Emerging approaches to study cell-cell interactions

Poulcharidis, D.

2

Chemical approaches to enhance cell-cell contact

2.1 Introduction to membrane component exchange in cells

Multicellular organisms form cell-cell interactions via direct interactions between cell membranes and play a vital role in cellular activity and development. Cells communicate with each other through these interactions and orchestrate the events that coordinate multicellular assembly.[1,2] Examples of cell-cell exchange include transfer via direct cell-cell contact,[3] exosome uptake[4,5] and membrane nanotubular formation.[4,6]

One particular method of cell-to-cell communication is the physical transfer of cell-surface components between adjacent cells, which plays a critical part in cellular communication responses. This was first described in the 1970s, when Bona *et al.* observed the transfer of lipopolysaccharide to lymphocytes from macrophages.[7] Recent examples have shown it to a be far more widespread phenomenon

than initially believed. In the immune system many cell-surface molecules are capable of being transferred between leukocyte subsets (Figure 1). For example, the trogocytosis[8] (from the ancient Greek word *trogo*, meaning 'gnaw') of co-stimulatory molecules of the antigen-presenting cell (APC) surface represents a key mechanism by which regulatory T cells attenuate APC function.[1,9] The trogocytosis process describes the plasma-membrane components transfer from one cell to the other. Furthermore, the same phenomenon between B-cells and lymph node-resident dendritic cells plays an important, yet poorly understood, role in the activation of humoural responses.[10,11] Membrane-component exchange is not exclusive to the immune system, with the exchange of membrane components also observed between endothelial cells and tumour cells as a further example.[12–15]

It has been shown that lipid components play an important role in cell-cell communications.[12,16,17] For example, lipid interactions were shown to be necessary for the intercellular communication between mesothelioma cells for exchanging cellular information and maintaining tumour organisation.[18,19] However, the exact role of (specific) lipids in the above-described trogocytosis events is relatively unknown.[20] In this chapter, membrane-exchange mechanisms will be discussed, as well as the chemical tools that have been developed to better study these processes.

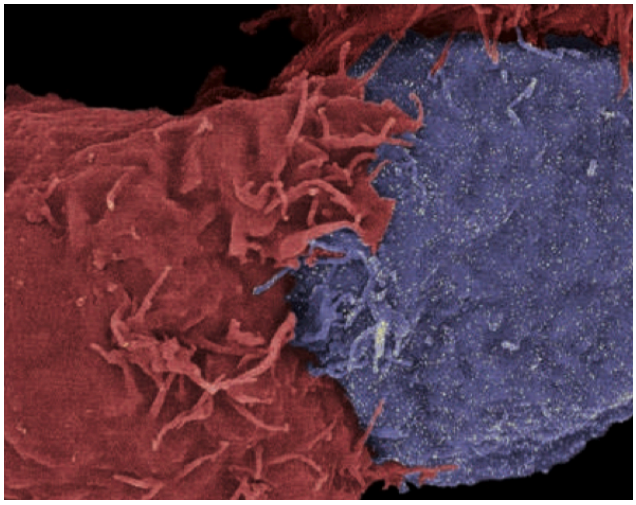

Figure 1 *Scanning electron microscopy (SEM) image shows a natural killer cell (red) grasping a target cell (blue). [Image reproduced from reference: D. M. Davis,* Nat. Rev. Immunol.*, 2009, **9**, 543–555]*

2.2 Mechanisms of intercellular transfer

The molecular mechanisms that control the transfer of membrane components remain unclear. At the cellular level, several mechanisms are thought to be responsible for facilitating the transfer of compounds between cells, such as a transfer via direct cell-cell contact, exosome exchange and nanotube formation (Figure 2).

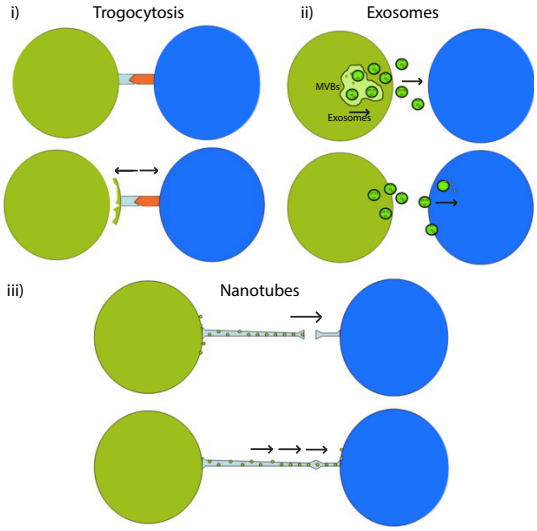

Figure 2 *Intercellular exchange of membrane components via trogocytosis transfer, exosomes and nanotubes. (i) Direct contact between cells allows the intercellular exchange of cell-surface-bound compounds. Different colours show membrane receptors that upon contact are "stealing" membrane pieces. (ii) Secreted exosomes carrying lipids, proteins and their signals can be transferred from one cell to another [where MVBs refers to multi-vesicular bodies]. (iii) Long membrane nanotubes extended between bordering cells support contact-dependent membrane component transfer from one cell to another. [Image reproduced from reference: O. Rechavi, I. Goldstein and Y. Kloog,* FEBS Lett.*, 2009,* ***583****, 1792–1799]*

Most forms of membrane component transfer appear to require cell-to-cell contact, suggested by the evidence that most transfer events are suppressed when cells are physically separated with a semi-permeable trans-well set-up.[21–23] This shows that direct contact facilitates the rapid transfer of cell-surface components (Figure 3).[24] For example, the formation of an integrin-mediated high-affinity synapse between cytotoxic T lymphocytes (CTLs) and a target cell is stabilised by the cytoskeleton.[25–27] Due to direct contact formation, membrane protein transfer between CTL and target was reported.[27]

In the immune system, this process was shown using live cell imaging. Wetzel *et al*. demonstrated that T cells, while immediately detaching from APCs, commonly acquire major histocompatibility complex (MHC) peptide complexes straight from the APC-side of the immunological synapse (IS).[28] B cells too can acquire proteins from T cells following synapse formation,[29] and even some tumour cell lines can take up patches of autologous membranes through synapse-like structures.[30] The transfer is rapid, with exchange occurring within minutes of synapse formation,[26,31] and the transferred molecules, such as lipids and proteins, commonly appear at the cell surface and are fully functional.

