Hep G2 behaviour in 3D electrospun scaffolds as in vitro cancer inhibitor model

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Hepatocytes, which perform the major functions of the liver, are commonly isolated and cultured in vitro, e.g. for hepatic tissue engineering studies and drug screening (Lee et al., 2007). Unfortunately, under standard culture conditions, hepatocytes loose the specific liver functions (i.e., albumin secretion, urea production and cytochrome P450 activity) just after three or five days of culture. Hepatocytes cultured in an extracellular matrix (ECM) called three-dimensional culture (3D) show a different behaviour in terms of survival and pharmacological response, when compared to hepatocytes cultured on flat surface (Guarino et al., 2015). Several studies have demonstrated that the use of 3D scaffolds concurs reproducing an hepatic-like microenviroment, better than culture plate, providing more comprehensive and relevant information of molecular mechanisms in cancer cell lines culture (Chien et al. 2014). It is well established also that cancer cells cultured on 3D scaffold show differences in functional behaviors such as differentiation, proliferation, and gene expression, when compared to cancer cells cultured on a flat surface (two-dimensional (2D), (Erler et al. 2009). Here we propose a comparative study of Hep G2 – a cell line derived from hepatocellular carcinoma - behaviour in culture plate (2D model) and polymeric scaffolds fabricated by electrospinning technique (3D model). In particular, the aim of the present investigation is to evaluate the effect of fibres made of Polycaprolactone (PCL) on an experimental in vitro model of hepatic cancer in the presence or in the absence of doxorubicin at different concentration (0.4 and 0.8 μM). Hep G2 plated onto PCL nanofibers and on flat surface were grown in Williams' E medium and cell viability was assessed using MTT assay after 1, 3, 7 and 14 days of cell culture. The morphological features of fibres were preliminary evaluated by SEM/AFM investigation. Hep G2 cells were seeded onto PCL electrospun fibers, fixed and examined by SEM to observe cell adhesion. For evaluation on proliferation mechanisms, cells were plated onto electrospun PCL or in plate and were treated with doxorubicin (0.4 and 0.8 μM) for 7 and 14 days; cell proliferation was analized by Brdu ELISA kit. Here we demonstrate that PCL nanofibers reduced Hep G2 cell viability after 3 and 7 days of culture compared to plate control. SEM clearly showed Hep G2 cell adhesion onto electrospun PCL fibers after 1, 3, 7 and 14 days of culture. Brdu assay suggested that PCL without doxorubicin significantly inhibited Hep G2 proliferation after 14 days of cell culture compared to plate control, but did not improve the effect of doxorubicin on Hep G2 proliferation. Hence, it was concluded that PCL fibres exert an anti-proliferative effect on Hep G2 cells thus confirming the influence of 3D network on biological response in terms of cell proliferation. In view of understanding PCL action mode, these 3D electrospun scaffolds might be worthy of consideration for future evaluations of new therapeutic strategies in hepatocarcinoma treatment.

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