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Michalopoulos G K & Pitot H C. Primary cultures of parenchymal liver cells on collagen membranes—morphological and biochemical observations. *Exp. Cell Res.* 94:70-8, 1975.
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Modification of the substrate of attachment affects the viability and differentiation of cells in culture. This manuscript was the first to show that prolonged differentiation of hepatocytes can be achieved by culturing them on collagen gels. The findings of this paper prompted further studies with hepatocytes and other cells in which modification of the attachment substratum resulted in enhanced cell differentiation and/or growth. [The *SCI*® indicates that this paper has been cited in more than 530 publications.]

The In Vitro Quest for the Differentiated and Growing Hepatocyte

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At the time when this work was performed, cell culture experience in most laboratories derived mainly from established lines of connective tissue cells. The breakthrough of producing millions of viable hepatocytes by collagenase perfusion of the liver had been achieved by M.N. Berry and D.S. Friend in 1969.[1] Hepatocytes, however, were mainly studied in cell suspensions until D.M. Bissell et al. in 1973[2] first introduced hepatocytes in primary monolayer culture. These cultures were the first in which a dispersed population of fully differentiated epithelial cells was transferred directly from its natural location and maintained in an in vitro environment. This type of culture is commonly used today for many other cell types. At that time, however, the attempt received a mixed reaction.

Hepatocytes were losing their characteristic differentiated functions within days. Furthermore, despite attempts to use all growth supplements available at the time, hepatocytes did not grow in numbers or synthesize DNA. Cell line aficionados continued advocating the advantages of cells with stable differentiation, whereas others focused their attention on different epithelial cells growing out of liver preparations, even though the latter cells had no markers relating to hepatocytes and their cell of origin in the liver was not (and still is not) clearly established.

The findings of our paper showed that it was possible to affect the differentiation of complex epithelial cells by modifying their substrate of attachment. Hepatocytes stayed more or less differentiated much longer on collagen gels than on plastic plates or dry collagen layers. This dispelled the notion that the loss of differentiated functions in hepatocytes was an inexorable process and led to more studies to stabilize their phenotype in culture. In work that followed, hepatocyte differentiation was also stabilized by culturing on biomatrix extracted from the liver,[3] in 2 percent DMSO,[4] in high laminin gels,[5] etc. Furthermore, our findings with collagen gels were soon used by investigators on other cell types.

To date, collagen gels have been used to grow epithelial cells from mammary ducts, prostate, epidermis, colon, vagina, bronchi, submandibular glands, endometrium, eye lens, and Sertoli cells of testes. Connective tissue cells such as endothelial cells, fibroblasts, and chondrocytes, as well as primary cells from several cancers, have also been cultured using this method. While differentiation of hepatocytes in culture can be maintained by several methods, regulation of their growth proved much more difficult. It was soon realized that remedies required to maintain differentiation of hepatocytes tended to inhibit their response to mitogens. The best response to mitogens is seen in hepatocytes maintained in short-term conventional cultures on plain plastic or dry collagen. The latter cultures were the ones used as bioassays by us and many others for the identification of EGF, TGFa, HGF/HPTA, aFGF, and bFGF as hepatocyte mitogens, and of TGFβ and IL-6 as mitoinhibitors.[6]

Now that the mitogens for hepatocytes have been identified, the quest for the differentiated and growing hepatocyte continues in a more meaningful manner. Perhaps this is the best time to reexamine the effect of the mitogens on hepatocytes in complex substrates. Collagen gels do not inhibit DNA synthesis as the other complex substrates seem to do and may now have a more meaningful role to play to define the ideal culture conditions for hepatocytes. Given all the recent emphasis for the use of hepatocytes for gene therapy and replacement of hepatic function, the pursuit of the differentiated and growing hepatocyte cultures is becoming even more fun and likely to be joined by many other adventure seeking hepatomaniacs. The quest is still on!

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