

Advances in polymer scaffolds for tissue engineering

Two very different, but equally inventive, methods of preparing drug-containing porous materials have been reported by Professor Kevin Shakesheff of Nottingham Universities' School of Pharmaceutical Sciences. Along with co-workers from the University of Nottingham and the University of Southampton, Shakesheff has developed polymer networks which contain biologically active macromolecules in their pores. Such materials are currently finding use in the field of tissue engineering, where the delivery of nutrients and growth factors is crucial to the success of treatment. A major obstacle so far in the preparation of these types of polymer materials has been the sensitivity of biological intercalates to heat and/or organic solvents. The methods described by Shakesheff *et al* aim to overcome these problems.

The authors' first approach, published in *Advanced Materials* in December 2002, uses supercritical fluid techniques developed by Steve Howdle's research group in Nottingham's Chemistry department. In the supercritical phase, molecules such as CO₂ and argon are exceptional solvents and are more 'innocent' than traditional organic media. The use of supercritical CO₂ (sc-CO₂) to mix biological factors into biodegradable polymers is not new, however previously published methods were not applicable for loading trace quantities and some current tissue engineering treatments require ppb levels of drugs.

In this new work, a solvent-sensitive biological factor is first deposited on top of a polymer 'scaffold' – in this case a rhodamine-tagged protein; Avidin, is deposited on top of racemic polylactide (PLA). Supercritical CO₂ was then used to redistribute the factor evenly within the scaffold. Because the biological factor used in this example is tagged with a fluorescent dye, the extent of distribution inside the material can be followed using confocal fluorescence microscope. After initial deposition, the majority of the fluorescence is found on the surface of the material, but after treatment with sc-CO₂, however, this luminescence becomes homogenous, indicating a uniform distribution of Avidin throughout.

By replacing the labelled Avidin with the well-known enzyme ribonuclease A, it is possible to gauge the level of biological activity retained after supercritical-fluid treatment. By following the rate of reaction of a ribonuclease specific substrate (cytidine-2',3'-monophosphate) it was established that, within experimental error, 100% of the free enzyme's activity is retained inside the scaffold.

Using supercritical fluids to incorporate a molecule into PLA is an example of the formation of an inclusion compound from a pre-existing host lattice. In Shakesheff's second

paper, published in February (*Advanced Materials* 2003) this concept is turned on its head with the polymer lattice being built up *in situ*, around pre-existing biomolecules and cells. The resulting materials have the characteristics of classic porous matrices and the formation mechanism of polymer hydrogels.

Microparticles of poly(lactic acid)-poly(ethylene glycol)-biotin are mixed with a suspension of cells and growth factors. This mixture is then injected into the area being treated, along with a cross-linking agent - in this case the protein Avidin. Almost immediately, the Avidin starts to bind to the biotin portions of the microparticles, crosslinking them and forming a scaffold. The end result is a three-dimensional, bio-degradable polymer network containing the cells and bio factors necessary for tissue growth. Confocal microscopy images of a crosslinked network containing tagged cells

shows a bimodal pore distribution, created by the imperfect packing of the microparticles.

The PLA-PEG-biotin/Avidin combination seems to be an ideal candidate for implantation into the body. The biological compatibility of the scaffold has been demonstrated *ex vivo*. Wedge-shaped defects were made in the femurs of chick embryos, and these were then filled with cell-free polymer support. After seven days of culture, vascularisation; the development of capillaries from the chorioallantoic membrane surrounding the embryo, to the femur/scaffold construct was observed, indicating good compatibility.

The technology has

the potential to be widely applied in the area of tissue engineering with the goal of incorporating stem cells into the scaffold, along with trigger molecules which could begin propagation and differentiation processes, ultimately leading the formation of specific new tissues.

Both technologies illustrate how profitable interdisciplinary collaboration can be, incorporating work from researchers in chemistry, biology, pharmacy and medicine.

Incorporation of proteins into polymer materials by a novel supercritical fluid processing method. Michael S Watson, Martin J Whitaker, Steven Howdle and Kevin M Shakesheff *Adv Mater*, 2002 **14** 1802-1804.

Porous polymer and cell composites that self-assemble in situ. Aliasger K Salem, Felicity R A J Rose, Richard O C Oreffo, Xuebin Yang, Martyn C Davies, John Mitchell, Clive J Roberts, Snjezana Stolinik-Trenkie, Saul J B Tendler, Phil M Williams and Kevin M Shakesheff *Adv Mater*, 2003, **15**, 210-21.

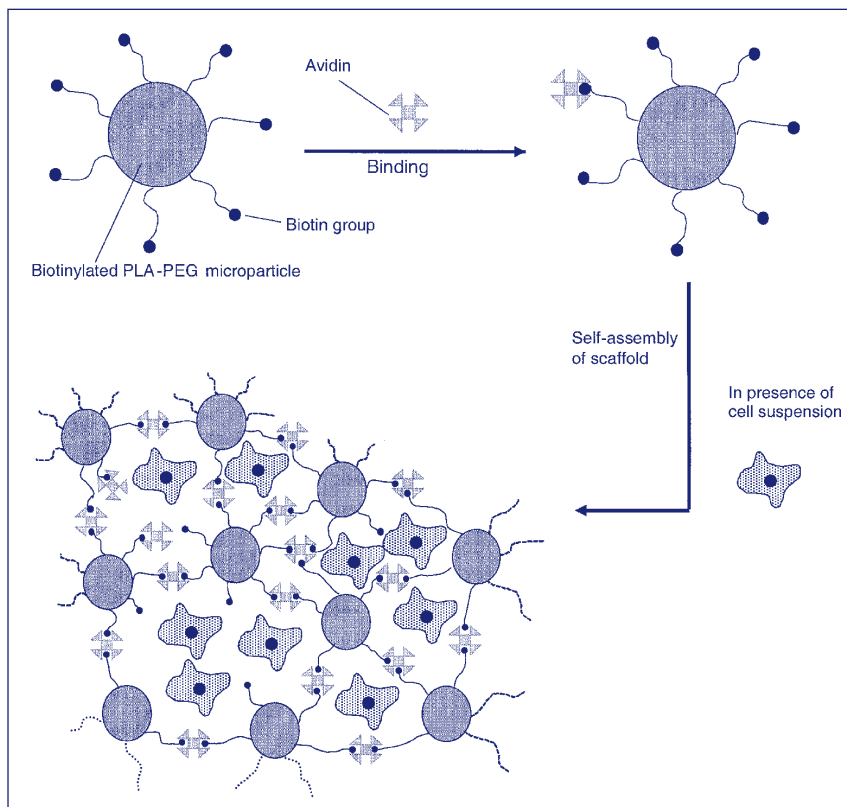


Figure 1: A schematic representation of scaffold self-assembly. Reproduced with permission from *Adv Mater*, 2003, **15**, 210 © 2003 Wiley-VCH