A different type of synapse, the 'stromal synapse', has also been reported in the immune system. It has an opposite function, namely to dampen the immune response in tissue.[32] It was observed between interstitial/stromal cells and was shown to establish close contacts. The exact role and importance of this immune dampening synapse are as yet unknown. What is known is that it can be triggered by costimulatory molecules and/or be dependent on the activation state of the donor and the acceptor (recipient) cells.[25,33,34]

Aside from the above-described trogocytic mechanisms, cells can also communicate through gap junctions (GJs). This mechanism – which is the formation of intercellular connections – allows not just the exchange of membrane components but also of cytosolic materials.[35–37] It plays a vital role in tissue maintenance and homeostasis. In the immune system, for example, it is one of the proposed mechanisms by which APCs acquire exogenous antigen.[38–40] However, it can be detrimental too, with this same gap junction-transfer between

melanoma and endothelial cells resulting in the killing of healthy neighbouring cells by autologous cytotoxic T lymphocytes.[41]

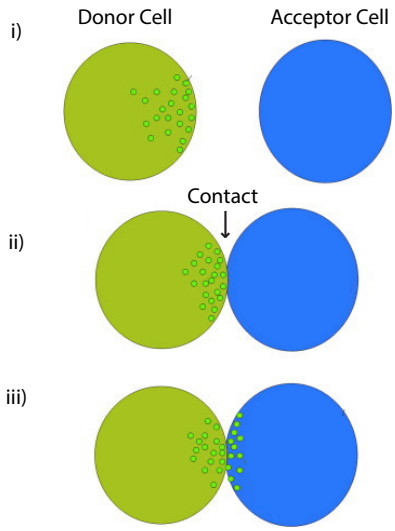

Figure 3 *Intercellular transfer of cell compounds. (i) A donor cell is expressing active compounds. (ii) The donor and the acceptor cell form a contact. (iii) During contact formation, the active membrane component is transferred to the plasma membrane of the acceptor cell from the plasma membrane of the donor cell. [Image reproduced from reference: O. Rechavi, I. Goldstein and Y. Kloog,* FEBS Lett.*, 2009,* ***583****, 1792–1799]*

A third cell-contact dependent method of membrane transfer between immune cells is through transient membrane nanotubular networks. Nanotubes are long, membranous tethers formed between cells either at the termination of the IS as the cells dissociate, or through an extension of membrane from one cell fusing to another,[42] although it is likely that these two processes are not mutually exclusive events. The formation of nanotubes has been detected in a wide variety of cells (Figure 4).[27,34,43] Rustom *et al.* have reported that membrane compounds can transfer directly between cells linked by tunnelling nanotubes.[43] In recent literature, these processes are mostly described interchangeably using the terms 'tunnelling nanotubes' (TNTs) and 'membrane nanotubes'.[34,44] Although the functional role for nanotubes is still under debate, studies have shown that nanotubes between myeloid cells can

mediate intercellular calcium alterations and thus cause phenotypic changes.[45] They can also enable the exchange of cell-surface membrane components as well as cytoplasmic content.[20,46] Studies using time-lapse imaging have shown membrane nanotube formation between B cells (B721.221) and T cells (Jurkat) following prolonged cell-cell contact, resulting in Ras-membrane protein transfer.[47]

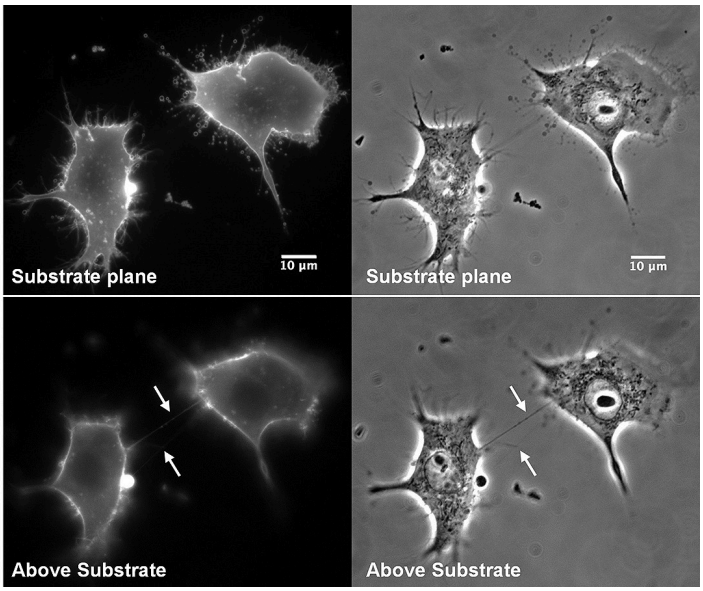

Figure 4 *Images are showing the formation of tunnelling nanotubes (TNT). The membrane of RAW/LR5 cells stained with the membrane dye FM1-43FX. The above images show cell substrates, and the lower images show the cell substrates where TNTs are formed. The arrows indicate the formed TNTs. Scale bars 10 µm. [Image reproduced from reference: K. McCoy-Simandle, S. J. Hanna and D. Cox,* Int. J. Biochem. Cell Biol.*, 2015, **71**, 44–54]*

Scanning electron microscopy (SEM) of cell-to-cell channelling showed that phaeochromocytoma (PC12) cells could form cellular TNTs[43] that provided a direct path for transporting signalling molecules between cells without escape into the extracellular milieu. The TNTs can be very long, even exceeding cellular dimensions, but their diameter is narrow (20 to 200 nm).[34] There are no known

biochemical markers for labelling tunnelling nanotubes. They can, however, be detected using high-resolution light and electron microscopy.[6]

Nanotube formation requires that cells be in close contact for a considerable length of time – if cells dissociate too quickly, nanotube formation does not occur.[48] Live cell imaging has shown that TNTs are ephemeral structures that last from minutes to hours.[49] For example, when immature neurons and astrocytes are co-cultured, after five hours, tunnelling nanotubes are formed, which disappeared after 24 hours.[49] This may reflect the amount of time required to create intercellular contacts of strong avidity which can withstand the forces required to draw out surface membranes as cells dissociate.[44] As a result, this likely eliminates the involvement of nanotubes as a mechanism for the fast transfer of membrane components between cells.

