Express delivery

Good things come in small packages!

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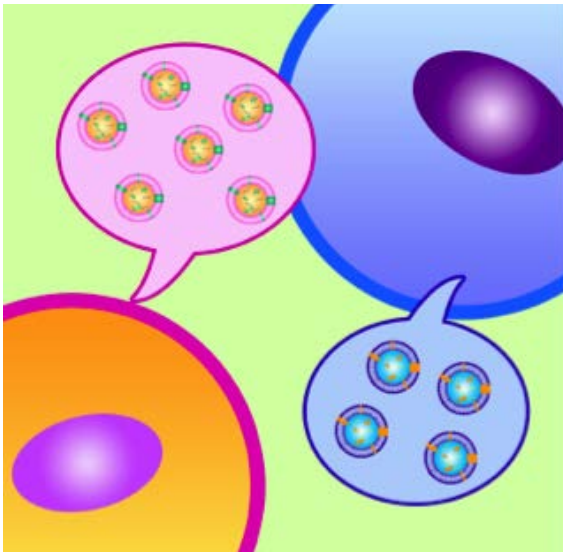
Key words
cells
communication
communication
microscopy

Have you ever waited all day for a parcel to arrive? Looking out of the window every five minutes in hope of a glimpse of the postman, you refuse to leave the house and watch the clock as the minutes slowly tick by. Parcels are also delivered to cells inside the body, but your cells do not wait all day; little packages, scientifically referred to as exosomes, are constantly being delivered to cells. The time between release of exosomes from the cells where they are produced to their delivery to recipient cells can be as short as 30 minutes. Exosomes are carried in biological fluids, most commonly in the blood.



Different types of cell produce different types of exosome.

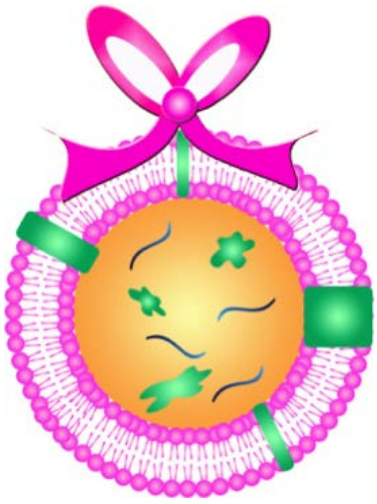
Small packages

In order to maintain life it is vital that all 37 000 000 000 000 (37 trillion) of our cells communicate with each other. There are many processes through which this occurs. Hormones and neurotransmitters are the best described forms of intercellular communication; however exosomes also participate substantially in cell-to-cell signalling. Unfortunately, due to their excellent communication ability, exosomes also support disease development.

Exosomes are approximately 30-120 nm in diameter; this is 20 times smaller than bacteria, about the same size as a virus. In order to examine exosomes they are extracted from biological fluids by ultracentrifugation for at least 60 minutes at 100 000 *g* (*g* = acceleration due to gravity).

Wrapping paper

Exosomes are delivered to neighbouring cells or distant organs in the same way that packages can be distributed both nationally and internationally. In same way that we might wrap a parcel with packaging suitable for the recipient – for example, (excuse the stereotypes!) pink wrapping paper for girls and blue wrapping paper for boys – exosomes do the same. They encase their cargo in a lipid shell which displays different proteins on the surface to ensure interaction with the correct recipient cell.



An exosome is a package delivering material from one cell to another. Its outer membrane is a double layer of lipid molecules.

Unlike most good delivery services though, it is likely that only a small percentage of exosomes reach their target cell. For this reason excessive numbers of exosomes are released to maximise the chance of signal transfer. Some exosomes may carry messages suitable for receipt by more than

one cell type so their surface may be less specific; comparable to Christmas wrapping paper – suitable for all.

It is also important that the contents of the parcel are protected. The exosomal membrane is less fluid and more stable than the cell membrane despite being made of the same components. This is due to the rigidity of the exosomal membrane caused by enrichment in cholesterol and lipid raft domains which are compact structures made of densely packed lipids and proteins; they reside on the plasma membrane (which is much more fluid structure) much like boats (or rafts, hence the name) out at sea. This is thought to help exosomes stay intact and maintain their spherical structure, even in harsh conditions, such as high or low *p*H, that they often become exposed to during transit.

