White Paper

Functional Stability of exVive3D™ Liver, Bioprinted Human Tissues

The native human liver consists of parenchymal hepatocytes and non-parenchymal cells, including hepatic stellate cells (HSC), endothelial cells (EC) and Kupffer cells (KC). Normal tissue functions and response to injury rely on cross talk and interplay between these cell types, thus in vitro systems that incorporate multiple cell types within a 3D architecture offer advantages over simple monocellular systems with respect to predictive modeling.

Additive manufacturing strategies, such as 3D bioprinting, have been applied to generate small-scale tissues in vitro using human primary cells as building blocks. Organovo's exVive3D™ Liver, bioprinted human tissues are generated with a proprietary tissue fabrication platform, yielding 3D tissues comprised of primary human hepatocytes, HSC, and EC, with spatially defined zones enriched for specific cell types (Figure 1).

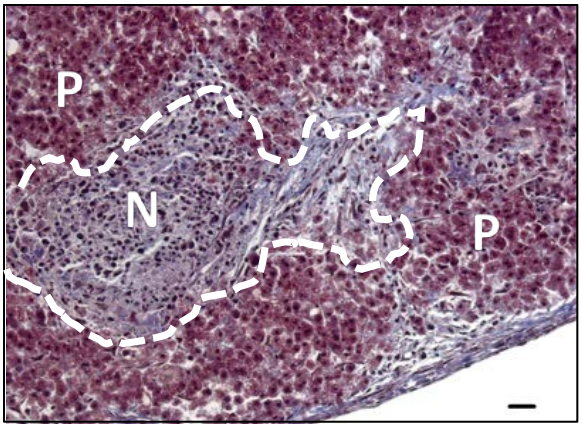

Figure 1: exVive3D™ Liver, Bioprinted Human Tissue, cross section subjected to Masson's Trichrome stain*, showing clearly demarcated zones enriched for either non-parenchymal (N) or parenchymal cells (P, hepatocytes).*

The stability and consistency of bioprinted human liver tissues can be appreciated by measuring ATP production – an indicator of cell viability and tissue health – and secretion of the liver-specific protein albumin over time (Figure 2). The expected inherent differences in the magnitude of ATP production or albumin secretion from hepatocytes derived from unique human donors is preserved in exVive3D Liver tissues, while the trends of increasing viability and function over time are remarkably similar. Thus, one can anticipate that key features of a particular donor will be observed in the 3D bioprinted tissues while the overall trends and functional performance of tissues over time will be very consistent.

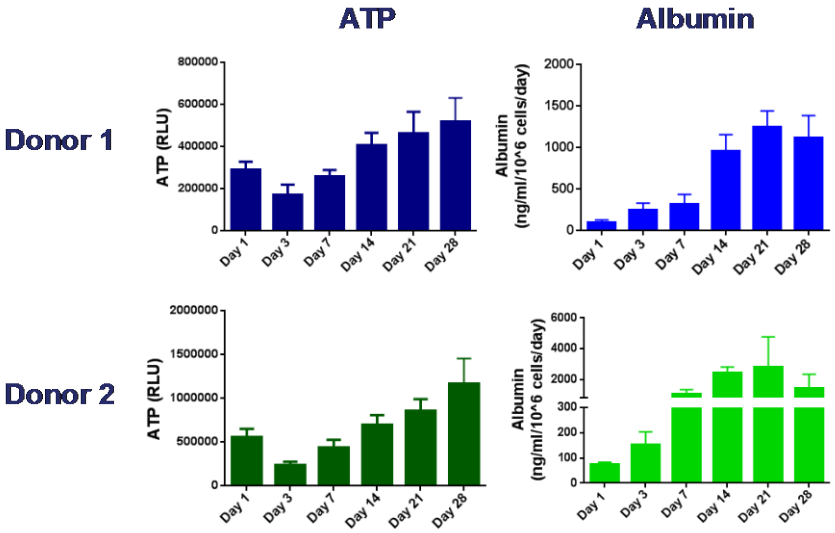

Figure 2: exVive3D™ Liver, Bioprinted Human Tissue are durable in vitro and remain viable for weeks after manufacturing. Cell viability and tissue health as measure by ATP production per tissue using Cell-Titer Glo® (Promega), stabilizes over time. Production of human albumin, assessed by ELISA, increases after fabrication, stabilizing by day 14. Data shown is the average +/- standard deviation for independent tissues generated from two separate hepatocyte donors.

