

A new trick for an old delivery system

Yvonne Perrie and Alan Smith, at the medicines research unit, Aston University, and Jonathan Harris and Richard Shelton, at biomaterials unit at the school of dentistry, University of Birmingham, explain why they have been developing photosensitive liposomes

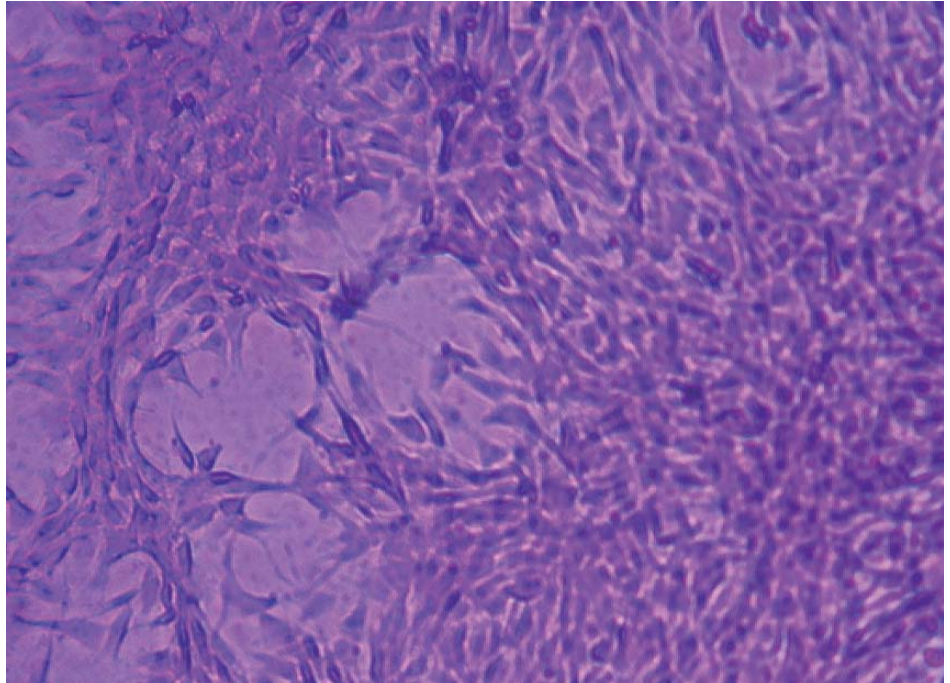
The use of liposomes has spread wider than drug delivery to more unusual settings, such as ripening cheese, producing hydrolysed-lactose milk and extracting oil from wells (*PJ*, 24/31 December 2005, p 809). Now liposomes are being applied to tissue engineering — growing tissue from cells, using engineering materials and biochemical factors.

With the shortage of donor organs, tissue engineering offers the opportunity to create functional replicas of failing or damaged tissues and, perhaps, the possibility of overcoming problems of tissue rejection. To grow tissue, a small sample of cells can be harvested from a patient and cultured in the laboratory. These cells are then implanted (seeded) into an artificial structure — called a scaffold — where the tissue is grown. This regrown tissue can then be used in the patient. In this way, tissue engineering has been used to replace tissues, such as skin and cartilage, and this year the *Lancet* (2006;367:1241–6) reported that scientists had successfully tissue engineered autologous bladders for patients needing cystoplasty.

A range of materials has been tested as possible scaffolds for growing tissues, including hydroxyapatite, bioglass and various porous polymers. These materials need to allow cell attachment and migration, and diffusion of nutrients and waste products, so they need to have a high porosity. In addition, they need to be biodegradable, so that they do not need to be removed once the tissue has grown and, ideally, they should degrade at a rate that matches the rate of tissue formation so that it continues to provide structural integrity until the new tissue is able to do this. It is also important for the scaffold to allow the new tissue to grow in a way that means it is fit for purpose. For example, bone cells must be encouraged to grow into an organised structure that can bear mechanical loads appropriately. In some cases where a piece of new bone tissue may be needed, the scaffold is made into the desired shape, seeded with cells and growth factors, and implanted in the patient.

Unfortunately, the harsh processing conditions associated with constructing scaffolds from many of the materials above mean that cells must be seeded after the scaffold has been formed. For example, some processes require temperatures that cause cell death. This is a significant limitation because, at this stage, it is difficult to achieve an even distribution of cells throughout a scaffold.

Recently, however, hydrogels (such as alginates) have been shown as effective cell culture substrates for three-dimensional structures. Alginates are linear, water-soluble



Bone marrow cells growing on 3 per cent G-type alginate after six days, stained with toluidine blue

polysaccharides extracted from brown seaweed and are composed of alternating blocks of 1–4 linked α -L-guluronic and β -D-mannuronic acid residues. Alginates are anionic and form viscous solutions at low concentrations in water and, in the presence of di-valent metal ions such as calcium, they form transparent gels.

The advantage of using a hydrogel as a scaffold, compared with other materials already described, is that cells can be seeded within a solution of the hydrogel. Gelation can then be triggered in a controlled manner that does not compromise the cells, resulting in the creation of cell population immobilised within a 3D-structure rather than on a surface.

Use of liposomes

For their role in site-directed drug delivery, liposomes have been designed to release drugs in response to stimuli (eg, light), and at different pHs or temperatures. To develop 3D-cell constructs, similar liposomes can be triggered to release metal ions to stimulate cross-linking and gelation of the alginate.

Of the trigger-sensitive liposome systems available, light activation is attractive because it provides a broad range of adjustable parameters (eg, wavelength, intensity, duration). A range of excipients can be incorporated within the bilayer of liposomes to make them photo-reactive. For example, the photochromic phospholipid 1,2-bis(4-(n-butyl)-

phenylazo-4-phenylbutyryl)phosphatidylcholine (Bis-Azo PC) has a stable trans-isomer conformation that can be easily incorporated within a closely packed liposomal bilayer. When hit with long wavelength ultraviolet light the phospholipid changes to its bulky cis-isomer (photoisomerisation) and this destabilises the liposomal bilayer sufficiently to cause the release of trapped solutes.

Using Bis-Azo PC, we have been able to formulate calcium loaded photosensitive liposomes that can be mixed with an alginate solution containing cells. By stimulating the photosensitive lipid using a light emitting diode we can trigger release of liposome-entrapped calcium chloride, resulting in cross-linking of the alginate and immobilisation of bone-derived cells over a range of seeding densities (1.25×10^6 to 5.0×10^6 cells per ml), approximately 97 per cent of which remains viable for up to 14 days in culture.

New drug delivery systems continue to be derived and investigated for a plethora of applications, yet liposomes remain a key interest for many pharmaceutical scientists. The technical aspect of drug entrapment within liposomes has always been simple; the difficult part appears to be getting them off the bench and developed into usable products. With the ever increasing developments within the various scientific fields, however, it is clear that there is still much that liposomes have to offer.