Packing a parcel

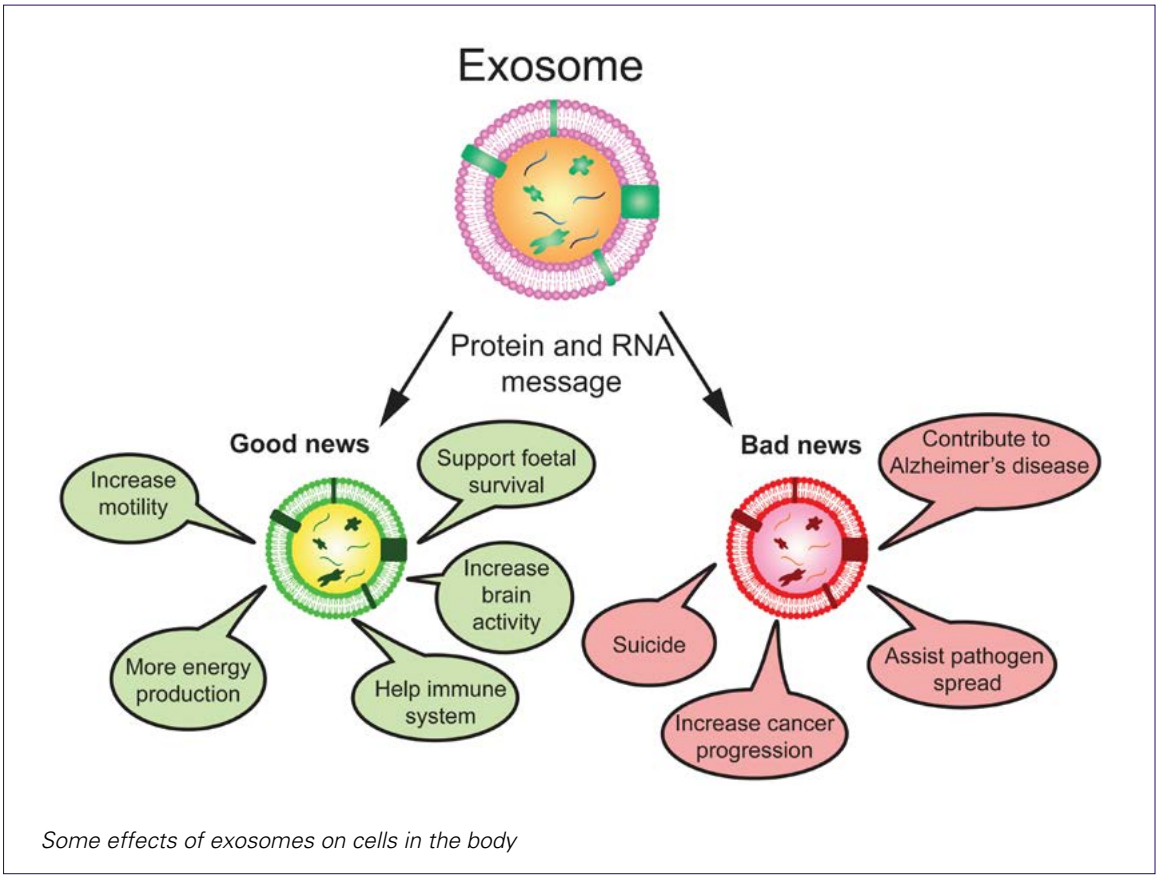
Exosome assembly is not a process that occurs at random. Exosome cargo is carefully selected during biosynthesis. Proteins including Alix and heat-shock protein 70 (HSP70) are exosomal markers. Markers are proteins that are used to identify biological structures because they are known to be associated with that structure. The contents are often representative of the cell of origin, but are also tailored to the recipient cell.

Once the exosome content has been selected, the endosomal sorting complex required for transport (ESCRT) machinery and associated proteins begin to fold the endosomal membrane inwards, encasing the selected cargo. This process forms intraluminal vesicles (immature exosomes) that remain held inside the endosome (a component of the cell responsible for transportation of molecules). When the endosomal membrane fuses with the plasma membrane, exosomes are released into the external environment, ready to interact with target cells.

Good news, bad news?

Our actions, as a result of receiving a parcel or letter, differ, depending upon its contents. In the same way, a cell changes its activity depending upon the exosome message it receives. Exosomes carry messages in the form of proteins and ribonucleic acids (RNA). These molecules have the potential to alter gene expression, and hence protein synthesis, in the recipient cell which can dramatically change its characteristics. For example, the cell may decide to move or make more energy or even commit suicide in response to an exosomal message.

Exosomes deliver good news in many situations. For example, they assist with identification of invading microbes in the immune response, contribute to brain development, and support foetus survival in pregnancy. However exosomes can also deliver bad news. They facilitate development of numerous diseases. Exosomes encourage cancer progression by promoting tumour development growth. They also assist pathogen spread during infection and contribute to the development of Alzheimer's disease. Additionally, exosome concentration in the biological fluids of disease sufferers is elevated. This increase in exosome numbers also contributes to development of disease and hence may one day assist with disease diagnosis.