A key function associated with the liver is the metabolism of xenobiotics, through the actions of the cytochrome P450 (CYP450) enzyme family. CYP3A4 is a hallmark CYP enzyme that is responsible for metabolizing a broad range of drugs; unfortunately, it is one of the most challenging CYPs to maintain in hepatocytes cultured in vitro and is also characterized by significant species differences between humans and other vertebrates[1].

While short-term studies of metabolite formation can be conducted in isolated microsomes, hepatocytes in suspension, or freshly plated hepatocytes, there is a growing interest in conducting longer-term studies that involve drug uptake and metabolite formation with the ability to distinguish between intracellular metabolite accumulation and metabolite excreted into the media[2].

Studies have been conducted to examine the ability of exVive3D Liver tissues to uptake and metabolize the midazolam, a benzodiazepine that is metabolized primarily in the liver and gut by CYP3A4 to the pharmacologically active metabolite, 1-hydroxy-midazolam[3]. Metabolite formation was detectable even in the basal state, with the capacity for 1-hydroxy-midazolam production increasing over two weeks in both the tissue homogenates (tissue accumulation) and the culture media (efflux and excretion) (Figure 3). CYP3A4 expression and function were significantly induced by Rifampicin, with an observed increase in inducibility over the two-week time course.

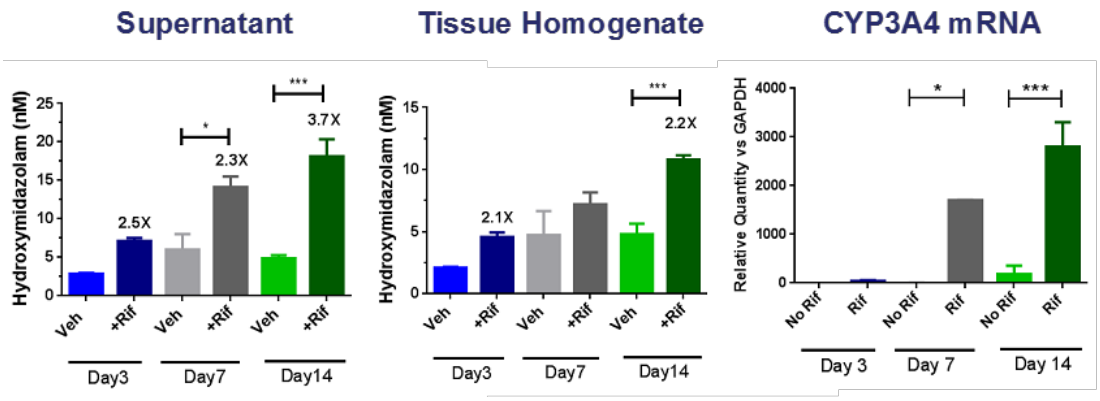

Figure 3: The capacity of exVive3D™ Liver, Bioprinted Human Tissues for CYP3A4-mediated metabolism increased over time and displayed a 2-3X induction response to Rifampicin. exVive3D Liver tissues were treated for three days +/- Rifampicin (10µM) starting on the culture day indicated, followed by a 24-hour exposure to midazolam (10µM). Levels of 1-hydroxymidazolam were measured in either tissue homogenates or culture media by GC/MS (SciAnalytical Strategies, Inc.). Relative expression of CYP3A4 mRNA was quantified by qRTPCR following induction, normalized to GAPDH. Data shown is the average +/- standard deviation for independent tissues; *(p,0.05), **(p,0.01), ***(p,0.001), one way ANOVA.

Many drugs are hepatotoxic and cause damage to one or more cell types in the liver, resulting in necrosis, inflammation, steatosis, and/or fibrosis[4]. Multicellular 3D tissues, such as exVive3D bioprinted human tissues offer a unique mode of assessing both biochemical and histologic end points in tandem to better determine the safety or efficacy profile of a drug. Bioprinted human liver tissues, manufactured with hepatocytes derived from three independent human donors, have been used successfully to detect toxic tissue damage after multi-day exposure to mildly toxic, moderately toxic, or extremely toxic treatments (Figure 4).

