MATERIALS SCIENCE

Radicals promote magnetic gel assembly

Engineering complex tissues requires high-throughput, three-dimensional patterning of materials and cells. A method to assemble small gel components using magnetic forces from encapsulated free radicals could be just the ticket.

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The ability to control the micrometre-scale assembly of water-swollen polymer networks known as hydrogels has great potential in many disciplines. It might, for example, advance the development of robots from soft materials, of lab-on-a-chip diagnostic devices and of tissue-engineering constructs. Writing in *Nature Communications*, Tasoglu *et al.*[1] describe just such an ability. They have used paramagnetic forces (the attraction of certain materials to an external magnetic field) to guide the two- and three-dimensional assembly of micrometre-sized hydrogel subunits called microgels. Like the blocks in the game Tetris, these subunits can be turned and manipulated to assemble a desired structure. Because the technique can simultaneously accommodate several types and compositions of material in the presence of cells, it is highly appealing for tissue-engineering applications.

Tissue engineering depends on a precise interplay between cells and their surroundings, and on the hierarchical organization of three-dimensional (3D) tissues. Typical behaviours of cells, such as proliferation, differentiation and migration, are controlled in part through spatially and temporally distributed signals from the local surrounding matrix (the cell niche), as well as by soluble signals that diffuse through it. Researchers have used a variety of fabrication and patterning approaches to introduce these signals into synthetic materials in an attempt to understand and recapitulate cell–matrix interactions on multiple size scales.

Scaffolds for tissue engineering are produced by either encapsulating cells within, or seeding them into, materials. Historically, such scaffolds had uniform composition and did not replicate the complexity of, for example, developing tissues or the wound-healing response. Many researchers have therefore expanded their methods to incorporate top-down design — the production of a uniform material that is subsequently patterned. In one such approach (photopatterning), spatially controlled exposure of materials to light generates changes in biomolecule presentation[2], mechanical cues[3], or both[4], to control cell behaviour through interactions with the matrix.

Although top-down approaches provide some control over uniform cell distributions in a single material, many tissues are highly non-uniform in matrix composition and cell distribution. Bottom-up construction methods, in which tissue constructs are assembled from smaller components, may thus be better suited for replicating biological complexity. 3D printing, in which the direct deposition of material creates precise 3D structures, embodies this strategy. Recent advances in technology have allowed 3D printing of tissues through the deposition of cellular aggregates or cell-laden materials[5]. However, these processes still rely on repeated layer-by-layer deposition — a method[6] reported in 1986 — and thus require long fabrication times that currently limit construct size.

Assembly of preformed microgel components may offer an alternative method, because this would allow rapid assembly and therefore the potential formation of tissue constructs at clinically relevant scales. So far, the assembly of such components has been achieved largely through passive thermodynamic processes[7] or direct serial manipulation[8]. To improve efficiency, attention has recently turned to methods that drive assembly through external forces. In their approach, Tasoglu and colleagues used a chemical component that can be easily incorporated into nearly any hydrogel through diffusion (Fig. 1), and which contains a stable free radical (an unpaired electron). When exposed to an external magnetic field, the encapsulated radicals exert a paramagnetic force, which enables hydrogel components to be easily and rapidly manipulated into a desired location.

The authors show that the magnetization process can be temporally controlled and is compatible with cell viability and proliferation. Moreover, the radical-containing hydrogels are magnetically responsive for up to 24 hours after encapsulation of the radicals. However, the materials can be quickly rendered magnetically inactive by treatment with the antioxidant vitamin E, which acts as a radical scavenger. The researchers observed that vitamin E treatment tended to improve the viability and proliferation of encapsulated cells when compared with untreated gels.

