growth factors. This considerably facilitates the transition to 3D. Most importantly, cells growing this way mirror cells growing in vivo and are thus valuable for biomedical research.