PhD in Bioengineering Final Defense

POLITECNICO MILANO 1863

PhD Carlotta Mondadori

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Aula Seminari – Schiavoni

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PhD student:	**CARLOTTA MONDADORI – XXXII Cycle**
Thesis title:	Development of a microfluidic model of the articular joint to screen multiple anti-chemokine agents for the treatment of osteoarthritis
Advisor:	**Prof. ALBERTO REDAELLI – SILVIA LOPA – MATTEO GIOVANNI MORETTI**

Abstract:

Osteoarthritis (OA) is the most common type of arthritis and affects millions of people worldwide. OA induces the progressive degeneration of the articular cartilage and of the surrounding tissues leading to the loss joint functionality and to a high disability. Nowadays, the current OA therapies are based on symptomatic drugs that do not counteract the disease and, at later disease stages; surgical joint replacement represents the only possible option. Recently, the inflammation of the synovial membrane, called synovitis, has been shown to play a crucial role in the onset and progression of OA pathology, with OA synovial membrane showing a consistent infiltration of macrophages. In this context, the search for new therapies targeting inflammation and focusing on the macrophagic cell component may represent a promising approach for the treatment of this inflammatory disease.

In this context, the use of advanced 3D models mimicking the interactions between joint tissues and the complex mechanisms of OA pathology represents a promising tool in view of findings novel OA therapeutic approaches. To this aim, in Chapter 3, we designed and fabricated a microfluidic platform that includes the compartments representing all the main tissues involved in synovitis: the vascularized synovial membrane, the articular cartilage, which is the main target of inflammatory processes, and the synovial fluid that contains pro-inflammatory molecules secreted by tissue-specific cells in the joint. In the design phase, our attention was specifically focused on the dimension of the channel mimicking the synovial venule, which was sized to mimic the shear stress reached in the synovial venule in vivo. The developed microfluidic platform was used in Chapter 4 to recapitulate the mechanism of monocyte extravasation that leads to the abnormal accumulation of macrophages in the synovial membrane. The idea was to reproduce monocyte extravasation in view of targeting this process to develop novel strategies for OA treatment.

Synovial fibroblasts and articular chondrocytes were isolated from human OA tissues to model, respectively, the synovial membrane and the articular cartilage. These tissue compartments were separated by a channel for OA synovial fluid injection. The endothelialized channel comprised in the synovial compartment was stimulated by fluid flow and by a pro-inflammatory factor to mimic the in vivo situation. The developed osteoarthritic joint-on-a-chip model was then used to reproduce monocyte extravasation in response to chemoattractant factors. We demonstrated that monocytes specifically extravasated only in the presence of chemokines and that extravasation was enhanced when the endothelium was pre-activated by both fluid flow and inflammatory stimulus. Following the hypothesis that OA synovial fluid contains inflammatory molecules that can promote the recruitment of monocytes, we also provided for the first time a direct evidence that OA synovial fluid induces monocyte extravasation.

Besides therapies targeting monocyte extravasation, another approach to regulate inflammation is represented by the modulation of macrophage phenotype once macrophages infiltrate the synovial membrane. Based on environmental stimuli, macrophages can polarize toward an Ml or an M2 phenotype, thus exerting pro-or anti-inflammatory properties, respectively. The idea was to exploit the anti-inflammatory potential of M2 macrophages to modulate inflammation. Besides biochemical factors, also biophysical cues have been shown to play a role in influencing macrophage behavior. Specifically, scaffolds characterised by specific 3D geometrie cues have recently emerged as a promising approach to control macrophage phenotype. To this aim, in Chapter 5, we exploited the Melt Electrowriting (MEW) technique to generate Polycaprolactone (PCL) scaffolds with different 3D architectures to study their influence on macrophage response in terms of cell morphology, surface marker expression and secretion of pro- and anti-inflammatory proteins. Different architectures based on different pore geometries were generated: square, triangle and rhombus. Macrophages showed a different morphology when in contact with different scaffold architectures in correspondence of angles: macrophages formed "bridges" in the square and triangle scaffolds, while in the rhombus this did not occur. On the contrary, rhombus enhanced the elongation of cells along fibers. Among all architectures, the rhombus was the only one that enhanced the secretion of all the analyzed anti-inflammatory proteins (ILIRA, ILl0, IL13, CCL22, CCL24), with a significant difference for ILl0 compared to PCL films.

To summarize, in the present PhD thesis, we presented two different approaches that can be pursued for the development of novel therapies counteracting OA-related inflammation. In one case, therapies aiming at blocking monocyte recruitment to the synovial membrane can be developed to prevent the negative consequences caused by the abnormal infiltration of monocytes. In the other case, therapies aiming at exploiting the anti-inflammatory properties of macrophages can be developed to promote a tissue healing-friendly environment.

PHD COMMITTEE

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