Krzysztof Wrzesinski, Pharmacoeconomics 2018, Volume:3
DOI: 10.4172/2472-1042-C1-004

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

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. Ultrastructurally, 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!

Fig. 1 Relationship of the changes in the proteome following its adaptation from 2D to 3D culture with structural and physiological properties.

Recent Publications

1. Wrzesinski K and Fey S J (2015) From 2D to 3D - a new dimension for modelling the effect of natural products on human tissue. Current Pharmaceutical Designs. 21(38): 5605-5616.
2. Fey S J and Wrzesinski K (2012) Determination of drug toxicity using 3D spheroids constructed from an immortal human hepatocyte cell line. Toxicological Sciences. 127(2):403-411.
3. Wrzesinski K and Fey S J (2013) After trypsinisation, 3D spheroids of C3A hepatocytes need 18 days to re-establish similar levels of key physiological functions to those seen in the liver. Toxicology Research. 2(2):123-135.
4. Wrzesinski K et al. (2013) HepG2/C3A 3D spheroids exhibit stable physiological functionality for at least 24 days after recovering from trypsinisation. Toxicology Research 2(3):163-172.
5. Wrzesinski K et al. (2014) The Cultural divide: exponential growth in classical 2D and metabolic equilibrium in 3D environments. PLOS One. 9(9):1-15.

Biography

Krzysztof Wrzesinski is CSO and Co-founder of CelVivo Ivs, a company dedicated to 3D cell culture technology. He received his PhD in 2005 from the University of Southern Denmark, SDU. After receiving a Postdoctoral grant from the Danish Biotechnology Instrument Centre (DABIC), he has been working together with Olympus Denmark A/S on developing a new Laser Micromanipulation System specially designed to operate on live 3D tissues and tissue like structures. From 2009, he was headhunted to DrugMode ApS and in 2010 he became General Manager. In 2011, he became the Associate Professor of the Department of Biochemistry and Molecular Biology, SDU and co-founded a new research group, Tissue Culture Engineering Laboratory (TCEL group). He is currently focusing on further improvements and detailed physiological characterization of 3D tissue like structures and establishing human *in vitro* systems for pre-clinical development of drugs and toxicological assessment of chemical compounds.

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