Abstract of the thesis

Injectable hydrogels have emerged as promising systems for tissue engineering, regenerative medicine, and pharmacology applications, owing to their high-water content and the capability of encapsulating and transferring their payload to the surrounding tissues. In this scenario, hydrogels based on the Schiff-base chemistry are attractive tools to engineer new advanced therapeutic platforms. Furthermore, the integration of nanocarriers (e.g., nanogels) into hydrogels has also emerged as a strategy to improve drug bioavailability and to achieve a combined drug release.

In the first part of the Ph.D. work, highly tunable hydrogels relying on the Schiff-base crosslinking of *ad hoc* customized poly(ether urethane)s (i.e., PEUs) have been developed, as promising drug delivery systems. Firstly, a library of water-soluble poly(ether urethane)s with suitable molecular weight and number of primary amines and aldehyde groups along the polymer chains was successfully developed. A high molecular weight PEU bearing primary amines along its chains, referred to as SHE3350 ($\overline{M_n}$ 24 kDa, D 1.7), was synthesized by reacting poly(ethylene glycol) ($\overline{M_n}$ 3350 Da), 1,6 hexamethylene diisocyanate (HDI) and N-Boc serinol, through an optimized one-step synthesis procedure followed by a deprotection treatment resulting in *ca.* 1.96 x 10^{20} -NH_2/$g_{SHE3350}$. Conversely, low molecular weight PEUs with aldehyde-end groups, referred to as AHE1500 ($\overline{M_n}$ 4 kDa, D 1.5), AHE6000 ($\overline{M_n}$ 9 kDa, D 1.2) and AHE600 ($\overline{M_n}$ 1.6 kDa, D 1.4) were synthesized by end-capping the isocyanate-terminated prepolymers, based on PEG ($\overline{M_n}$ 1500 Da, 6000 Da, and 600 Da, respectively) and HDI, using 4-hydroxybenzaldehyde. The exposed -CHO groups were quantified to be *ca.* 3.94 x 10^{20}, 9.85 x 10^{19}, and 1.18 x 10^{21} total -CHO units per gram of AHE1500, AHE6000 and AHE600, respectively. Subsequently, hydrogels based of Schiff-base crosslinking were successfully engineered from PEU SHE3350 and aldehyde-terminated PEUs. Firstly, hydrogels coded as SHE3350 – AHE1500 were developed and characterized. The effective presence of covalent imine bonds within the hydrogel network was confirmed by Carbon-13 and Proton Solid State NMR Spectroscopies, whereas rheological characterization proved the formation of gels characterized by high resistance to applied strain values (up to *ca.* 1000%). In addition, hydrogels possessed remarkable fluid absorption ability in physiological-like conditions, with an increase in wet weight of 270% at 27 days of incubation. In contact with an acidic pH aqueous environment, they exhibited enhanced buffer absorption and hydrogel dissolution (dry weight loss of 70% at 27 days of incubation), due to the hydrolysis of the Schiff-base bonds at low pH. Furthermore, the hydrogels displayed high permeability to a model molecule (i.e., fluorescein isothiocyanate dextran, FD4 $\overline{M_w}$ 4 kDa), whereas they were characterized by a controlled, sustained and pH-triggered molecule release up to 17 days of incubation. Lastly, hydrogels displayed easy injectability, self-healing ability and good cytocompatibility, according to the ISO 10993:5 regulation. Hydrogels with tunable mesh size were also formulated from SHE3350 PEU and AHE6000 as such or a mixture of AHE6000 and AHE600 (hydrogels coded as SHE3350 – AHE6000 and SHE3350 – AHE6000+AHE600, respectively). The rheological characterization confirmed the formation of gels with modulated structural parameters. Indeed, the mesh size was estimated to be *ca.* 17.9 nm for SHE3350 – AHE1500, 50.7 nm for SHE3350 – AHE6000, and 32 nm for SHE3350 – AHE6000+AHE600. The diversified hydrogel structures resulted in hydrogel physical properties modulation. Due to its larger mesh size, SHE3350 – AHE6000 possessed increased buffer absorption in contact with a physiological-like environment (wet weight change of 517% for SHE3350 – AHE6000 and 430% for SHE3350 – AHE6000+AHE600, at 27 days of incubation), and it also exhibited higher instability in contact with acidic pH environment. Furthermore, the correlation between structural properties and model molecules release was investigated. The release mechanism of fluorescein isothiocyanate dextran molecules at different molecular weight (i.e., $\overline{M_w}$ 4, 10 and 70 kDa)

