191d Modulation of Liver Functions Expressed by Hepg2 Cells by Limiting Oxygen Diffusion

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Tissue engineered replacements for the liver, either for clinical or in vitro research purposes, tend to exhibit only a limited subset of the wide array of liver functions. One explanation is that the functional unit of the liver, the acinus, is divided into two zones with distinct sets of metabolic functions. It is therefore desirable to develop a culture system that replicates both of these zones. While systems have been devised for maintaining the zonation of primary hepatocytes for short periods in vitro, zoned liver functions have not before been observed in a sustainable, continuous cell line. The two zones observed in the liver correspond to high (periportal) and low (perivenous) levels of dissolved oxygen, and it is thought that the oxygen level induces expression of one phenotype or the other. Therefore, many attempts to maintain zonal function have relied upon maintaining separate incubators with different oxygen levels. We have devised a culture system that uses diffusive barriers to control the amount of oxygen that reaches the cells. HepG2 human hematoma cells grown in this system exhibit zonal activity of multiple liver functions, including glucose metabolism and detoxification through cytochrome P450. Measurement of glucose consumption showed that high-oxygen (periportal) cells produced small amounts of glucose while low-oxygen (perivenous) cells consumed significant amounts of glucose. As measured by the ethoxyresorufin-O-dealkylase assay, low-oxygen cells exhibited a P450 1A1 activity roughly twice that of high-oxygen cells. Further, P450 activity displays a bimodal response to oxygen concentration, with a maximum near the perivenous oxygen level. These results agree with observations in the literature of zonal liver activity, indicating that limiting oxygen diffusion can indeed replicate zonal liver functions.