The final type of exchange mechanism that will be discussed does not rely on cell-cell contact. Instead, cells may use the secretion and uptake of exosomes to exchange material. Exosomes are tiny membrane vesicles with a diameter between 50-100 nm and are secreted by a variety of different cells.[4,50] They are formed by inward budding of endosomal membranes or multi-vesicular bodies (MVBs) within the cell and are secreted when the MVB fuses with the outer cellular membrane (Figure 5).[51] Exosome-mediated transfer is a relatively slow process but can occur over substantial distances.

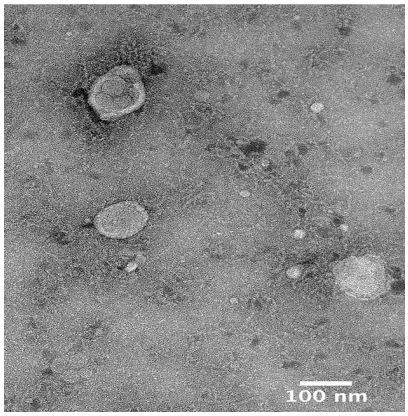

Figure 5 *Exosomes purified from mononuclear phagocytes as depicted by a transmission electron microscopy. Scale bar indicates 100 nm. [Image reproduced from reference: K. McCoy-Simandle, S. J. Hanna and D. Cox,* Int. J. Biochem. Cell Biol.*, 2015,* ***71****, 44–54]*

Exosomes can transfer membranes and encapsulating molecules that can be acquired and reprocessed by the cells that take them up.[52] For example, MHC-II-deficient dendritic cells can acquire MHC class II molecules from dendritic cell-derived exosomes, integrate these molecules into their membranes and subsequently trigger the proliferation of antigen-specific $CD4^+$ T cells *in vitro*.[53] Recently, it was demonstrated that exosomes could be secreted by T cells upon synapse formation with APCs resulting in miRNA transfer.[54]

In summary, it appears that tight junctions or intercellular nanotubes formed between two cells play an essential role in the transfer of membrane compounds, with the strength of the interaction often determining the amount of membrane transferred.[55,56] However, the exact mechanism may vary with the nature of the cells and intercellular exchange process involved.

2.3 Biological effects of intercellular membrane component transfer

The exchange of membrane components can result in biological effects either because the donor cells are stripped of molecules from their membrane or because the acceptor cells get components which they can use. In both ways, cells acquire or lose components which may alter their cell-cell interactions.[1] For instance, the regulatory activity of lymphocytes might be changed when, during cell-cell communication, cellular components are exchanged. Moreover, the exchange of highly energetic components such as lipids has been reported to affect cell proliferation.[57] Kedl *et al.* have shown that dendritic cells acquire antigenic-MHC complexes from T cells through a direct membrane-exchange mechanism affecting the T-cell maturation.[58] Moreover, the antigen-complexes exchange between dendritic cells can also reactivate memory T cells.[59] Finally, another consequence of cell-membrane component exchange has been reported by Qureshi *et al.* is the transfer of costimulatory molecules from T cells to dendritic cells which regulates the T-cell response.[60]

The exchange of lipids can also play an essential role in the development of diseases. For example, the cholesterol-glycolipids and the cholesterol from *Borrelia burgdorferi*, the causative agent of Lyme disease, are vital for bacterial life cycle and can control the interactions between the eukaryotic host's cells and the bacteria itself (Figure 6).[61] Benach *et al.* demonstrated that *B.burgdorferi* stripped off the cholesterol and cholesterol-glycolipids from the membrane of eukaryotic HeLa cells via a contact-dependent mechanism.[61] This was also shown by Norris *et al.* for *Treponema pallidum*, the agent responsible for syphilis. It may directly obtain specific membrane components from host cells, such as lipids.[62] These are important examples of lipid-exchange because neither of these bacteria can

synthesise these fatty acids.[63,64] In both examples, the two-way lipid exchange between pathogens and host cells can occur either through the release of exosomes or by direct contact between the bacteria and eukaryotic cell.

