Abstract of the Thesis

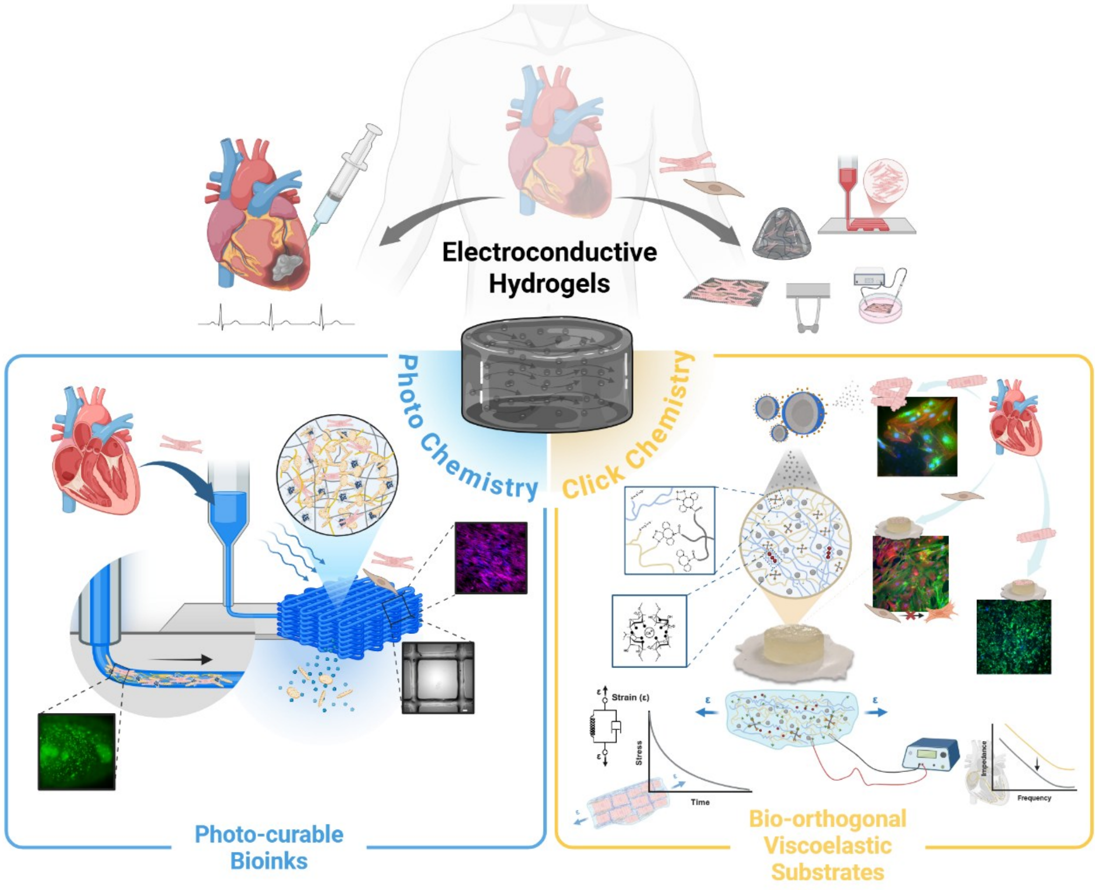
Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide, with myocardial infarction (MI) accounting for the majority of deaths. MI leads to irreversible loss of cardiomyocytes (CMs) and fibrotic remodeling, which impair conduction and contribute to heart failure. Despite therapeutic advances, heart transplantation is the only regenerative treatment for end-stage disease. Cardiac tissue engineering (CTE) aims to restore cardiac function by combining biomaterials, cells, and bioactive cues to develop regenerative therapies and advanced in vitro models. Hydrogels have emerged as promising scaffolds, but they often lack electrical conductivity and fail to reproduce the complex biochemical and mechanical microenvironment of the myocardium. Electroconductive hydrogels (ECHs) have been found to improve post-MI repair and CM functional maturation in engineered cardiac tissues (ECTs). However, **ECHs integrating biomimetic biochemical and physical cues, biocompatibility and controlled biodegradability are still missing and required with the aim to mimic the complex cardiac microenvironment.**

This **PhD project** addressed these gaps through a bottom-up strategy, based on the design of **hydrogel platforms** with a **modular, scalable, and biomimetic design, for synergistic integration and independent tuning of physical and biochemical features.**

