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Controlling Cell Fate In Vivo

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Consider the injection of a small biomaterial device under the skin that could trigger the immune system to attack cancer, treat inflammatory disease, or slow the progression of age-related tissue degeneration. The most recent advances at the interface of chemistry, material science, cell biology, and medicine suggest that this concept is becoming a reality—the key relies on the design of appropriate functional materials to control cell trafficking and cell fate directly in the patient's body.

Biomaterials, with well-defined chemical, biological, physical, and structural property/function relationships, have been explored as the basis for tissue engineering, cell therapy, and regenerative medicine.[1–3] Traditionally, biodegradable polymers are used as scaffolds on which cells are seeded and sometimes matured (that is, proliferated and differentiated) in vitro; this is followed by implantation to replace diseased and damaged tissues or organs.[4] The use of biomaterials as cell carriers has certain advantages compared to the direct injection of cells for cell therapy.[5,6] In the case of direct cell infusion or injection (which represents the most commonly utilized cell delivery strategy in the clinic—that is, bone marrow transplantation), the cell delivery process often leads to: 1) massive cell death of the transplanted cells due to mass transport limitations of oxygen and nutrients (in the case of local injection[1b]), 2) extremely poor (typically $<3\%$) homing/engraftment efficiency to a the target tissue (in the case of systemically infused cells[1c]), and 3) the loss of control over the fate of the transplanted cells.[5] By contrast, the use of biomaterials as cell carriers provides a substrate that can be used to promote cell survival and can provide the necessary physical constraints to better localize cells. The biomaterial can also serve as a template for guiding the formation of new tissue and for promoting engraftment with the host.[6] In particular, the third generation "bioactive" materials[1a] integrated with drugs, proteins, peptides, genetic materials, and other biomolecules can provide control over transplanted cells with respect to cell adhesion, proliferation, migration, differentiation, and production of paracrine factors or extracellular matrix. However, such in vitro procedures require the isolation of primary cells and ex vivo cell manipulation; this results in potential activation of cells or modification of their phenotype, immune rejection following implantation, high cost, and other scale-up issues and substantial regulatory issues.[5,6] Moreover, ex vivo cell manipulation, which depends largely on culture conditions, might lead to the loss of cell therapeutic capabilities and variations between trials.[7]

In a potentially ideal scenario (Figure 1), one might dream of a biomaterial that, once implanted into a patient, can recruit native cells, program them (using the cues that are loaded on or within the materials) and then send the cells throughout the body to treat injuries or diseased tissues. The most recent advances from Mooney and his colleagues at Harvard University showed this dream is well on its way to becoming a reality.[7–10] The new generation of biomaterials, when properly integrated with cell adhesion molecules, growth factors and genetic material, can now provide exquisite control over the in vivo fate of host cells—without ex vivo manipulation—and this could lead to more effective regenerative therapeutics.

Mooney and co-workers first asked how cells could be recruited in vivo by using a biomaterials approach.[8,9] The

