Public summary of PhD-thesis of Valentina Bonito
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Regenerating blood vessels and heart valves by directing wound healing immune cells

Macrophages, a specific class of immune cells, give salamanders the amazing ability to regrow entire limbs and organs. This recent discovery has led to research focused on these cells for human tissue regeneration. Valentina Bonito investigated how we can exploit macrophages for heart and blood vessel regeneration. She developed biodegradable scaffolds that can be implanted in the human body, guide macrophage response and new tissue formation, while fully degrading over time. Bonito discovered several ways to control the differentiation of macrophages into the important anti-inflammatory and wound healing M2-phenotype and to guide macrophage-driven scaffold degradation.

Over the last decades, tissue engineering within the body ("*in situ*") has emerged as a promising approach to treat cardiovascular diseases. This approach is based on biodegradable prostheses (or "scaffolds") for the replacement of damaged or malfunctioning cardiovascular tissues, such as blood vessels and heart valves. Once implanted, the scaffolds are infiltrated by cells and hence gradually develop into living, functional parts of the cardiovascular system. The secret ingredient lies in the inflammatory response of the patient, and in the regenerative power of macrophages.

The role of macrophages is determined by their different "phenotypes", being the pro-inflammatory "M1" phenotype and the anti-inflammatory and wound healing "M2" phenotype. While M1 macrophages are the protagonists of the early stages of the inflammatory response to the implanted scaffold, anti-inflammatory M2 macrophages should appear at later stages, to dampen the inflammatory response and promote the formation of new, functional tissue. Besides their immunological role, macrophages also 'eat' the implanted scaffold, preventing persistent scaffold presence and, thus, a long-lasting inflammatory response, possibly leading to implant failure.

Given the multifaceted roles of macrophages, the development of scaffolds targeting their response is an appealing strategy for cardiovascular regeneration. In this PhD project, macrophage response to biodegradable scaffolds was studied, with the ultimate goal of providing design criteria for the next generation of cardiovascular implants. A multi-disciplinary approach was used, addressing scaffold design considerations, such as scaffold microstructure, degradation and bio-activation, and the influence of the patient's body.

A previous study showed that in presence of cyclic straining mimicking the pulsatile blood flow, scaffolds subjected to low strain levels induce the differentiation of the infiltrating macrophages towards the M2 anti-inflammatory phenotype. Bonito showed that this differentiation is independent of fiber size, one of the key scaffold characteristics. These results can be used to improve scaffold design. In fact, for a specific range of fiber diameters, the strain will always be the driving force guiding macrophage differentiation. Also, by choosing a more or less stiff scaffold material, the strain experienced by the cells might be tuned to trigger a specific anti- or pro-inflammatory macrophage response.

Bonito also investigated macrophage response and macrophage-induced scaffold degradation, for scaffolds differing in both fiber diameter and fiber alignment. Bonito showed that scaffolds with different microstructures undergo completely different degradation pathways, with scaffolds with big and oriented fibers having the most pronounced macrophage-driven degradation. This emphasizes the necessity to always choose an appropriate scaffold microstructure based on the implant location and the required degradation rate.

Lastly, the possibility to directly push macrophages into a specific phenotype was investigated by incorporating into the scaffolds interleukin-4 (IL-4), a protein normally produced by the human body to activate M2 macrophages. Tubular scaffolds with IL-4 were implanted in an animal model as abdominal aorta replacements. Impressively, abundant colonization of the scaffolds by M2 macrophages was observed within the days following implantations, followed by promising early signs of cardiovascular tissue regeneration and scaffold degradation after a few months.

Title of PhD-thesis: Immunomodulatory materials for in situ cardiovascular tissue engineering. Supervisor: Carlijn Bouten, TU/e; Co-supervisor: Anthal Smits, TU/e. Other main parties involved: SupraPolix, Utrecht University and Central Lab Animal Research Facility (CLARF).