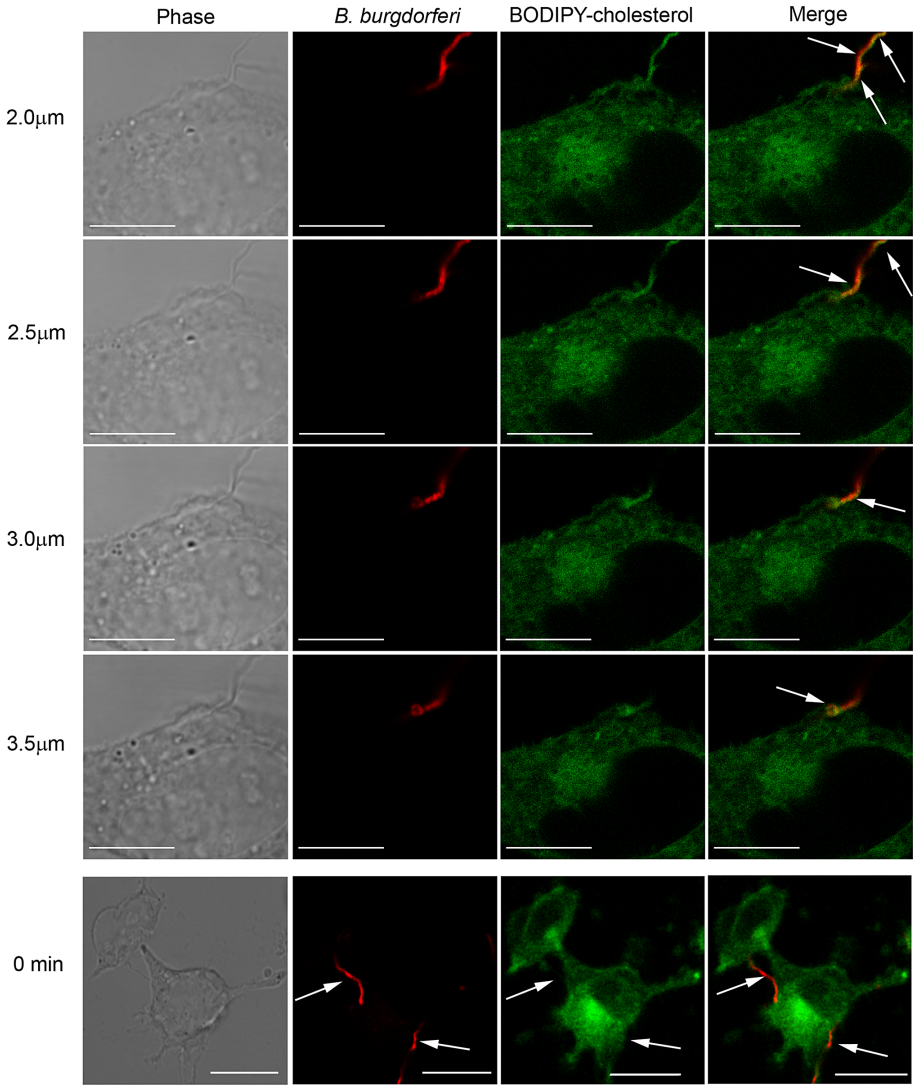

Figure 6 *Borrelia burgdorferi (red) was incubated with bodipy-cholesterol labelled HeLa cells (green). Confocal microscopy showed the co-localization (yellow, arrows) of bodipy-cholesterol (green) and Borrelia burgdorferi (red) at the point of attachment after 1 h of incubation. Scale bars = 10 μm. Confocal microscopy images taken at the beginning of the experiment, 0 mins (bottom), showed no colocalization (yellow) of*

bodipy-cholesterol (green) between labelled HeLa cells and Borrelia burgdorferi (red). The arrows point to the lack of co-localization. Scale bars = 20 µm. [Image reproduced from reference: J. T. Crowley, A. M. Toledo, T. J. LaRocca, J. L. Coleman, E. London and J. L. Benach, PLoS Pathog.*, 2013, **9**, e1003109]*

2.4 Chemical strategies to enhance cell-cell contacts

Due to the importance of cell-cell interaction and exchange in the eukaryotic life cycle, various methods to chemically control cell-to-cell contacts have been developed. These are based on approaches such as genetic engineering, chemical cell modification, metabolic engineering and many others, and will be discussed below.

Genetic modification has been used to alter cell surfaces to affect extracellular communication. For example, Mrksich *et al.* created a new specificity for the binding of the αβ integrin-receptor to the extracellular matrix (Figure 7). They created a chimeric receptor containing β1 integrin, an extracellular domain from fractalkine, and a carbonic anhydrase IV (CAIV) domain at the end (the CAIV protein binds explicitly to benzenesulfonamide (BzS) ligands in order for the whole complex to bind in an extracellular matrix).[65,66] As a result, cells expressing the modified receptor could spread and attach selectively to immobilised substrates.

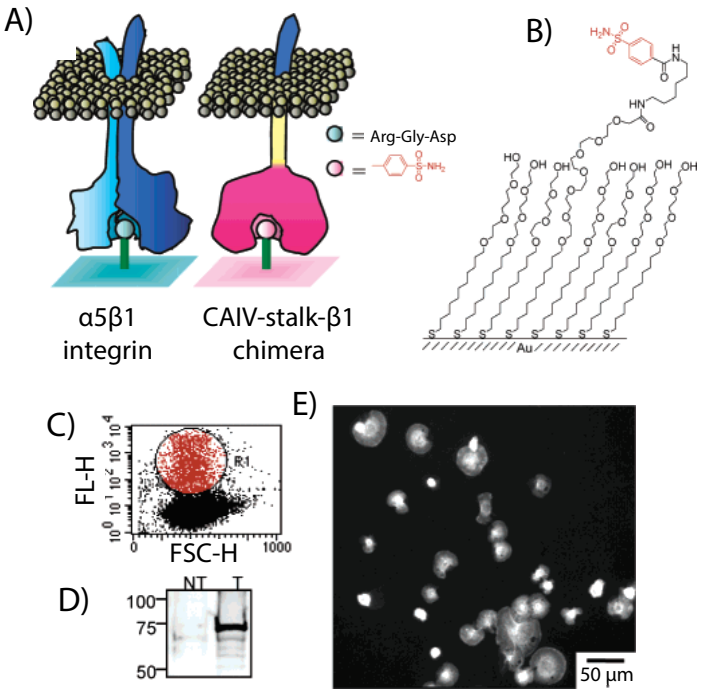

Figure 7 *Schematic illustration of the native and chimeric integrin. (A) The native α5β1 integrin (left) binds the Arg-Gly-Asp ligand. A chimeric receptor (right) was created to keep the transmembrane and intracellular domain of the β1 integrin (blue) and to contain extracellularly an enzymatic domain of carbonic anhydrase IV (CAIV) (pink) which is targeted by a benzenesulfonamide. (B) a modified gold surface presenting benzenesulfonamide. (C) A flow cytometry plot of cells expressing the chimeric receptor (red dots). (D) Western blot analysis with an anti-CAIV antibody. (E) Cells (stained with phalloidin-TexasRed) expressing the chimeric receptor attach on a modified gold surface containing benzenesulfonamide. [Image reproduced from reference: M. Kato and M. Mrksich,* J. Am. Chem. Soc.*, 2004, **126,** 6504-5]*

Chemical handles have also been introduced using a genetic engineering approach. Ting *et al.*, for example, produced cells expressing the epidermal growth factor receptor (EGFR) fused to a biotin ligase recognition sequence.[67,68] This peptide (GLNDIFEAQKIEWHE) – upon addition of a biotin ligase – could be site-specifically biotinylated, which in turn allowed a subsequent cross-linking with ligation to streptavidin.