To demonstrate the general applicability of their method, Tasoglu *et al.* have used it to form constructs containing many material types, densities and porosities. They also show that, following the assembly of hydrogel components into a desired configuration, another hydrogel may be added as a precursor

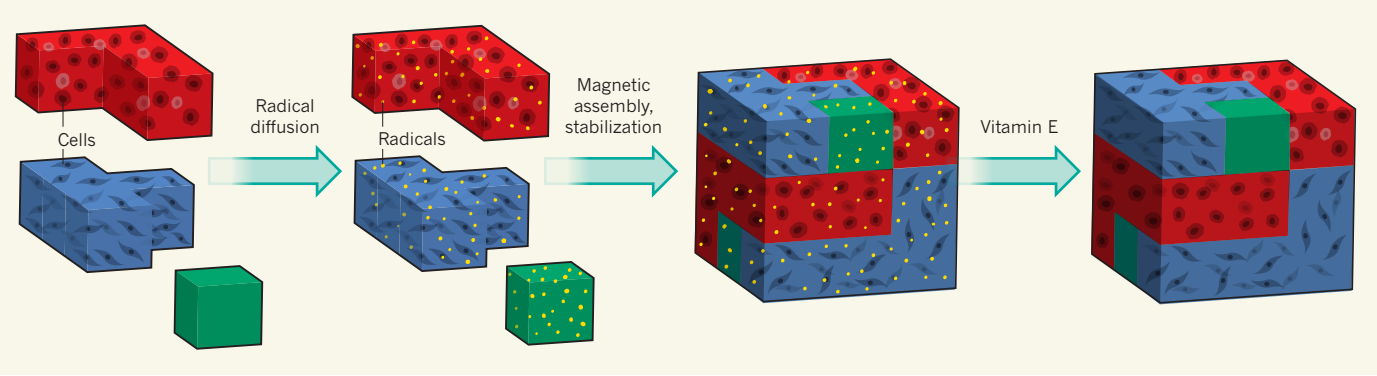

Figure 1 | Radical magnetic microgel assembly. Tasoglu *et al.*[1] describe a method for assembling micrometre-sized hydrogels (water-swollen polymer networks) into larger constructs with defined compositions and structures. The microgels are formed with or without encapsulated cells, and then a stable radical compound (yellow circles) is allowed to diffuse into them. The radicals generate forces in the presence of an external magnetic field, driving microgel motion and assembly. The resulting constructs are stabilized by the addition of a solution of a hydrogel precursor that polymerizes on exposure to ultraviolet light. Finally, the radicals are quenched (converted to non-radical products) by the addition of vitamin E, to prevent them from damaging the encapsulated cells.

solution that is solidified by ultraviolet-light-initiated polymerization — a process that stabilizes the assembled construct. Although not demonstrated, the method should also be able to control the assembly of components that provide different stiffnesses, biochemical cues or other biologically relevant features. Such generality is important for tissue-engineering applications.

Although tissues are inherently 3D, most microgel-assembly methods have been limited to manipulation in two dimensions. By contrast, the authors demonstrate that they can levitate many microgel components, and simultaneously drive the assembly of truly 3D structures of up to a few millimetres across by arranging external magnets in appropriate orientations. Massively parallel assembly in three dimensions will be needed to achieve the formation of larger tissue constructs.

In principle, the new method addresses several major challenges in tissue engineering. However, practical applications will be realized only when guided assembly of constructs can occur on the tissue scale. Incorporating molecular recognition between hydrogel subunits[9], to automate the stabilization of 3D assemblies, might help to achieve this. Moreover, it will probably be necessary to pattern multiple cell types and to introduce a means of perfusing thicker constructs to provide oxygen and nutrients for long-term cell viability.

