Abstract of the Thesis

Advanced tissue-engineered *in vitro* models are emerging as relevant tools to preclinically study the translational potential of new therapies, supporting their advancement from the bench to the bedside. Integrating predictive experimental *in vitro* models in the drug development pipeline enables the selection of safe and effective drug candidates at the pre-clinical stage, prior to clinical trials, reducing animal experiments in agreement with the "3Rs" principle (Reduction, Refinement and Replacement). Traditional 2D cell-based systems fail to replicate the complex *in vivo* tissue environment, and animal models show genetic variabilities and limited drug safety predictability for humans. In this context, biomimetic tissue models, offer controlled physical and chemical microenvironments that mimic human tissues closely. The extracellular matrix (ECM) is essential for cell processes, tissue development, and homeostasis and changes in its fiber composition can mis-regulate signaling pathways and trigger disease. Recently, fibrous scaffolds have been extensively studied for their ability to mimic the native ECM nano- and micro-architecture accurately. **In this PhD work fibrous scaffolds were explored for the engineering of 2D and 3D models with application in cardiac and skeletal muscle tissue modelling.** Adverse cardiac tissue remodelling, results in the formation of a fibrotic scar, loss of anisotropy and electrical coupling impairment. Following myocardial infarction, replacement fibrosis is hallmarked by the macroscopic deposition of structural and profibrotic non-structural ECM proteins, and the activation of resident cardiac fibroblasts into myofibroblasts. **To mimic the architecture of fibrotic myocardial matrix, in this PhD thesis, polycaprolactone-based scaffolds with randomly oriented nanofibrous structure were proposed for *in vitro* engineering of human cardiac scar tissue. Such scaffolds were surface grafted with different types of proteins to explore their use for human cardiac scar tissue modelling.** Electrospun fibers were surface modified with gelatin, a collagen-derived protein, exploiting polydopamine mussel-inspired approach (PCL/polyDOPA/G) to support cell adhesion and modulate surface stiffness. Following substrate characterization, human ventricular cardiac fibroblasts were cultured (> 21 days), and cell phenotype and produced biomatrix in response to substrate cues were deeply investigated. Immunostaining for the fibrogenic markers α-smooth muscle actin and discoidin domain receptor 2 demonstrated the differentiation into myofibroblasts, showing very organized stress fibers. The deposition of abundant collagen and fibronectin enriched matrix was assessed by advanced microscopy. The engineered PCL/polyDOPA/G scaffolds appeared promising for cardiac scar tissue engineering, to be exploited as model for the validation of new cardiac regenerative therapies targeting fibroblasts (e.g., direct reprogramming of fibroblasts into cardiomyocytes). To enhance model biomimicry and boost its industrial translation, the model was further improved. Specifically, biomimetic fibrous scaffolds were designed based on randomly oriented polycaprolactone nanofibers, surface grafted with human type I collagen and human fibronectin (70:30, PCL/polyDOPA/C1F), binary a blend closely mimicking human cardiac scar extracellular matrix composition, based on a literature analysis. Human ventricular cardiac fibroblasts were cultured up to 7 days, with and without transforming growth factor-β administration. The predictivity of such model was investigated by treating cells with an antifibrotic commercial drug (Tranilast), which mitigates transforming growth factor-β signalling. Thanks to their protein-based coating, PCL/polyDOPA/C1F scaffolds in wet conditions closely matched fibrotic cardiac tissue-like stiffness. PCL/polyDOPA/C1F biomimetic cues (random architecture, biomimetic surface composition and mechanical stiffness) triggered human cardiac fibroblast activation after only 7 days, even without an external biochemical stimulus. Upon validation with Tranilast, a significant decrease

in fibrotic hallmarks (α-smooth muscle actin expression, and cell synthesis of type I collagen and fibronectin) was observed, confirming model robustness for drug screening purposes and perspective industrialization. By tuning substrate topography and protein coating composition, and introducing cardiomyocytes, other sub-types of cardiac fibrosis may be easily implemented. **In parallel the *in vitro* engineering of skeletal muscles was explored.** Skeletal muscles are soft tissues responsible for voluntary movement and postural maintenance, highly susceptible to damages, such as contusion or strains, congenital diseases or invasive tumour ablation. Although skeletal muscle tissues are endowed with good plasticity, severe muscle deficiencies cannot be restored. *In vivo*, each muscle fiber (or myocyte) is made up of elongated and multinucleated cells, anisotropically organized into a repetitive arrangement of sarcomeres, actin, and myosin filaments (myofibrils). This hierarchical structure permits efficient force generation. **The engineering of biomimetic anisotropic constructs represents a valid solution for the restoration of extensive tissue loss and for the *in vitro* study of myogenesis and pathogenesis. Herein, 2D and 3D models of skeletal muscle tissue were developed based on gelatin nanofibers in 2D or novel 3D arrangements obtained through a new biofabrication approach.** 2D anisotropic membranes were fabricated through solution electrospinning of gelatin (A-Gel). Nano-surface cued A-Gel scaffolds were seeded with C2C12 cells and myotube fusion and maturation were investigated after 10 days of culture. Such anisotropic scaffolds were able to quickly promote the differentiation of C2C12 in the absence of medium supplement stimulus, showing aligned and elongated myotubes with sarcomere-like structure, overcoming typical detachment issues of traditional monolayer cultures. Besides anisotropy for contact guidance, construct hierarchy and mechanical compliance are key biophysical cues regulating skeletal muscle cell functionality. Although electrospinning technique offers evident advances for biomimicry, the engineering of anisotropic 3D electrospun structures for cell-embedding is challenging. In this context, fiber-reinforced hydrogels are emerging in soft tissue engineering for the ability to form three-dimensional fibrous microstructures with biomimetic mechanical behaviour. In this Doctorate thesis, a method to achieve 3D anisotropic hierarchical structures was finely tuned. In detail, A-Gel were fragmented and homogenously dispersed into a C2C12 cell-laden interpenetrating network of Alginate and Gelatin, as a novel bioink for 3D bioprinting. Fibers within 3D printed parallel filaments were oriented by shear-stresses during micro-extrusion. Fiber reinforcing improved bioink viscosity and construct mechanical and stability behaviour. The embedding of fragmented nanofibers enhanced C2C12 differentiation and functional organization even in 3D not printed hydrogels, highlighting the importance of a 3D microenvironment combined with biomimetic mechanical properties for reliable *in vitro* modelling. C2C12-derived myotubes fused and organized anisotropically in the 3D printed constructs with aligned pattern as well, showing high biomimetic functional organization. In the future, by shifting from using C2C12 cells to human myoblasts, the herein proposed 2D and 3D models of skeletal muscle tissue could be exploited for studies on muscle disease mechanisms and drug validation, representing a significant innovation respect to current Matrigel-based skeletal muscle models. Furthermore, by using patients' cells from muscle biopsies or derived from induced pluripotent stem cells, skeletal muscle diseases could be reproduced *in vitro*. Overall, findings from this PhD thesis suggest that fiber-based scaffolds can be exploited to develop robust and physiologically relevant cardiac and skeletal muscle tissue models, with the ability to overcome current limitations in preclinical testing of therapies. Once validated and translated from research to industrial application, developed models will impact drug research and development, providing robust tools for preclinical studies in agreement with 3Rs Principles and with potential application in personalized medicine.