Covalent conjugation of functional groups on the cell surface has also been used to alter cell-cell contacts. Sulfhydryl, amines, carboxyl and carbonyl groups, and carbohydrate groups have all been used for functionalisation. The chemical reactions on the cell surface should be orthogonal and performed under moderate conditions. There are a well-established and wide variety of protocols for the chemical modification of amine groups under mild conditions and with readily available linkers. For example, a popular reaction to modify primary amines is the reaction with N-hydroxysuccinimide (NHS) ester, which has been used extensively for surface modification with molecules such as PEG[69] and biotin[70,71].

The latter has been used extensively to manipulate cell-cell contacts. The cells are biotinylated with an NHS-biotin conjugate before the addition of streptavidin (Figure 8). The streptavidin serves as a ''bridge'' to connect the cells with a biotinylated ligand, thereby altering the behaviour of the cell. In this manner, cell surfaces have been functionalised with antibodies recognising epithelial growth factor receptor (EGFR)[72,73], as well as P-selectin-binding aptamers.[74,75] These surface-modified cells were then able to interact with EGFR-expressing HEK293T and HeLa cells, [72] and selectin-expressing cells *in vitro*[74], and *in vivo*[75,76]. Streptavidin has also been used to cluster and layer cells in specific orientations.[77–79]

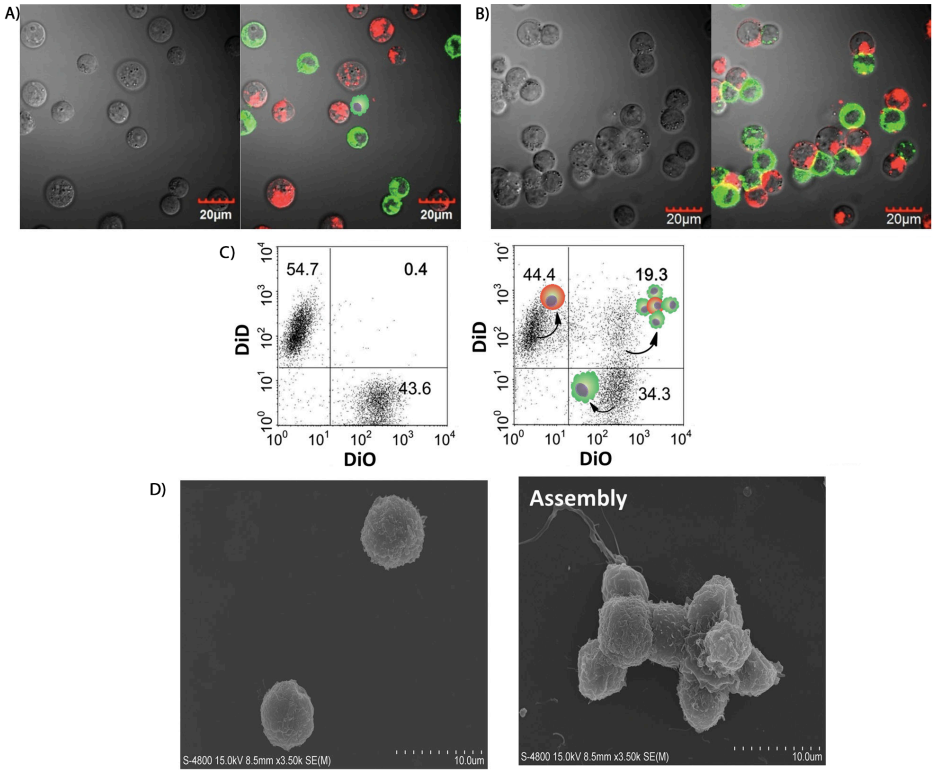

Figure 8 *Examples of surface modification using NK-streptavidin with Jurkat-biotin. Confocal laser scanning microscopy (CLSM) images of non-assembled cells (A), and of (B) assembled cells after surface modification and mixing Jurkat-biotin cells with NK-streptavidin at a 1:1 ratio. (C) Flow cytometry scatters. NK cells were labelled with DiO fluorescent dye, and Jurkat cells were labelled with DiD dye before and after modification and assembly. (D) SEM images of Jurkat-biotin cells and a multicellular cluster (mixed at a 1:1 ratio). [Image reproduced from reference: B. Wang, J. Song, H. Yuan, C. Nie, F. Lv, L. Liu and S. Wang,* Adv. Mater., *2014,* ***26****, 2371–2375]*

Although these chemical approaches are encouraging, it is still challenging to govern cell-cell interactions in time and space. To overcome these concerns, there are opportunities for enhancement by designing novel protein engineering, bioconjugation, material science and chemistry approaches for cell-surface engineering.[80–82]

One set of approaches relies on the fusion of liposomes with cells.[83–85] In order to present functional groups on the cell surface, specific liposomes containing new functional groups are incubated with cells, leading to fusion of the liposomal membrane and the cell membrane. This methodology was used, for example, to fuse biotinylated liposomes with the membrane of mesenchymal stem cells (MSC) to yield biotinylated MSCs.[76] Yousaf *et al.* used the approach to introduce a selectively ligatable handle into the cell: liposomes consisting of either O-dodecyloxyamine or dodecanone were fused with cell membranes to yield membranes carrying oxyamine or ketone functionalities, which could be ligated through oxime formation (Figure 9).[84,86]

Carbohydrates have also been used to modify the cell surface with selectively ligatable functionalities in a liposome system. Chen *et al.* used liposomes loaded with modified sugars, which are metabolically incorporated into cell-surface glycans that would allow specific modification of the surface glycans.[65,87–89]

