
Abstract

Tissue engineering approaches aim to the design of three-dimensional constructs that can support cell viability, growth and differentiation. Among the wide range of biomaterials available, hydrogels offer many advantages for different applications thanks to their watery nature and viscoelastic properties. Additive manufacturing has been massively investigated as tissue engineering approach because of its high technological versatility and the possibility to build three dimensional constructs with high resolution and printing fidelity as well as building speed.

Ploxamer 407 is a Food and Drug Administration approved triblock copolymer; its water solutions show a thermo-responsive behavior with micelle formation and packing with increasing temperature. Despite their reported advantages (e.g. ,non-toxic, able to form gels at room temperature at a concentration of 20 %w/v), such hydrogels are characterized by fast dissolution in aqueous environment and weak mechanical strength, limiting their *in vivo* application.

In this work, Ploxamer 407 (P407) were exploited as building block in the synthesis of amphiphilic copolymers suitable for the design of thermo-sensitive hydrogels for cell/drug carrier applications and bioprinting technology. In detail, P407 was chain extended to increase its molecular weight and enhance the hydrophobic interactions and hydrogen bonds of its chains in water environment, by exploiting polyurethane (PUR) versatile chemistry. An amphiphilic PUR (NHP407) was thus synthesized starting from P407, an aliphatic diisocyanate (1,6-hexanediisocyanate) and an amino acid derived diol (N-Boc serinol). NHP407-based solutions in water-based media were able to form biocompatible injectable thermo-sensitive hydrogels with faster and more efficient gelation kinetics, enhanced stability as well as mechanical properties, compared to P407-based ones. The application of such hydrogels as

biomolecule/drug carriers was evaluated by studying the encapsulation and the release of different hydrophobic antioxidant drugs (i.e., dexamethasone, curcumin, resveratrol.) and hydrophilic model proteins (i.e., bovine serum albumin, horseradish peroxidase). However, NHP407-hydrogels were not suitable for bioprinting approaches due to their relatively low stability in the shape of thin filaments. In order to overcome this drawback different strategies were explored in order to provide the designed PUR-based hydrogels with the potential to be chemically crosslinked. Among all the tested approaches, the addition of acrylate moieties within the hydrogels showed the best results in term of increase of stability in water environment. Hence, three different families of thermo- and photo-sensitive hydrogels were designed and characterized. The first formulations involved the use of the previously designed PUR (NHP407) blended with a water-soluble acrylate polymer (e.g., poly(ethylene glycol) acrylate - PEGDA-) that upon UV/Vis irradiation forms a mesh entrapping the PUR-based micelles. The second approach, instead, dealt with the design of a new family of amphiphilic PURs exposing acrylate moieties (HHP407 and PHP407 synthesized by end-capping an isocyanate-terminated P407-based prepolymer with hydroxyethyl methyl acrylate or Pentaerythritol triacrylate, respectively) along their backbone, allowing the formation of a mesh of chemically cross-linked micelles upon light irradiation. Eventually, the last formulations involved the use of the latter PURs blended with an acrylate polymer (e.g., PEGDA) in order to obtain a double degree of crosslinking upon UV/Vis irradiation. The addition of chemical crosslinks within the hydrogel structure made it possible to design hydrogels with tunable stability in water environment as well as mechanical properties.

In order to select the best formulations to be applied in bioprinting approached, all the designed hydrogels were characterized in terms of thermo- and photo-sensitivity. Based rheological characterization, the NHP407/PEGDA- and HHP407/PEGDA-based formulations were

selected as bioinks. In order to 3D print PUR-based bioinks a commercially available bioprinter (Inkredible +, CELLINK) was modified and a custom-made printing process was designed.

Both the printing and photo-crosslinking procedures were studied separately in order to select the best parameters and conditions that allowed printing resolution and cell viability maximization. The best conditions of each procedure were finally combined to 3D print cellularized scaffolds in the shape of circular multi-layered constructs with a grid pattern showing prolonged stability and cell viability up to one month.

Moreover, the bicomponent nature of the designed bioinks allowed to finely tune both the bioink thermo- and photo-sensitivity in order to match different physico-chemical properties of soft tissues.