Abstract of the thesis

Environmental sustainability is a pressing issue across all sectors, including bioengineering, stimulating a growing interest in the design of green biomaterials and technologies aligned with the circular economy and UN 2030 Sustainable Development Goals.

Oxidative stress arises in a wide range of pathological conditions, such as ischemic heart disease and chronic wounds, and is characterized by the overproduction of reactive oxygen species (ROS), severely impairing tissue regeneration. Plant-derived antioxidants have emerged as promising treatments against oxidative stress. However, their clinical potential is hampered by poor solubility and bioavailability, demanding for efficient drug delivery systems (DDSs) for improved drug pharmacokinetics and release profiles. Nanoparticles (NPs) are versatile DDSs for the encapsulation of hydrophilic and/or hydrophobic drugs, but face challenges in terms of long-term stability and rapid systemic clearance. In contrast, hydrogel-based DDSs enable localized and sustained drug release, although their hydrophilic nature limits efficient loading of hydrophobic drugs. Hence, hybrid DDSs, based on NPs/hydrogel, have gained increasing attention for sustained delivery of hydrophobic drugs, including plant-derived ones, at injection/implantation sites, enhancing drug efficacy. To date, only a limited number of studies have explored hybrid NPs/hydrogel DDSs to quench ROS, but they did not focus on the sustainability of employed biomaterials and technologies.

This PhD thesis aimed at the development of sustainable hybrid NPs/hydrogel DDSs from bio-based materials for natural antioxidant delivery: implantable patches for wound healing and injectable DDS for soft tissues.

Irregular wounds have driven growing interest in designing customizable hydrogel dressings by 3D bioprinting technology, which operates under mild, eco-friendly conditions. In this context, methacrylated polymers, allowing photo-crosslinking, have been widely used for the 3D printing of reproducible and structurally complex constructs. Herein, pectin, a bio-based polysaccharide derived from fruit peels, was methacrylated (PECMA; 65% degree of methacrylation) and used for the 3D microextrusion printing of hydrogels loaded with mesoporous silica nanoparticles (MSNs) loaded with natural anti-oxidant compounds. Such custom-designed MSNs-loaded patches were developed for treating irregular wounds. PECMA hydrogel-based patches (3% w/v) were double-crosslinked (by UV-irradiation and calcium ions complexation), as confirmed by rheological analysis, showing degradability and *in vitro* indirect cytocompatibility with human fibroblasts and keratinocytes. Moreover, printability studies showed bioink versatility to reproduce complex geometries, suggesting the potential adaptability to irregular wound topographies. MSNs were prepared via eco-friendly ball milling technique and loaded with natural antioxidant *Rosa canina* (R/MSNs) and *Harpagophytum procumbens* (H/MSNs) extracts (10%w/v of dry extracts). MSNs embedding into PECMA bioink significantly improved hydrogel stiffness (~3-fold) without altering UV photocrosslinking kinetics (<1 min) and printability. Dose-dependent *in vitro* cytocompatibility tests were performed to assess the safe and effective drug-loaded MSNs

concentrations (100 and 30 mg/mL for R/MSNs and H/MSNs, respectively). The novel 3D-printed PECMA hydrogel loaded with therapeutic MSNs provides a customizable and bio-based strategy for personalized wound treatment of local oxidative stress impairing healing.
Another major challenge in wound healing is the frequent occurrence of bacterial infections, in cases such as burn wounds, which exacerbate inflammation and elevate ROS production, further impairing the healing process. To address the multiple factors contributing to delayed wound healing, we developed a dual-functional sustainable patch for controlled release of natural antioxidant and antibacterial agents. A bacterial cellulose (BC) patch was produced by bacterial fermentation and then loaded with the antibacterial rhamnolipid R89, produced by bacteria, and bio-based zein NPs encapsulating antioxidant curcumin (CurZNPs). Initially, BC pellicles were produced by *Gluconacetobacter xylinus* fermentation and strategies for scaling up production were evaluated. CurZNPs were prepared by nanoprecipitation, to minimize the use of organic solvents: they showed high encapsulation efficiency (~85%), antioxidant and anti-inflammatory behavior. CurZNPs/BC (containing 5 µg/mL of curcumin) exhibited controlled release over 28 days, high cytocompatibility up to 7 days and antioxidant activity 24 h after H_2O_2-induced oxidative stress in human keratinocytes. Then, R89 biosurfactant was loaded into BC pellicles and the optimal concentration (0.5 mg/mL) was assessed based on cytocompatibility studies. The combined R89/CurZNPs/BC patch showed effective simultaneous loading of both compounds (66% and 85% CurZNPs and R89 loading, respectively), exhibited high cell viability up to 7 days and antibacterial and antibiofilm activity against *Staphylococcus aureus*. The combined therapeutic properties of R89/CurZNPs/BC patches make them promising as bio-based DDSs for infected wounds.
Injectable self-healing systems offer a minimally invasive alternative to implantation to reach deep tissues, reducing costs, discomfort, and associated risks. Injectable and self-healing oxidized pectin-based hydrogels were designed for the delivery of antioxidant CurZNPs. Pectin was oxidized into pectin dialdehyde (PDA, oxidation degrees of 2.3% and 4.5%) and PDA was blended with carbohydrazide-modified gelatin (G-CDH) to develop injectable PDA/G-CDH hydrogels by Schiff base reaction. Two compositions, i.e. PDA_2.5/G-CDH (70:30) and PDA_5/G-CDH (50:50), were selected for their tunable rheological properties and higher stability over 21 days (26% and 38% weight loss, respectively). Hydrogels showed injectability and self-healing behavior and high cytocompatibility with cardiac and foreskin fibroblasts, for potential soft tissue applications, e.g. wound healing and cardiac repair. CurZNPs/PDA_2.5/G-CDH (70:30) DDS exhibited the most prolonged CurZNPs delivery, therefore it was further characterized showing the ability to restore fibroblast viability after induced oxidative stress and to reduce NO production in macrophages after induced inflammation for 7 days. Overall, PDA/G-CDH hydrogels represent a promising injectable platform for *in situ* sustained release of CurZNPs.
In conclusion, this PhD thesis demonstrated the potential of bio-based materials and green technologies in developing a new generation of bio-based hybrid NPs/hydrogel DDSs for treating ROS-related conditions, potentially exploitable in a range of different clinical areas.