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Non-local models for the formation of hepatocyte–stellate cell aggregates

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ABSTRACT

Liver cell aggregates may be grown *in vitro* by co-culturing hepatocytes with stellate cells. This method results in more rapid aggregation than hepatocyte-only culture, and appears to enhance cell viability and the expression of markers of liver-specific functions. We consider the early stages of aggregate formation, and develop a new mathematical model to investigate two alternative hypotheses (based on evidence in the experimental literature) for the role of stellate cells in promoting aggregate formation. Under Hypothesis 1, each population produces a chemical signal which affects the other, and enhanced aggregation is due to chemotaxis. Hypothesis 2 asserts that the interaction between the two cell types is by direct physical contact: the stellates extend long cellular processes which pull the hepatocytes into the aggregates. Under both hypotheses, hepatocytes are attracted to a chemical they themselves produce, and the cells can experience repulsive forces due to overcrowding. We formulate non-local (integro-partial differential) equations to describe the densities of cells, which are coupled to reaction–diffusion equations for the chemical concentrations. The behaviour of the model under each hypothesis is studied using a combination of linear stability analysis and numerical simulations. Our results show how the initial rate of aggregation depends upon the cell seeding ratio, and how the distribution of cells within aggregates depends on the relative strengths of attraction and repulsion between the cell types. Guided by our results, we suggest experiments which could be performed to distinguish between the two hypotheses.

1. Introduction

At present there are few treatments for chronic liver disease, organ transplant being the most successful. However, a lack of suitable donor organs means that interest is turning to the development of liver support devices. As a result, increasing research effort is being focused on the *in vitro* engineering of liver tissue for such devices, as well as for drug testing and, potentially, for transplantation (Green et al., 2009). Approximately 80% of a healthy liver is composed of hepatocytes (Mitaka, 1998), cells which perform most of its biological functions (Selden et al., 1999). The liver contains at least four other cell types including stellate cells (also known as Ito cells), which are thought to play an important role in hepatic regeneration *in vivo*; hence there is interest in their potential use in liver tissue engineering *in vitro* (Bhandari et al., 1997; Riccalton-Banks et al., 2003; Thomas et al., 2005). A number of studies suggest that cell–cell contact between hepatocytes, and between hepatocytes and other cell types, is key to maintaining the viability and functionality of liver tissue grown *in vitro*. Such contacts are promoted by culture techniques that result in the formation of spheroidal cell aggregates (Abu-Absi et al., 2002; Riccalton-Banks, 2002; Richert et al., 2002; Thomas et al., 2005). Our aim in this paper is to use mathematical modelling to investigate the effect of hepatocyte–stellate cell interactions on the aggregation process.

When hepatocytes and stellate cells are co-cultured, cell aggregates form more rapidly and retain liver-specific functions (such as albumin production and cytochrome P450 activity) for a longer period than when the hepatocytes are cultured alone (Krause et al., 2009; Riccalton-Banks et al., 2003; Riccalton-Banks, 2002; Thomas et al., 2005). One mechanism that may contribute to enhanced aggregation is chemotaxis. Hepatocytes are known to respond chemotactically to hepatocyte growth factor (HGF) *in vitro* (Stolz and Michalopoulos, 1997), and stellate cells from rats produce HGF when stimulated with hepatocyte-conditioned medium (Skrtic et al., 1999). In fact, stimulation with just one

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component of the hepatocyte-conditioned medium, insulin-like growth factor-1 (IGF-1), was sufficient to cause the stellate cells to produce HGF. Another study, by Gentilini et al. (2000), reported that IGF-1 is a chemoattractant for human hepatic stellate cells. Hence there may be a feedback loop between the two cell types: the hepatocytes produce insulin-like growth factor-1 which attracts the stellates, and stimulates them to produce more HGF. HGF then acts as a chemoattractant for the hepatocytes, leading to the formation of heterogeneous cell aggregates.

An alternative explanation has been put forward by Thomas et al. (2006). Time-lapse video footage reveals that stellates extend long processes, which, on contact with an hepatocyte, appear to pull the cell into the nascent aggregate (Fig. 1). We speculate that this physical contact between the two cells types promotes aggregation. It is possible that the action of the processes is provoked by a chemical factor secreted by the hepatocytes, as mono-cultured stellates stimulated with hepatocyte-conditioned medium retracted their processes (as they do when pulling heptocytes into an aggregate), whilst this did not occur when the conditioned medium was absent. Furthermore, aggregates formed more slowly, and were less well defined, when the stellates were co-cultured with cells of the Hep G2 cell line (hepatocellular carcinoma cells) rather than hepatocytes, suggesting an interaction specific to these particular cell types. However, the stellates exhibited the same contractile response to hepatocyte fragments as to whole cells, which suggests that the retraction of the processes is not solely due to the secretion of chemical factors by hepatocytes. Thomas et al. (2006) suggest that the attraction between stellate cells is negligible. Stellate cells in monoculture were found to have low cell motility compared to those in co-culture, and did not form aggregates (*e.g.* see Thomas et al., 2006, Fig. 4).

