Emerging approaches to study cell-cell interactions

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Summary

This thesis describes approaches to study cell-cell interactions, and explores the exchange of membrane components. In the first part, the rate of lipid exchange was studied, both during natural cell-cell contacts and during prolonged enforced contacts. In the second part, lipidated coiled-coil peptides were used to study the behaviour and potential fusogenic behaviour of these compounds on cell-wall-deficient L-forms of bacteria.

Chapter 2 provides a thorough review of membrane component exchange in cells as well as chemical approaches to enhance cell-cell contacts. Examples of mechanisms of intercellular transfer, biological effects of intercellular membrane component transfer and chemical strategies to enhance cell-cell contacts are described and their strengths and limitations discussed.

Components from the cell membrane can relocate between cells both *in vitro* and *in vivo*. This exchange of molecules plays an essential role in the regulation of cellular interactions and responses to the cell's environment. However, the exact mechanism and functional outcome of these exchanges remain to be elucidated in many cases.

The dynamics and kinetics of membrane compound exchange critically impacts many different biological activities in cells including cell-cell recognition, energy production, signal transduction and conversion, cell adhesion and foreign molecule identification. In **Chapter 3** an adaptable strategy utilising flow cytometry is presented, in which labelled membrane components are used to quantify their exchange rates between mammalian cells in co-culture, investigate the interactions between glycans and membrane lipids, and understand the exchange mechanism.

Using this approach, the rate of sterol exchange was shown to be highly cell-line dependent and the rate of sialic acid containing component exchange significantly slower than that of the sterolic lipids. Moreover, in a different experiment, a non-exchanging cell line was forced to exchange both sterols and carbohydrates when brought into prolonged close proximity using complementary coiled-coils.

Chapter 4 provides future implications for and directions towards quantification and identification of membrane lipid exchange between immune cells. It presents an adaptable strategy based on trogocytosis assays in which fluorescent and clickable lipids are used to determine the membrane component exchange between antigen-presenting and T cells in co-culture. A range of sterols and aliphatic acids were screened and their effect on trogocytosis was determined. It is generally accepted that cell membrane lipids might not be equally transferred during cell-cell contact. However, there does appear to be a specific rate of selectivity in the classes of cell membrane components transferred from antigen-presenting cells to T cells.

The precise mechanism of exchange between immune cells has yet to be discovered, but likely requires the formation of a synapse after direct contact between donor and acceptor cells. These findings might lead to alternative approaches of the exchange of membrane lipids in immune cells, focusing on manipulating either the targeting lipids for restricting undesirable processes, in order to understand immunotherapies

Chapter 5 deals – for the first time – with the surface modification of living cell-wall-deficient bacteria (i.e. L-form), as a first step towards bacterial fusion, using cell-compatible coiled-coil peptides. The L-forms used in this study have been generated from actinomycetes, by inhibiting crucial steps in the biosynthesis pathway of the cell wall. A supramolecular method was developed, based on a complementary

coiled-coil-forming peptide pair, to functionalise the membranes of L-form bacteria in an efficient manner. Besides L-form surface modification, this chapter also concerns the viability and liposome docking as well as potential membrane fusion.

Due to the small size of the bacteria as well as light scattering limitations, it was difficult to distinguish aggregated from fused cells. To overcome these limitations in the current experimental set-up, a double antibiotic selection assay could be designed to select for fused L-forms. Additionally, coiled-coil peptides with different PEG-spacers, peptide length or lipid moieties might have a positive effect in L-form bacterial fusion. It is expected that this method will be able to induce fusion between L-form cells of distinct species, whereby the chemistry of two living cells is merged into a new cell. Successful fusion and regeneration of different actinomycetes strains at high frequency would facilitate the future discovery of new antibiotics and microbial strain improvement.

Chapter 6 presents the synthesis of a fluorescent alkyl phospholipid ether analogue and an initial study into whether this modified phospholipid is a potential tool for targeting prostate cancer membranes.

This phospholipid is used with current fluorescence imaging devices to visualise prostate cancer *in vitro*. For *in vivo* studies, a zebrafish xenograft model was used. Zebrafish xenograft models are suitable for live-cell imaging, allowing the examination of circulation and distribution of labelled compounds in real time. However, further studies should be undertaken including different cancer models as well as kinetic experiments to shed light on the docking mechanism of this ether phospholipid analogue.