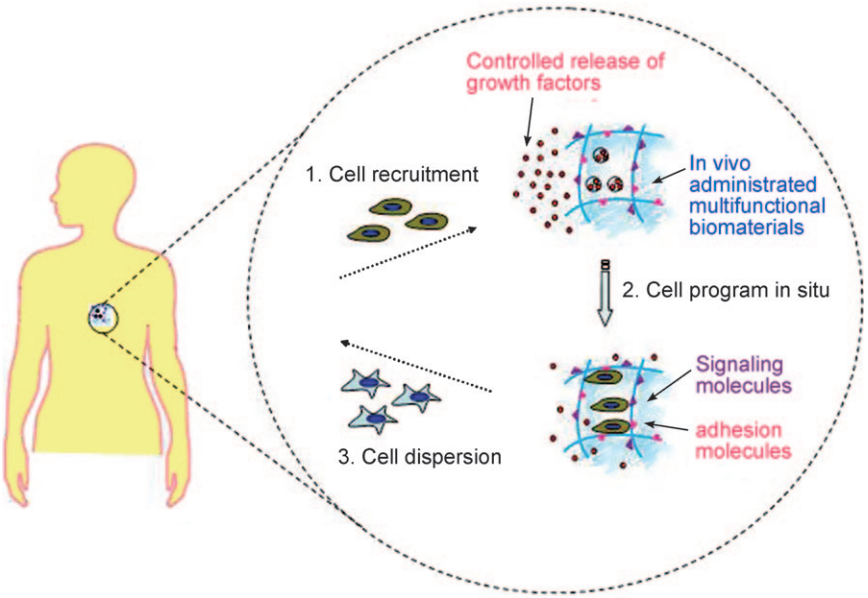

Figure 1. New generation biomaterials are capable of recruiting, programming, and dispersing host cells in situ to target locations for tissue maintenance, regeneration, or destructive purposes.

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trick is to use the materials to send "messengers" called growth factors (i.e., vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)) to cells such as endothelial cells, which line blood vessels in a process called angiogenesis.[11] The porous polymer scaffold made from poly(lactide-coglycolide) (PLG), a Food and Drug Administration (FDA)-approved polymer, was fabricated using a high-pressure carbon dioxide fabrication process[12] during which one or multiple growth factors, either in lyophilized form or in pre-encapsulated PLG particles,[13] can easily be incorporated.[8] Importantly, the release kinetics can be controlled distinctly for each individual growth factor simply by altering the degradation rate of the polymer by using various polymer formulations and molecular weights.[8] Typically, the PLG scaffold sustains a continuous release of growth factors that remain viable for up to several weeks. The material is then tested for its ability to recruit local blood-vessel-forming cells in an in vivo angiogenesis assay. After implantation in a relevant animal model, the growth-factor-doped scaffold promoted rapid formation of a mature vascular network and section imaging clearly showed that the scaffold loaded with growth factors recruited more cells than the blank scaffold.[8] Notably, the dual delivery of VEGF and PDGF resulted in a denser and mature vascular network than delivery of VEGF or PDGF alone. In addition to the recruitment of local cells, Mooney and colleagues showed in a subsequent report that the incorporation of interleukin-8 (IL-8), a cytokine that diffuses a greater distance than VEGF, delivered from such biomaterials can recruit distant bone marrow-derived progenitor cells that effectively mediate vascularization at the implanted scaffold/host tissue interface.[9]

The next task is to control the release or dispersion of cells from a polymer scaffold.[10] In this case, Mooney et al. designed a macroporous alginate scaffold coupled with cell adhesion peptides (arginine–glycine–aspartic acid, RGD) by using carbodiimide chemistry. When RGD is conjugated at appropriate densities, distributions, and stiffnesses, the scaffold provides defined "niches," which send specific signals that control cell adhesion, proliferation, and migration.[14] Specifically, the incorporation of RGD adhesive ligands to scaffolds preloaded with endothelial progenitor cells resulted in an order of magnitude increase in cell migration out of the scaffolds as compared to those scaffolds without RGD.[10] The presence of RGD (to which cells adhere through their membrane receptors, i.e., integrins) is essential for cell migration, which requires sufficient attachment strength to allow traction for cells to pull themselves forward (although strong adhesion at a high RGD graft density inhibits cell migration).[14] The incorporation of growth factor VEGF in the alginate scaffold was shown to further promote cell emigration as the subsequently released VEGF molecules attract cells out of the scaffold. When such scaffolds were preloaded with cells and then implanted into the mouse model, the cells migrated out of the scaffold to rapidly repopulate and revascularize the surrounding host tissue. Ultimately this approach was shown to prevent the death of cells in the toes and feet of mouse limbs starved of blood flow.[10]

