# Stimuli sensitive polyurethane-based hydrogels for the controlled and triggered release of anti-inflammatory drugs and anti-bacterial ions.

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**Introduction:** Skin lesions are a global healthcare problem: more than 40 million people suffer from chronic wound worldwide (8 million in EU and 6.5 million in US in 2009), most commonly caused by poor blood supply to the lower limbs. Chronic wound is a frequent and very severe problem in patients with diabetes mellitus, a pathological condition involving more than 285 million people globally. The design of injectable hydrogels for the targeted release of anti-inflammatory drugs and/or ions with antibacterial properties can be a promising tool in the treatment of these pathologies. Moreover, hydrogel chemistry can be easily tuned to exploit the chemical environment that characterizes chronic wounds, i.e. their pH value, as a smart stimulus to tune the release kinetics of previously encapsulated biomolecules. In this work, a new library of stimuli-sensitive hydrogels was developed by chain extending the commercially available triblock copolymer Poloxamer P407 with an aliphatic non-toxic diisocyanate and a commercially available diol, with the final aim of overcoming the well-known drawbacks of Poloxamer P407 hydrogels (i.e. poor stability in aqueous environment, lack of pH sensitive moieties, slow gelation kinetics at body temperature).

**Methods:** Poloxamer P407 was chain extended with an aliphatic non-toxic diisocyanate (1,6-hexamethylene diisocyanate) and a commercially available diol, namely an amino-acid derived diol containing BOC-protected amino-groups (N-Boc Serinol) or a cyclic diol (1,4-cyclohexanedimethanol), through a two steps procedure (Boffito M. Polym. Int. 2016;65:756-769). To provide the developed systems with a more pronounced pH sensitivity, a protocol for the exposure of amino or carboxylic groups on polymeric chains was optimized. The synthesized and functionalized polyurethanes (PUs) were characterized by Size Exclusion Chromatography (SEC), Fourier Transformed Infrared (FTIR) Spectroscopy, Proton Nuclear Magnetic Resonance ($^1$H-NMR) and colorimetric assays. The thermo-sensitivity of PU aqueous solutions with concentration in the range 10-20 %w/v was investigated by tube inverting test, gelation time test at 37 °C and rheological analysis (strain sweep tests, frequency sweep tests and temperature ramp tests). The capability of the hydrogels to transmit the pH of the surrounding environment through their thickness was studied by placing them in contact with buffer solutions at different pH (4÷8). The effects of pH changes on hydrogel network were studied by rheological measurements. The release profile of anti-inflammatory drugs, i.e. ibuprofen, resveratrol and curcumin, and antibacterial ions, i.e. $Ag^+$, $Ce^{3+}$ and $Cu^{2+}$, was also obtained at different pH to assess to capability of the surrounding environment to tune the release kinetics.

**Results:** The successful synthesis of PUs incorporating Poloxamer P407 blocks was demonstrated by FTIR spectroscopy (appearance of bands at 1722 $cm^{-1}$ and 1675 $cm^{-1}$, assigned to the stretching vibration of free and bound carbonyl groups (C=O) respectively, and at 1530 $cm^{-1}$ due to N−H bending vibrations) and SEC ($M_n \approx 55000$ Da, D=1.3). PU aqueous solutions with concentration in the range 10-20 %w/v showed a gelation temperature varying between 26 and 32 °C, with a complete sol-to-gel transition at 37 °C within 5 minutes. Data derived from tube inverting tests and rheological temperature ramp analysis were in good agreement: some small differences were probably due to the different analysis conditions, as tube inverting test does not apply deformations while temperature ramp test is performed under constant strain. The injectability of the designed systems was demonstrated at different temperatures (5, 25 and 37 °C), using syringes equipped with G18 needles. The developed systems turned out able to transmit the pH of the surrounding aqueous environment through their thickness: a pH change of at least the 70% of the entire gap between the pH of the polymer solutions and the pH of the buffers used to simulate the pathological environment was observed within the first hour of observation. pH indicators (bromocresol purple and phenol red) were also added to the hydrogels to easily display the progression of the acid/basic gradient through their thickness. The anti-inflammatory drugs ibuprofen, resveratrol and curcumin, and the antibacterial ions, $Ag^+$, $Ce^{3+}$ and $Cu^{2+}$, were progressively released from the developed systems in a concentration-dependent manner (after one month, release of the 65 and 80 % of the encapsulated drug from gels with 20 and 15 %w/v concentration, respectively). Their release kinetics was proven to be affected by pH variation, as a result of hydrogel network changes, as demonstrated by rheological tests. Cytotoxicity tests performed on hydrogel extracts with HaCaT keratinocytes demonstrated no cytotoxicity (cell viability > 80%).

**Conclusions:** A platform of reverse thermo-responsive, cytocompatible polymeric systems was successfully developed starting from custom-made PUs based on Poloxamer P407, an aliphatic non-toxic diisocyanate and a commercially available diol, overcoming some of Poloxamer gel drawbacks. The developed hydrogels showed a fast sol-to-gel transition in physiological conditions and promising properties for tissue engineering as delivery systems. Moreover, the pH of the surrounding environment was proven to be a smart stimulus to finely tune the release kinetics of previously encapsulated anti-inflammatory drugs or antibacterial ions.

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