was studied through the Korsmeyer-Peppas model, resulting to be prevalently diffusive for FD4 and FD10 released from SHE3350 - AHE1500 and SHE3350 – AHE6000+AHE600 in physiological-like condition. Conversely, an anomalous transport characterized the FD4 and FD10 release from SHE3350 – AHE6000, besides the FD70 release from all the tested formulations. Furthermore, the molecule diffusion coefficients were theoretically and experimentally evaluated to study the influence of the polymeric network on molecule diffusion. Lastly, SHE3350 – AHE1500 hydrogel was further characterized as versatile delivery platform of drugs with different wettability. Ibuprofen sodium salt (IBUSS)-loaded hydrogels were prepared by IBUSS encapsulation as hydrophilic drug, while curcumin (Cur) was selected as hydrophobic drug to be loaded at high concentration (i.e., 200 μg/mL) by exploiting its complexation with α-cyclodextrins. Both IBUSS and Cur were released according to controlled, pH-triggered and sustained profiles up to 17 days.

In the second part of the Ph.D. work, nanoscale hydrogels were developed to be loaded within the bulk Schiff-base hydrogels, towards modulated and combined drug release applications. Natural polymer-based nanogels were successfully formulated from custom-made gelatin methacryloyl (i.e., GelMA). GelMAs with different degree of methacyloylation (i.e., DoM ranging between 35% and 96%) were successfully synthesized by reacting gelatin type A with a variable amount of methacrylic anhydride. GelMA with medium DoM (i.e., 60%) was selected to prepare nanogels (i.e., nanoGelMA) through an optimized synthesis procedure, consisting of a two-step desolvation followed by nanogels stabilization through photo-crosslinking. nanoGelMA average hydrodynamic diameter and polydispersity index turned out to be *ca.* 250 nm and 0.2, respectively, while the nanoGelMA production yield was measured to be around 30%. Furthermore, nanogels exhibited good stability in suspension in physiological-like and acidic pH conditions, up to 6 days of incubation at 37 °C. Moreover, nanoGelMA storage and easy re-dispersion from the freeze-dried form were demonstrated, without changes in their features or aggregates formation. Lastly, nanoGelMA showed high cytocompatibility, according to ISO 10993:5 regulation. Drug-loaded nanoGelMA were prepared by modifying the optimized two-step desolvation synthesis protocol to include the encapsulation of both hydrophilic (i.e., glycine, Gly) and hydrophobic (i.e., ibuprofen, IBU) molecules within the process, thus resulting in the simultaneous nanogel formation and drug loading. The encapsulation efficiency resulted to be *ca.* 20%, while the maximum released drug in physiological-like condition was quantified to be below 5% from both Gly-loaded and IBU-loaded nanoGelMA up to 6 days of incubation, probably due to the concurrent payload and nanoGelMA network interaction and high photo-crosslinked nanogel stability in watery environment. Lastly, as a proof of concept, nanoGelMA were loaded within the hydrogel SHE3350 – AHE1500. The combined system was characterized, demonstrating the maintenance of the physico-chemical and rheological properties, self-healing ability and cytocompatibility of the native formulation.

This Ph.D. thesis work successfully developed drug delivery systems based on chemical crosslinked hydrogels and photo-cured gelatin methacryloyl-based nanogels. In particular, the formulation of highly tunable hydrogels relying on both *ad hoc* customized poly(ether urethane)s and the Schiff-base chemistry was reported for the first time in literature, demonstrating their high potential towards the development of new therapeutic platforms in the biomedical field. Secondly, a protocol for the production of gelatin nanogels based on GelMA as constituent material has been developed and thoroughly optimized, by combining the two desolvation method and nanogel stabilization through photo-crosslinking.

The outcomes of this study have demonstrated the potential of the developed therapeutic platforms for modulating drug delivery, which could be applicable in minimally invasive procedures and whenever a prolonged *in situ* pH-triggered payload release is required. Moreover, the combination of chemically crosslinked hydrogels and natural polymer-based nanogels could be efficiently exploited to develop hybrid drug-loaded systems as advanced therapeutic treatments in the biomedical field.