In this paper, we use mathematical modelling to explore the two hypotheses outlined above for hepatocyte–stellate cell interactions. Our model accounts for chemical signalling between the two cell types, the forces exerted by the stellates' cellular processes, and the effects of overcrowding, which causes cells to repel each other when they become too densely packed. We adopt an approach which combines a Keller–Segel modelling framework to describe chemotactic movement with non-local (integro-differential) terms to represent cell–cell interactions due to overcrowding or the action of the stellates' processes on hepatocytes. Similar non-local terms have previously been used to model differential adhesion in cell sorting experiments (Armstrong et al.,

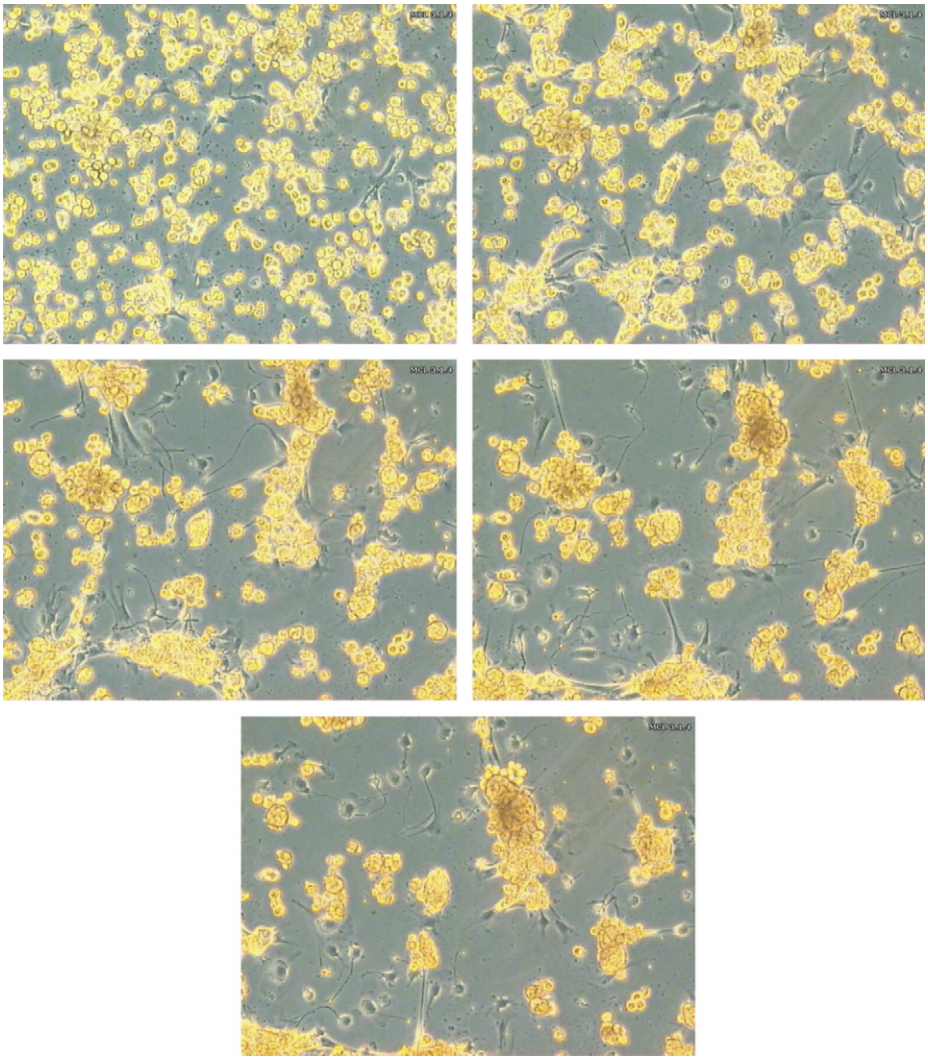

Fig. 1. Snapshots from time-lapse video of an hepatocyte–stellate cell co-culture during aggregation. The hepatocytes appear yellow, and have a rounded morphology. Stellates appear grey, but their long cellular processes are clearly distinguishable. The images show an area approx 1000 μm × 700 μm and were taken at approximately 5-hourly intervals (total time elapsed: approx. 21 h). (Images courtesy of Robert Thomas, Tissue Engineering Group. Similar images, including later timepoints, are shown in Thomas et al. (2006). Timelapse video footage is also available at: www.ecmjournal.org/journal/papers/vol011/vol011a03.php). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)