Dissecting fibrosis

Intravitral microscopy reveals how giant cells and neovascularization drive the fibrotic encapsulation of biomaterials implanted in live animals.

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Tissue injury associated with the implantation of biomaterials, medical devices or prostheses triggers a complex cascade of events, collectively known as the foreign-body response (FBR), that can cause implant failure. The FBR is a multistep process that starts with the absorption of a cohort of blood- and tissue-derived host proteins on the surface of the implant. These proteins form a provisional matrix that favours both the recruitment and the adhesion of a large repertoire of immune cells, which in turn elicit a phase of acute inflammation via the secretion of various chemoattractants. Then comes a phase of chronic inflammation, characterized by the formation of granulation tissue associated with the recruitment of macrophages and fibroblasts, neovascularization, and the formation of fibrous tissue that ends up encapsulating the implant. A detailed understanding of the machinery regulating the molecular interactions at the interface between the implant and the various tissue components is hence crucial to enabling the modulation of the FBR, and therefore to preventing the failure of implanted devices. Although the essential cellular and biomolecular players involved in the FBR have been identified, many details of the dynamics of this process, and the causal links among the various steps in vivo, remain to be elucidated. Reporting in *Nature Biomedical Engineering*, Peter Friedl and colleagues show that state-of-the-art intravitral microscopy enables the study of the progression of the FBR occurring on implantation of a porous 3D scaffold into the skin of live mice.

Friedl and co-authors exploited the use of multimodal nonlinear microscopy — which includes two-photon microscopy, and second- and third-harmonic generation microscopies — with a dorsal skinfold chamber, a technique and rodent model that have been previously developed to follow tumour progression in live mice for up to several weeks. The authors’ approach allows for the simultaneous, dynamic 3D view of the scaffold, the infiltration of different populations of immune cells, the development of the neovascularature and the assembly of collagen fibres.

The authors observed that migrating myeloid cells (predominantly macrophages of the M1 type) are one of the crucial components of the FBR and a possible therapeutic target. At an early stage, they are driven towards the implant and move rapidly, together with other mononuclear interstitial cells, along either existing collagen fibres or the surface of the scaffold. Later, they become immobilized on the scaffold/tissue interface and fuse to form giant cells. Notably, both macrophages and giant cells locally secrete vascular endothelial growth factor (VEGF), which initiates and maintains an immature neovessel network. These vessels show characteristics similar to those elicited during tumour-mediated angiogenesis (such as irregular shape and frequent branching points) and preferentially orient in parallel or orthogonally with respect to the scaffold, thus suggesting that the implant provides cues to guide their growth. Concomitantly to neovascularization, there is deposition of two subpopulations of collagen fibres, one encapsulating the scaffold and the other aligning in parallel with the scaffold fibres.

Moreover, the authors assessed the causal link between neovascularization and both giant cells and macrophages by depleting the two populations of immune cells through the administration of clodronate liposomes before scaffold implantation, and by the sequestration of the VEGF pool secreted into the tissue through administration of VEGF Trap (a VEGF blocker) at a regimen that was found effective for anti-angiogenic therapy in patients. Both treatments, alone
or in combination, resulted in substantial impairment of neovascularization and fibrosis, thus supporting a model in which macrophage and giant cells, being the sole source of VEGF in the tissue, play a major role in regulating the formation of new blood vessels, which in turn facilitates the further recruitment of macrophages and fibroblasts that is required for the deposition of collagen fibres. Notably, since both the depletion of myeloid cells and anti-angiogenic therapy are well tolerated and proven to be safe in clinical applications, they may form the basis of an effective therapy towards the successful integration of implanted devices into the host.

Through the use of a combination of sophisticated imaging modalities in a live animal and the rigorous use of quantitative imaging to tackle an important medical issue such as the FBR, Friedl and colleagues’ study is an elegant example of the power of intravital microscopy in pre-clinical settings. The approach has the potential to unravel more mechanistic details of the FBR process. For example, the response to the nature, shape and surface properties of the scaffolds could be further investigated by extending the imaging to the subcellular level, which will enable the visualization of the events occurring at the interface between the implant, the immune cells and the collagen fibres. Moreover, the use of mice expressing fluorescent markers in selected populations of immune cells will further help to characterize this process, particularly during the very early stages of the acute inflammatory phase. Nonetheless, there are still a few aspects of Friedl and colleagues’ experimental set-up that will surely be refined, such as the understanding of the contribution of the installation of the dorsal skin chamber to the immune response, and the extension of the window of observation beyond the reported two weeks. Overall, intravital microscopy is a formidable tool to complement the work performed in in vitro studies, to study processes that otherwise cannot be recapitulated in reductionist model systems, and to provide a tractable system to test the efficacy of new therapies.

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