The most remarkable example from this work is the development of an orchestrated biomaterials approach to create an infection-mimicking material that integrates in vivo cell recruitment, cell programming, and finally cell dispersion to desirable locations to treat disease.[7] Conceptually, Mooney and co-workers designed a porous polymer scaffold to first release inflammatory cytokines to recruit and house host dendritic cells (DCs).[15] Cancer antigens and danger signals conjugated on the scaffold can then activate the resident DCs. The activated DCs migrate out of the scaffold and then home to lymph nodes where DCs present antigens to initiate T cell-mediated immunity against tumor cells. Therefore, such infection-mimicking materials can potentially be used as a cancer vaccine. Specifically, a macroporous PLG scaffold encapsulated with inflammatory cytokine called GM–CSF (granulocyte–macrophage colony-stimulating factor) was fabricated by using a similar high-pressure CO_2 foaming process mentioned previously. The well-controlled, sustained release of GM-CSF from the scaffold for about two weeks was demonstrated in a mouse model.[7] Histological analysis at day 14 revealed that significantly high amounts of DCs were recruited to the scaffold compared with the control (scaffold without GM-CSF). In addition to recruitment of DCs, GM-CSF can also activate DCs in situ and subsequently release them to home to lymph nodes. To test whether such biomaterials can be used as a cancer vaccine, Mooney and colleagues further loaded the PLG scaffold with tumor antigens (melanoma tumor lysates) or a cytosine–guanosine oligonucleotide (CpG-ODN, a sequence uniquely expressed in bacterial DNA as a potent danger signal to stimulate DC activation and trafficking). Tumor lysates obtained by digestion of tumor biopsies were lyophilized and incorporated in the scaffold during fabrication. CpG-ODN was first condensed with polyethyleneimine (PEI) and subsequently lyophilized and incorporated in PLG scaffold. These PLG cancer vaccines were then implanted into a mouse model for 14 days to allow animals to develop protective antitumor immunity. Subsequently, these mice were injected with highly aggressive and metastatic melanoma cancer cells. Remarkably, implanted PLG cancer vaccine demonstrated 90% survival for animals that otherwise would die from the tumor within 25 days.[7] In addition to promoting a protective immune response, such biomaterials might also be applicable to other situations in which it is desirable to promote a destructive immune response (i.e., eradicate infectious diseases) or to promote tolerance (i.e., subvert autoimmune diseases).[7]

The use of biomaterials to program host cells in situ represents a new paradigm for therapeutic biomaterials and will create new opportunities to change the standard of care for patients in unprecedented ways. It complements other emerging cell-therapy-based technologies[19] that aim to improve delivery of an exogenous cell source or the mobilization of host cells.[19a] These biomaterial strategies not only bypass the complexities, cost of ex vivo cell manipulation and significant regulatory barriers, but enable control over the fate of cells in vivo which ultimately might lead to more effective treatment. Current ap-

proaches focus on angiogenesis, cancer therapy and immune disease,[7–10,20] but the concept can be broadly applied for the treatment of a variety of diseases, particularly those involving the programming/reprogramming of stem and progenitor cells in situ. In particular, recent breakthroughs to chemically or genetically reprogram somatic cells into induced pluripotent stem (iPS) cells and subsequently into differentiated cell types,[16,17] or into other differentiated cell types through trans-differentiation[18] (i.e., a single step approach) provides potential applications for the present biomaterials approach. For instance, one could envisage deriving mass numbers of particular cell types in vivo (without the need for cell culture) through loading a biomaterial construct with small molecules or genetic materials that guide an appropriate cellular programming or reprogramming process in situ.

Despite the initial encouraging results, the use of biomaterials to control cell fate in vivo, and ultimately achieve broad clinical utility, is still in its infancy. In particular, manipulation of cells in vivo requires a precise understanding of how cells function and interact with their niche. The design of appropriate biomaterials that mimic the complex signaling algorithms within biological niches cannot progress without enhanced understanding of the fundamental biology. However, state of the art in situ cell-programming technology provides a new paradigm for biomaterials research with the potential to generate novel therapeutics to overcome many of the translational barriers associated with cell based therapy and conventional tissue engineering approaches.

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