In one approach, photo-curable ECHs were optimized for 3D bioprinting applications in CTE. The system was based on blends of polyethylene glycol diacrylate (PEGDA) and unmodified gelatin, cross-linked via type II photopolymerization using riboflavin as photoinitiator. Gelatin served as both bioactive component and co-initiator, enabling its covalent incorporation into the hydrogel network without chemical modification. Physical properties could be modulated by the initial PEGDA:gelatin weight ratio. Particularly, hydrogel exhibited biomimetic stiffness (~5-30 kPa) and controlled degradation (~40-60% weight loss) after 2 weeks incubation in phosphate buffered saline at 37°C. The addition of PEDOT:PSS in the hydrogels enhanced photo-cross-linking kinetics, while increasing their electroconductive properties. Finally, *in vitro* tests with human cardiac fibroblasts (HCFs) hydrogels cytocompatibility. As a drawback, micro-extrusion bioprinting was still limited by unsuitable printability (low viscosity of hydrogels precursors) and the need for long UV exposure (~ 300 s) for cross-linking. As a solution, Laponite® nanoclay (2–4% w/v) was added into PEGDA-gelatin/PEDOT:PSS precursors to modulate rheological properties, yielding printable bioinks with enhanced rheological properties, including shear-thinning behavior, yield stress, and elastic recovery. Photo-cross-linking was performed by blue light irradiation, using riboflavin as type II photoinitiator, unmodified gelatin as co-initiator and PEDOT:PSS as catalyst. The process allowed efficient cross-linking with the integration of gelatin in the hydrogel network, significantly reducing gelatin release compared to photo-cross-linked hydrogels using type I photoinitiator. The optimized formulation (3% w/v Laponite; PEGDA:gelatin 1.5:1 wt./wt.; 0.26% PEDOT:PSS w/v) exhibited excellent printability, shape fidelity, and electrical conductivity, while maintaining stiffness within the physiological range (E ~ 13 kPa). As a proof of concept for therapeutic drug delivery, the hydrogels were loaded with hydrophobic Tranilast, either in free form or complexed with Laponite, enabling sustained *in vitro* release. The hydrogels supported *in vitro* adhesion and spreading of HCFs and H9C2 rat cardiomyoblasts and enabled the microfabrication of 3D-bioprinted constructs encapsulating viable H9C2 cells. Thus in the first approach, new multifunctional photo-cross-linkable hydrogel were designed able to integrate electroconductivity, mechanical tunability, bioactivity, and the ability for drug release, addressing key requirements for the design of next-generation cardiac patches and *in vitro* cardiac tissue models.

In a second approach, bio-orthogonal and viscoelastic ECHs were designed to replicate the dynamic microenvironment of native myocardium for ECTs. Alginate and gelatin were functionalized with azide groups and cross-linked by a 4-arm-dibenzocyclooctyne (DBCO) crosslinker using strain-promoted azide-alkyne cycloaddition (SPAAC) with 0.5:1 (R0.5) and 1:1 (R1) DBCO:azide molar ratios. Calcium ions were also introduced to obtain double cross-linked alginate-gelatin hydrogels (AG). Rheological analysis showed that hydrogels exhibited tunable stiffness and stress relaxation, closely mimicking the viscoelastic properties of native cardiac tissue. Stress-relaxing AG hydrogels promoted HCF spreading or enhanced asymmetric cell elongation, depending on their low or high stiffness, respectively, thereby showing substrate-mediated mechanosensing. HCFs cultured within 3D AG hydrogels for 7 days showed high viability. AG hydrogels, especially the double cross-linked R1 formulation, were able to integrate the hallmarks of native myocardial tissue mechanics. In order to impart electroconductivity, Ti_3C_2 MXene quantum dots (MQDs) were added to AG hydrogels. Initial MQDs characterization showed their autofluorescence and electroconductive properties, along with excellent cytocompatibility and effective intracellular uptake. Remarkably, MQDs at 50 μg/mL concentration significantly enhanced connexin-43 expression in 2D cultured differentiated H9C2 cells. MQDs were then integrated into AG hydrogels (AG_MQDs), improving electroconductivity, without altering cardiac tissue-like stiffness (~8 kPa) and time-dependent stress-relaxation behavior. Compared to elastic tissue culture plastic and polyacrylamide (pAAm) hydrogels, AG hydrogels demonstrated the ability to preserve HCF quiescent phenotype, reducing myofibroblast activation thanks to their viscoelasticity. Moreover, AG_MQDs hydrogels supported cell adhesion and spreading of both HCFs and H9C2 cells after 7 days of culture. Thus, in this second approach, a versatile and modular AG hydrogel platform, with tunable viscoelasticity, electroconductivity and bioactivity, was designed for the development of ECTs as therapeutic cardiac patches and/or *in vitro* cardiac tissue models.

In conclusion, this PhD thesis presents two complementary hydrogel systems that advanced the field of CTE by addressing key limitations of current ECHs. Through a rational, bottom-up design approach, the work demonstrates how electroconductivity, viscoelasticity, and biochemical functionality can be integrated in a modular and controllable manner within cytocompatible hydrogel networks. This strategy not only enabled the development of multifunctional and biomimetic materials, but also provided a conceptual framework for the systematic design of next-generation scaffolds for electroactive tissues. The proposed systems open new possibilities for improving the functional relevance of bioprinted cardiac constructs and/or ECTs, and for developing more effective regenerative approaches.



Graphical Abstract of the Thesis