Thesis title: Electrospun scaffolds incorporating nanoparticles for controlled drug delivery in post-infarction cardiac repair

Abstract

Circulatory diseases are the leading cause of mortality in Europe, with ischemic heart disease accounting for approximately one-third of deaths within this category. In myocardial infarction survivors, persistent inflammation and fibrosis drive adverse cardiac remodeling, often leading to heart failure. Due to the limited efficacy of conventional therapies, next-generation scaffolds have emerged as promising tools for locally supporting functional cardiac tissue repair throughout its various phases. Within this context, my PhD research focused on the development of a biomimetic electrospun scaffold engineered for the on-demand release of ibuprofen, intended to mitigate excessive inflammation while preserving its beneficial role in cardiac repair, and the sustained delivery of pirfenidone to modulate fibrosis. To achieve this, nanoparticles were engineered to release ibuprofen upon ultrasound (US) stimulation, chosen for its safety and non-invasive nature as a trigger for drug release. These nanoparticles were subsequently embedded, together with pirfenidone, into a piezoelectric polyvinylidene fluoride (PVDF) matrix.

First, ibuprofen-loaded mesoporous silica nanoparticles (MSNs) were engineered with a US-responsive coating to achieve on-demand control of inflammation. The initial strategy involved the Layer-by-Layer (LbL) deposition of chitosan and alginate onto a MSN core. Nanoparticles coated with either three or six polyelectrolyte layers were successfully fabricated and fully characterized to evaluate the influence of layer number on nanoparticle morphology and ibuprofen diffusion. However, despite extensive testing across a range of US frequencies (38 kHz-5 MHz) and intensities (250-2000 mW/cm^2), no significant enhancement in drug release was observed upon US stimulation, highlighting the need to explore alternative strategies for the development of US-responsive MSNs.

The alternative approach explored in this study involved covalently anchoring a crosslinked alginate coating onto ibuprofen-loaded MSNs, enabling on-demand drug release through reversible polymer structural changes upon US stimulation. The coating was optimized to prevent premature diffusion of ibuprofen from the silica pores without altering particle morphology, and the nanoparticles were thoroughly characterized at various stages of their preparation. A systematic investigation of various US conditions was conducted on the final nanoparticles (designated IBU@MSN-SA-Ca), revealing that optimized parameters (2000 kHz, 2000 mW/cm^2, 3 min) induced the release of 10% of the loaded drug following a

single US application. Building on the promising results, *in vitro* studies were conducted on human monocyte-derived macrophages (hMACs), confirming excellent cytocompatibility of IBU@MSN-SA-Ca at concentrations up to 1 mg/mL. Moreover, an indirect assay demonstrated that US stimulation significantly enhanced the anti-inflammatory efficacy of the nanoparticles, resulting in a twofold reduction in Prostaglandin E_2 (PGE_2) secretion.

Once the nanoparticles were optimized, the electrospinning of a PVDF scaffold co-loaded with IBU@MSN-SA-Ca and pirfenidone was investigated. Both blend (uniaxial) and coaxial electrospinning techniques were explored to design a three-dimensional biomimetic architecture for controlled drug delivery. Each configuration was optimized to maximize nanoparticle incorporation within the scaffold, and the resulting release profiles of ibuprofen and pirfenidone were compared.

Blend electrospinning enabled the stable processing of PVDF formulations containing 5% wt/vol of IBU@MSN-SA-Ca and 5% wt/vol of pirfenidone, while coaxial electrospinning was optimized to process a shell formulation enriched with nanoparticles (reaching a maximum concentration of 1.5% wt/vol in the PVDF solution) and a core solution containing pirfenidone at 1% wt/vol. Both resulting structures exhibited aligned fibers with a predominant presence of the piezoelectric β-phase of PVDF, effectively replicating the structural alignment and electroactive properties of native myocardial tissue. As intended, they displayed distinct release profiles for ibuprofen and pirfenidone. Incorporating pirfenidone into the core of coaxial electrospun fibers significantly reduced the initial burst release compared to the blend-electrospun scaffold. However, due to the reduced loading of both pirfenidone and nanoparticles, the overall release from the coaxial scaffold was approximately one order of magnitude lower than that observed with the blend-electrospun counterpart. Therefore, the scaffold produced via blend electrospinning was selected for *in vitro* testing, as it enabled a more comprehensive evaluation of the efficacy of the released drug concentrations through dilution experiments. The indirect in vitro assay confirmed excellent biocompatibility of the blend-electrospun scaffold on hMACs up to 6.2 mg/mL, while cytocompatibility on human cardiac fibroblasts (hCFs) was limited to 0.62 mg/mL, likely due to pirfenidone's burst release. At the optimal concentration of 0.62 mg/mL, the scaffold harnessed the synergistic effects of ibuprofen and pirfenidone to markedly reduce PGE_2 secretion in hMACs and attenuate fibrosis in hCFs, highlighting its potential as a multifunctional platform for targeted myocardial repair through modulation of key healing phases.