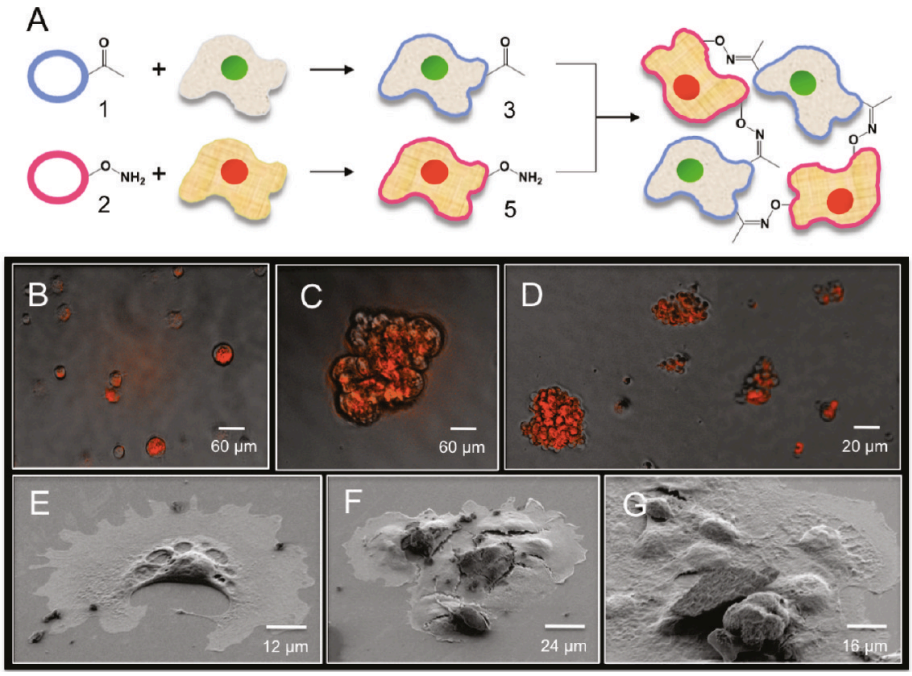

Figure 9 *Scanning electron micrographs and fluorescent images depicting the formation via liposome fusion of 3D populations. (A) Fibroblast populations were cultured separately either with ketone- (1) or oxyamine- (2) containing liposomes, resulting in membrane fusion and creation of ketone- (3) and oxyamine- (5) tethered fibroblasts. The nucleus of the oxyamine-tethered fibroblasts (5) was labelled with m-cherry, whereas the ketone-tethered fibroblasts (3) were unlabelled. After mixing these cell populations for 3 h, clusters and tissue-like forms occurred, due to oxime ligation. (B) Control experiments show the absence of cluster creation for cells which did not contain either oxyamine or ketone handles. (C and D) Images from co-cultured ketone- (3) and oxyamine- (5) tethered fibroblasts showing the formation of interconnected spheroid assemblies. (E-G) SEM images are displayed by control cells (E) and spheroid assemblies (F and G). [Image reproduced from reference: D. Dutta, A. Pulsipher, W. Luo and M. N. Yousaf,* J. Am. Chem. Soc., *2011,* ***133****, 8704–8713]*

A standard method for cell-surface modification is the treatment of cells with labelled metabolites, which are incorporated into the membrane. For example, Iwata *et al.* incubated cells with membrane lipids linked to DNA strand.[90] Introduction of a complementary strand on a second cell population created a forced interaction between the two cell types. Similarly, Gartner *et al.* manipulated the adhesive characteristics of cells using membranes modified with ssDNA oligonucleotides (Figure 10).[91] Bertozzi *et al.* also used this approach to

present synthetic glycolpolymers that mimic cell-surface glycoproteins. These are responsible for controlling cell-cell interactions.[92] The cell-assembly kinetics were controlled by adjusting the complexity of DNA sequences and enabled the formation of 3D microtissues with determined cell composition and ratio.

The glycans present on the surface of many cell types have also been used for introducing chemical functionality at the surface of cells. Bertozzi *et al.* described a method whereby they incubated cells with labelled precursors of sialic acids. These precursors containing either a ketone or azide functionality were taken by cells and incorporated to the cell surface glycome in order to get labelled.[93] This method was used by Francis *et al.* to introduce the ssDNA strands.[94,95] A modified oligonucleotide sequence (i.e. ssDNA) was first synthesised bearing a phosphine group. Via a Staudinger ligation the phosphine-ssDNA conjugation was then obtained with the azide to form the amide-link for further modification.[96]

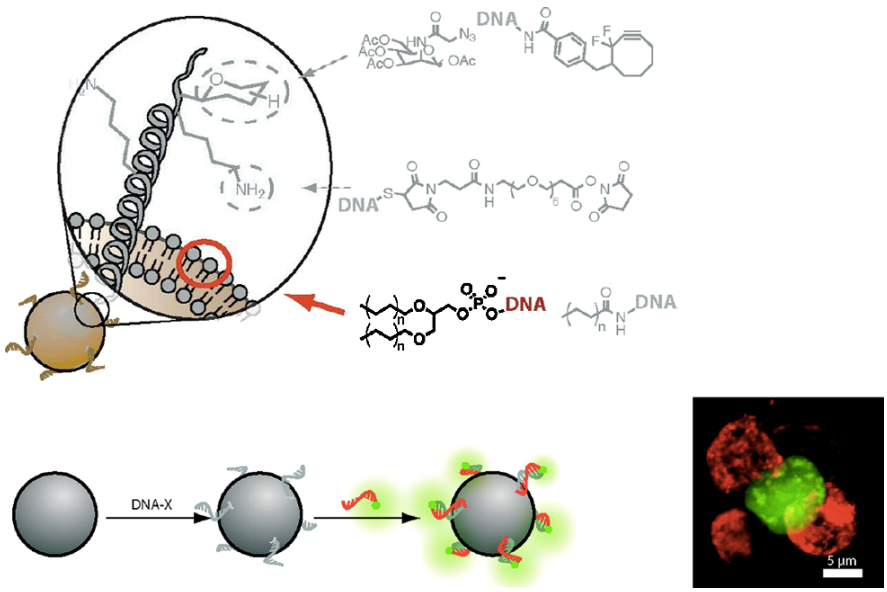

Figure 10 *Cell-surface glycans or protein lysine side chains or fatty acid amides are targeted for chemical remodelling and incorporation of oligonucleotides to cell surfaces. [Image reproduced from reference: N. S. Selden, M. E. Todhunter, N. Y. Jee, J. S. Liu, K. E. Broaders and Z. J. Gartner,* J. Am. Chem. Soc.*, 2012, **134**, 765–768]*

