

Development of an integrin stimulating polymer-peptide for dispersed non-adherent single-cell culture

ウエハラ ウエダ マリオ コウキチ

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氏 名 : UEHARA UYEDA Mario Kokichi
(ウエハラ ウエダ マリオ コウキチ)

論 文 名 : Development of an integrin stimulating polymer-peptide for dispersed
non-adherent single-cell culture
(非接着性単一細胞分散培養のためのインテグリン刺激ポリマー
ペプチドの開発)

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論 文 内 容 の 要 旨

The creation of recellularized liver grafts from decellularized xenogeneic liver tissue is expected to provide new alternatives in the fields of drug development and the treatment of liver-related diseases.

Decellularized liver tissue provides a natural scaffold that mimics the structure and microenvironment of the native liver, this includes its extracellular matrix (ECM), as well as its complex vasculature, necessary for the distribution of oxygen and nutrients to the infused cells.

Previous studies have identified that the infusion protocol used during recellularization plays a critical role for the distribution and survival of cells within the scaffold. It was hypothesized that improving the initial dispersability of freshly isolated hepatocytes could lead to a more uniform repopulation of the parenchymal space within the scaffold by preventing the blockage of the vasculature. However, under these conditions, the anchorage-dependent hepatocytes are at risk of anoikis, cell death derived detachment.

In this study a polymer-peptide conjugate comprised of polyethylene glycol and the cell adhesive peptide GRGDS (PEG-GRGDS) was developed and applied to freshly isolated hepatocytes. It was expected that the RGD motif within the conjugate is able to engage with the cell integrins and confer them with resistance to anoikis. On the other hand, PEG might provide cells a physical barrier that can promote the initial dispersability of cells. As a proof of concept, individual cells were treated with the conjugate (PG-treatment) and cultured within a microwell chip. The conjugate was characterized and the synthesis protocol optimized. The effectiveness of PG-treatment was evaluated for a variety of non-adherent culture conditions and its potential use for human hepatocytes was confirmed. The treated cells were then infused and cultured into a decellularized scaffold where

functionality was evaluated, showcasing PEG-GRGDS as a potential aid for recellularization applications.

In Chapter 1, the background, relevance, and concept for this study are presented.

In Chapter 2, the current state of hepatocyte cell culture techniques and recellularized liver constructs is detailed. Additionally, diverse mechanisms that result in cell death were introduced and the concept of PEG-GRGDS was compared to existing research.

In Chapter 3, the concept of integrin stimulation by PEG-GRGDS to reduce anoikis was tested. Treatment successfully improved the survival of dispersed non-adherent cells. Regarding the synthesis of the conjugate, coupling of the GRGDS peptide to the PEG polymer was confirmed and the efficiency of the reaction approximated to 70%.

In Chapter 4, PG-treatment was applied to cells in suspension and the treated cells were cultured under a variety of non-adherent conditions. Treated cells at low culture densities were found to remain well dispersed for up to 3 days. Fortunately, their ability to form spheroids was not affected. In addition, fluorescently labeled PEG-GRGDS was prepared and observed within live cells, and treatment to primary human hepatocytes was trialed to positive outcomes.

In Chapter 5, PG-treated cells were applied to decellularized liver tissue. Treated and untreated cells were seeded on model-size decellularized liver discs, where even after 24 h, mitochondrial activity was maintained for treated cells. When PG-treated cells were used for the recellularization of a miniature organ scaffold, the cells exhibited gene expression closer to that of healthy cells, showing that this technology could be applied to future studies aiming to improve the engraftment of cells into the liver template.

Finally, Chapter 6 summarizes the details of this study, providing a look into future prospects for this technology.