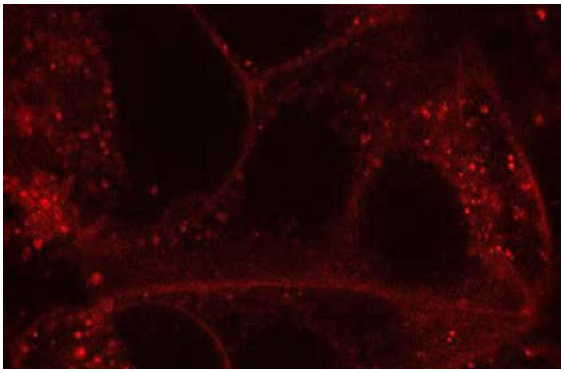


Some effects of exosomes on cells in the body

Despite significantly contributing to disease development it is important to note that no diseases to date have been found to be caused by 'bad' exosomes. It rather appears that 'bad' exosomes are a consequence of disease. Because of their unique durability and transport capabilities, exosomes show great potential as future therapeutic drug vehicles. I sincerely hope that one day it will be possible to isolate exosomes from a patient, manipulate their external appearance (to ensure they target specific tissues), and load them with relevant therapeutics that will fight disease upon re-administration to the patient.

What next?

In my current research, I am using fluorescent lipid stains to label the lipid-rich membranes of exosomes. Using a confocal microscope at ×630 magnification, I am able to watch exosomes enter cells (see Box). I plan to test a range of chemicals to see which ones are able to enhance or prevent exosomes from communicating with cells. Recently, for example, it was shown that heparan sulphate proteoglycans may help exosomes to enter mammalian cells.



Fluorescent exosomes inside cells, magnification ×630.

Summing up

Exosomes provide an 'express' delivery service from one cell to another; they can transport messages in just 30 minutes. They help regulate homeostasis which controls vital biological processes; in fact, it is highly likely that without exosomes human beings would not be able to maintain life, so we really have a lot to thank them for.

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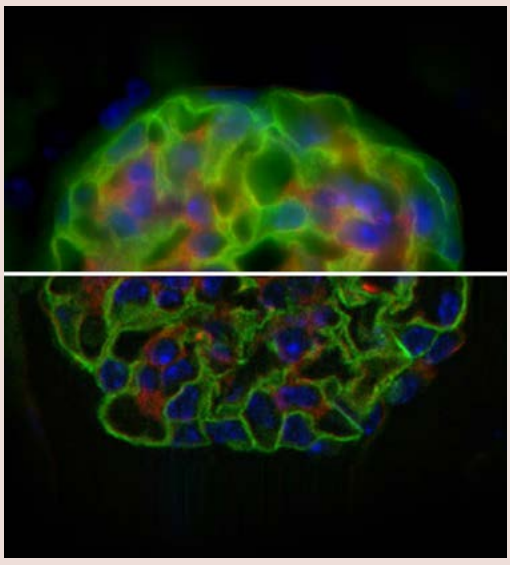
Confocal microscopy

Conventional microscopy often gives rise to blurred images due to light from regions outside the focal plane of the microscope. In fluorescence microscopy, where molecules are labelled in order to see where they end up, as here on a cell, it is important that the image shows only a restricted region. Conventional microscopy gives a haze around the image, whereas with confocal microscopy, this is largely removed, giving a much clearer image.



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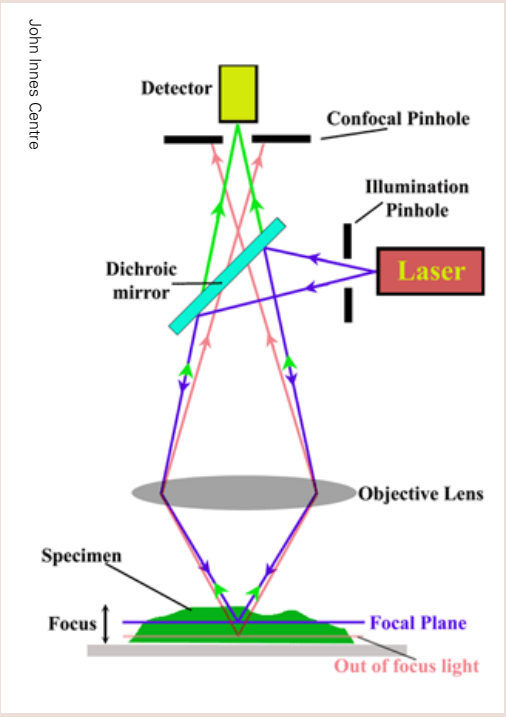
Mouse kidney section seen with a conventional light microscope (top half) and with a confocal microscope (bottom half). In this image, cell nuclei are blue.

How does a confocal microscope work? The object is viewed through a pinhole so that only a tiny area, at the focal point, is seen at any instant. Light coming from other areas of the object is blocked. The microscope scans across the object and gradually builds up a clear image on a screen.



How a confocal microscope works: only light coming from one point on the specimen (green rays) reach the detector. The confocal pinhole blocks light from out-of-focus points (brown rays).