Sugimoto *et al.* reported a different approach: by incubating cells with methacryloyl-modified N-acetylmannosamine (ManM), they could introduce methacryloyl groups in surface sialic acid of macrophages.[97] Via a light-assisted thiolene reaction, the ManM groups were coupled with a thiol-terminated nucleic acid aptamer, targeting protein tyrosine kinase-7 (PTK7). Upon co-culture of the cells with a PTK7-positive lymphoblastic leukaemia cell line (CCRF-CEM; Figure 11), cell adhesion was observed, and the cancer cell population was reduced.[97]

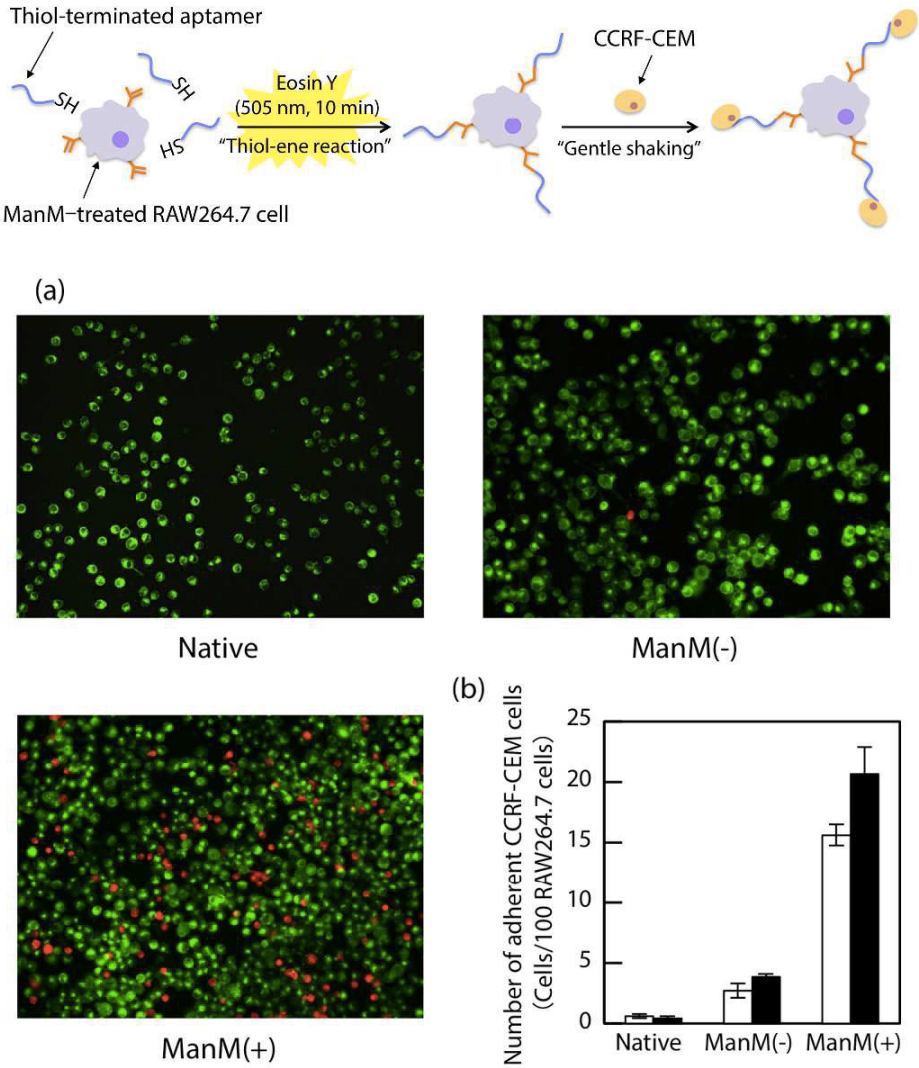

Figure 11 *The surface of RAW264.7 macrophages was modified with a nucleic acid aptamer and bound with a CCRF-CEM cancer cell. (a) Fluorescence images of co-cultured human CCRF-CEM leukaemia lymphoblasts (red) is adjacent to murine RAW264.7 macrophages (green). (b) Number of adherent T lymphoblasts on 100 macrophages. [Image reproduced from reference: S. Sugimoto, R. Moriyama, T. Mori and Y. Iwasaki,* Chem. Commun.*, 2015,* ***51****, 17428–17430]*

Supramolecular host-guest chemistry methodology has garnered widespread attention as a tool for controlling molecular recognition.[98] The aim is to create cell-cell contacts through the introduction of supramolecular interactions using host-guest motifs. Ideally, the guest molecules have a high affinity and selectivity for their host molecules and the pair make non-covalent, stable and inherent reversible supramolecular complexes in biological systems.[99] One such pair is based on phospholipidylated or cholesteryl-modified cyclodextrin. These guests could insert into phospholipid membranes and maintain the ability of the cyclodextrin group to receive guest molecules.[100-104] Alternatively, Peng Shi *et al.* modified the membrane of MCF-7 (human breast) cancer cells with β-cyclodextrin using a bioorthogonal labelling approach. Cell-cell interactions were then switched on by the addition of a divalent guest molecule that could bridge two different host molecules (Figure 12), or a cell decorated with guest molecules.[103,104] In the example mentioned above, the β-cyclodextrin decorated MCF-7 cells were co-cultured with azobenzene-decorated peripheral blood mononuclear cells (PMBCs), which resulted in the adherence of the PMBCs to the MCF-7 cells.