Nevertheless, Tasoglu and colleagues' method is likely to stimulate the growing interest in guided micro-assembly. The use of external driving forces, such as magnetism, allows previously inaccessible levels of parallel assembly and might therefore propel this bottom-up approach to clinical use. Before then, however, the method will probably have a more direct impact on the formation of smaller assemblies, such as lab-on-a-chip devices for diagnostic applications and organized co-culture systems for studies of cell–cell interactions. ■

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CARDIAC BIOLOGY

Cell plasticity helps hearts to repair

Fibroblast cells are known as key players in the repair of damaged heart structures. New findings show that injury also induces fibroblasts to become endothelial cells, helping to mend damaged blood vessels. SEE ARTICLE P.585

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Heart attacks caused by a blockage in the coronary artery induce severe injury to cardiac muscle cells, leading to cell dysfunction and death. The damage elicits repair and regenerative responses that provoke the removal of dying cells and cell debris, recruit immune cells and initiate the formation of new blood vessels to recover blood supply. Fibroblast cells play a central part in this repair response. In this issue, Ubil *et al.*[1] (page 585) describe how the plasticity of cardiac fibroblasts contributes to this process, by showing that fibroblasts can, in response to the activity of the transcription factor p53, convert into the endothelial cells that line the interior surface of blood vessels.

The heart consists of myocytes (muscle cells) and non-myocytes, which include cardiac fibroblasts and endothelial cells. The fibroblasts produce growth factors and extracellular matrix (ECM) proteins to maintain proper cardiac architecture, contraction and function[2]. They also interact with endothelial cells and myocytes to aid angiogenesis (blood-vessel formation) and maintain physiological homeostasis[3]. Following heart damage, cardiac fibroblasts are activated to produce ECM proteins and soluble factors to compensate for structural defects, contain the spread of damage, reinforce cardiac stiffness and prevent cardiac rupture. These activities, collectively referred to as fibrosis, aid in remodelling the heart musculature. Controlled fibrosis is crucial for restoring cardiac function after injury. However, excessive fibrosis is considered a pathological process that can lead to adverse effects, including reduced cardiac stiffness (diastolic dysfunction) and irregular electrical connectivity (arrhythmia).

Although endothelial cells in blood vessels are typically thought of as terminally differentiated cells, they can take on the characteristics of mesenchymal cells[4,5], which are generally mobile cells surrounded by interstitial ECM proteins. During this endothelial-to-mesenchymal transition (EndMT), the endothelial cells lose the tight junctions that hold neighbouring cells together, and gain the ability to move, produce ECM proteins and contribute to excessive fibrosis, while also depleting functional capillaries and the endocardium tissue layer. EndMT is induced in cardiac endothelial cells by signalling pathways that depend on the growth factor TGF-β1 and that can be reversed by the activity of the protein BMP7 (ref. 4).

Now, Ubil and colleagues show that, following acute cardiac injury, fibroblasts (which belong to the mesenchymal cell lineage) can undergo a reverse conversion — from mesenchymal to endothelial cells (MEndT) — and become components of blood vessels (Fig. 1). To study this plasticity, they used mice in which cells that gain or lose expression of cell-type-specific markers can be tracked by fluorescence, a technique called genetic fate mapping.

The authors induced ischaemia-reperfusion injury by blocking the coronary artery and then restoring blood flow in the hearts of these mice. Three days later, they found that 35% of fibroblasts in the injury zone expressed the endothelial marker VECAD and were located in the interior of the vessel. Of these fibroblast-derived endothelial cells, 41% took up acetylated low-density lipoprotein, which is suggestive of endothelial-cell functionality. Most of the cells undergoing this transition expressed the fibroblast markers Col1α2 or FSP1, whereas very few expressed αSMA, a marker shared by a subset of cardiac fibroblasts (myofibroblasts) and the mesenchymal cells generated through EndMT (Fig. 1). This finding highlights a functional heterogeneity of recruited fibroblasts in injured cardiac tissue with respect to their plasticity. Understanding this heterogeneity will require future studies using fate mapping of myofibroblasts[6].

Ubil and co-workers also found that the fibroblast-derived endothelial cells in the mice express increased levels of p53, a transcription factor known for its multiple functions, including regulation of the cell cycle, apoptotic cell death and DNA repair. To investigate the involvement of the p53 signalling pathway