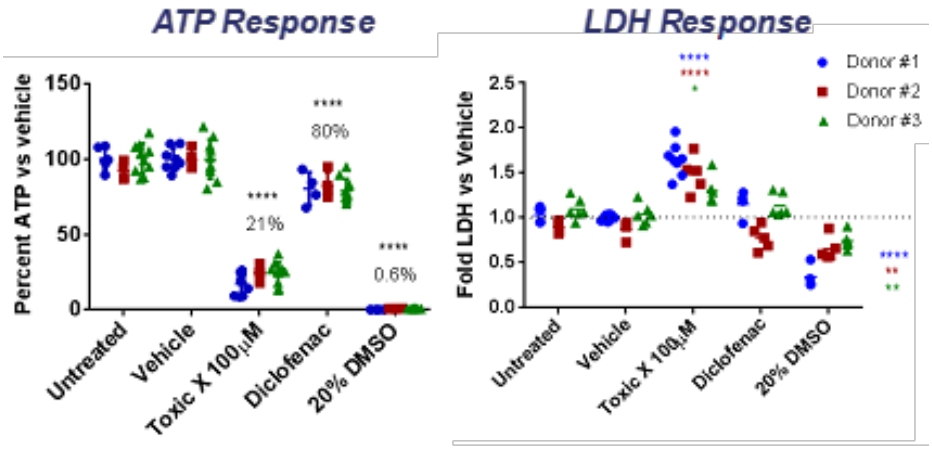

Figure 4: exVive3D™ Liver, Bioprinted Human Tissues can effectively model liver injury. exVive3D Liver tissues were manufactured from each of (3) hepatocyte donors and treated with vehicle, a known hepatotoxic compound (Toxic X), Diclofenac, or DMSO.ATP was measured in tissue homogenates (Cell-Titer Glo®, Promega), and LDH in supernatants (Abcam). Data shown is the average +/- standard deviation for independent tissues; *(p, 0.05), **(p, 0.01), ***(p, 0.001), one way ANOVA.

Treatment with dimethylsulfoxide (DMSO) resulted in severe tissue damage, with significant obliteration of both ATP production and lactate dehydrogenase (LDH) production, while treatment with a moderately toxic compound led to hallmark reductions in ATP and elevation in LDH signifying liver tissue damage.

Treatment with Diclofenac yielded mild but significant decreases in ATP production in all donors, without elevations in LDH. Outcomes were highly reproducible among replicate tissues from a single donor, and from tissues manufactured with hepatocytes from independent donors. Figure 5 highlights histological outcomes of necrosis and steatosis – two common forms of drug-induced liver injury – in the tissues.

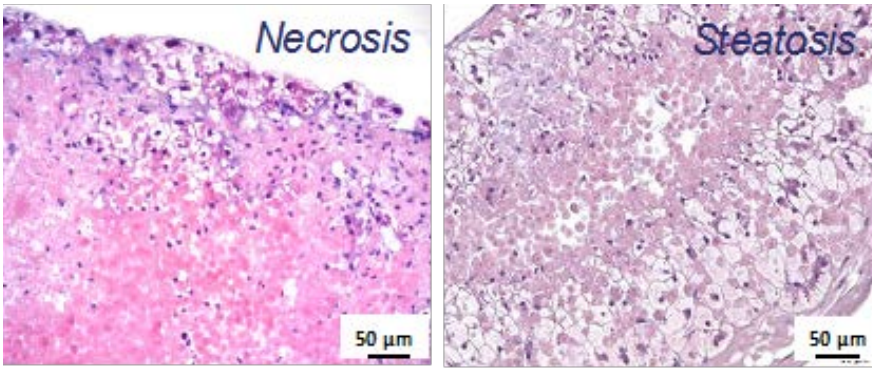

Figure 5: exVive3D™ Liver, Bioprinted Human Tissues were exposed to cytotoxic or steatogenic conditions, cross-sectioned and subjected to H&E stains. Necrotic damage was accompanied by loss of membrane integrity, cellular swelling, and loss of nuclei. Steatotic damage was accompanied by intracellular accumulation of lipid and prevalent vacuolization.

In summary: exVive3D™ Liver, bioprinted human tissues provide a highly reproducible, multi-cellular, fully human model for acute and chronic assessment of liver function, metabolite formation, and drug response.

1. Graham, M. J. & Lake, B. G. Induction of drug metabolism: species differences and toxicological relevance. Toxicology 254, 184-191, doi:10.1016/j.tox.2008.09.002 (2008).
2. Chu, X. et al. Intracellular drug concentrations and transporters: measurement, modeling, and implications for the liver. Clinical pharmacology and therapeutics 94, 126-141, doi:10.1038/clpt.2013.78 (2013).
3. Fabre, G. et al. Characterization of midazolam metabolism using human hepatic microsomal fractions and hepatocytes in suspension obtained by perfusing whole human livers. Biochemical pharmacology 37, 4389-4397 (1988).
4. Kmiec, Z. Cooperation of liver cells in health and disease. Advances in anatomy, embryology, and cell biology 161, III-XIII, 1-151 (2001).