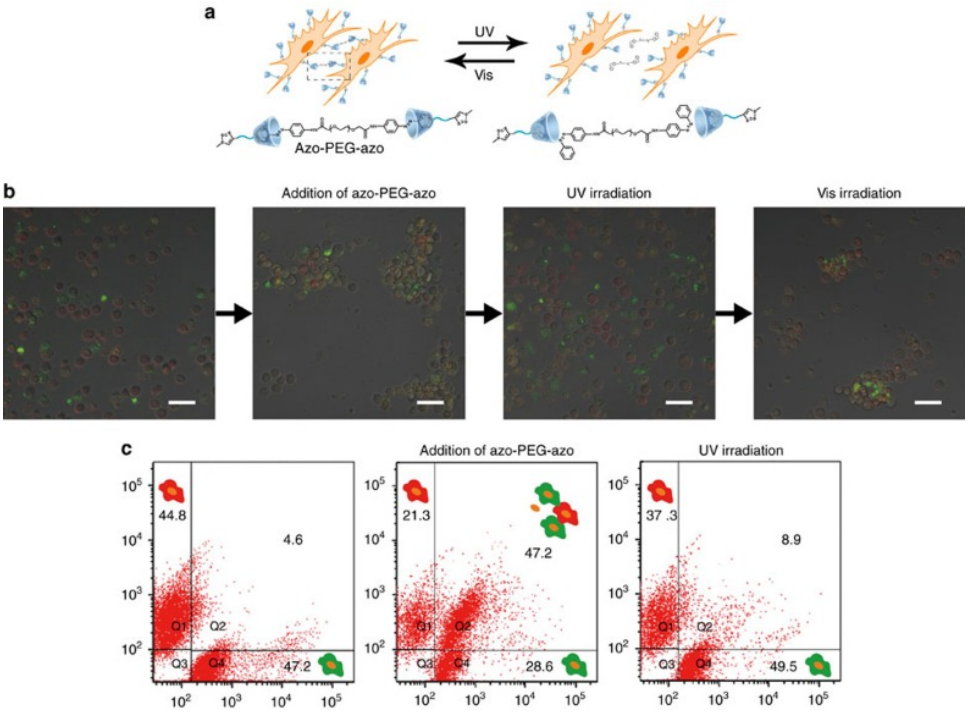

Figure 12 *(a) Schematic representation showing reversible cell-cell contacts. (b) Confocal microscopy images and (c) flow cytometry analysis of β-CD-modified cells under different conditions. Scale bars, 50 μm. [Image reproduced from reference: P. Shi, E. Ju, Z. Yan, N. Gao, J. Wang, J. Hou, Y. Zhang, J. Ren and X. Qu,* Nat. Commun.*, 2016, 7, 13088]*

Coiled-coil motifs are comprised of interacting α-helical peptides. On a subcellular level, this is one of the most common connecting motifs in nature.[105–107] These α-helices are stabilised by hydrogen bonds along the polypeptide chain due to a patterned arrangement of charged and hydrophobic residues. In nature, coiled-coil peptides present in around 10% of the eukaryotic proteome[108] and control a variety of cellular behaviours, such as transcription,[109] protein complexes assembling,[106] intracellular trafficking[110] and viral infection[111,112]. Coiled-coil motifs therefore offered a potent possible target for mediating intercellular interactions. Kros *et al.* have reported the

incorporation of lipid-modified coiled-coil forming peptides K and E (Figure 13) into eukaryotic membranes *in vitro*, but also in living zebrafish larvae.[83,113,114]

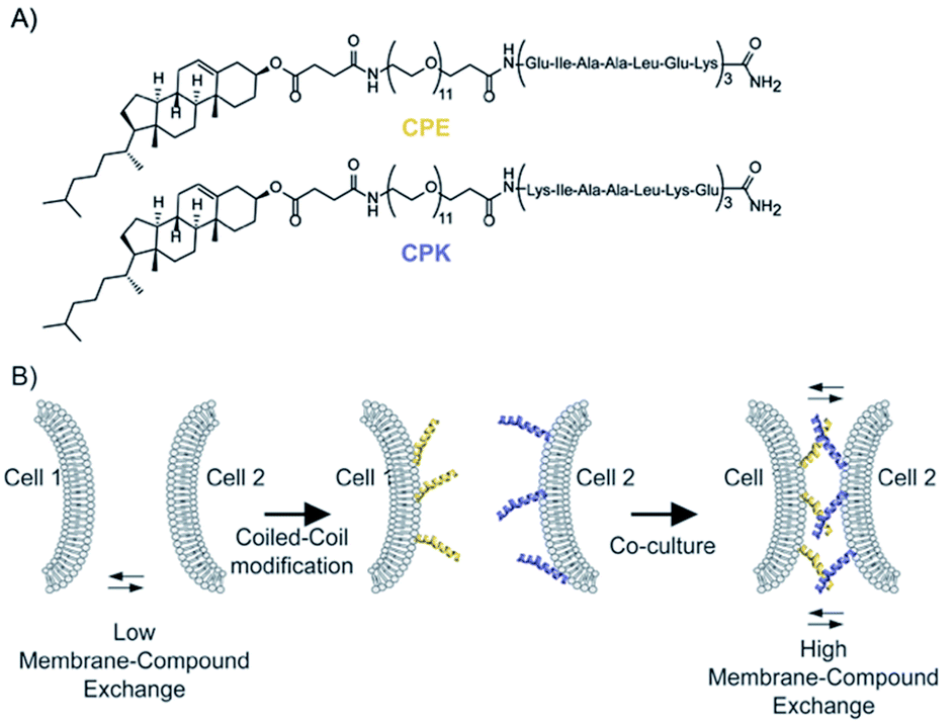

Figure 13 *(A) Structures of cholesterol-modified E_3 (CPE) and K_3 (CPK) peptides. (B) Schematic representation of coiled-coil formation and its use to prolong cell-cell contact, thus enhancing membrane component exchange.*

2.5 Conclusions

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

Chemical approaches to control such cell-cell interactions have been developed, which will aid in the understanding of the role of these contact and exchange mechanisms between cells. However, in the area of cell surface engineering, many challenges remain, such as the development of mild, cell-compatible reactions and interactors. The extremely dynamic nature of the cellular membrane also complicates factors. Nonetheless, cell membrane engineering appears to be the most robust methodology to manipulate cell properties and is associated with developing beneficial present and future cell-based processes.

2.6 References

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