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Sommario	The development of Nano Drug Delivery Systems (NDDS) is a promising approach for developing intelligent therapeutic systems, which will bring significant advances in the diagnosis and treatment of disease, with the challenges to maximize therapeutic activity and to minimize undesirable side effects. The aim of this thesis was focused on the design of two kind of self-assembled nanocarrier for the delivery of hydrophobic drugs: Hyaluronan based nanohydrogel and pNIPAAm based micelles that both show hydrophobic internal domains and a surrounding hydrophilic shell. To achieve such self-assembled nanostructures, amphiphilic polymers were synthesized and extensively characterized. Hyaluronan (HA) and the thermosensitive di-block copolymer of methoxy poly(ethylene glycol) -b-(N-isopropylacrylamide)-co-(2-azidoethyl) methacrylate (mPEG-b-p (NIPAAm)-co-AzEMA) constituted the starting polymers on which riboflavin 2',3',4',5'- tetrabutyrate (Rfv), due to its interesting chemical-physical proprieties and due to its particularly biocompatibility, was conjugated as hydrophobic moiety via azide-alkyne click chemistry reaction. The HA-Prop derivatives were synthesized in order to provide the polymer of alkyne groups and make it versatile for âclickâ reaction. In this way, the azido-

hexyl derivative of riboflavin tetrabutyrate was synthesized and covalently coupled to the propargyl derivative of hyaluronic acid by Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC), due to the high efficacy and selectivity of this kind of reaction. Sterile self-assembled NHs were obtained in one-step by thermal treatment in autoclave of the aqueous dispersion of the HA-c-Rfv polymers. Size and polydispersity of the obtained NHs resulted be influenced from the Mw and from the degrees of derivatization of the starting Hyaluronan. Micellar nano-assemblies composed of thermosensitive amphiphilic block polymers were formed by heating the aqueous solutions of the resulting poly(NIPAAm) block copolymer, in order to obtain temporal control of the release of the encapsulated drugs. For this purpose, mPEG-b-p(NIPAAm)-co-AzEMA block copolymer was synthesized by free radical polymerization and subsequently, a propargyl derivative of riboflavin (Rfv-Prop) was synthesized and covalently coupled to the azide modified diblock copolymers by Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC). The nanosystems thus formed were intended to be used for the delivery of hydrophobic drugs, thus taking advantages of the lipophilic core represented by riboflavin. Moreover, the potential ?-? stacking interactions between the aromatic rings of the riboflavin in the core of such nanocarriers, may bring more stable nanostructures able to provide increased loading capacity. Highly hydrophilic and biocompatible nanocarriers based on polysaccharide hydrogels (nanohydrogels, NHs) were shown to be promising systems for drug delivery applications. Inspired by these emerging and promising drug carriers for therapeutic applications, in this work we aimed to develop self-assembled hydrogel nanoparticles based on amphiphilic derivative of hyaluronic acid (HA). For this purpose, new HA-Riboflavin (HA-c-Rfv) derivatives were synthetized by âclickâ Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction, requiring a previous HA derivatization with alkyne moieties and Riboflavin modification with azide groups. The resulting amphiphilic product was able to form nanohydrogels in aqueous environments by suitable treatments, in particular by an innovative method by using an autoclave cycle. Various HA molecular weights (Mw) and derivatization degrees of the starting polymers have been used in order to assess the effect of different parameters on the NHs formation. The derivative HA220-c-Rfv (Mw 220kDa, 40% of propargylic portions and 40% of Rfv moieties), was chosen as the most interesting NHs forming system; NHs were 150-200 nm in size and showed a ?-potential in the range -40 / -50 mV, depending on the experimental conditions adopted. NHs resulted to be stable in water and at physiological pH. Moreover, by the addition of a suitable cryoprotectant, the NHs suspension can be freeze-dried and recovered by re-suspension in water. The developed system is intended to be used for the delivery of hydrophobic drugs, such us dexamethasone, piroxicam and paclitaxel, used as model drugs. Drug encapsulations were performed by hydrating drugs film with polymers aqueous suspensions, followed by an autoclave treatment, resulting in a high encapsulation efficiency (EE%). Moreover, the HA-propargyl backbone with 60% of propargylic portions and partially linked to Rfv, was capable to react with other molecules bearing an azide group, opening the route to a wide spectrum of functionalization opportunities: in this direction, PEG-N3 have been tested as model molecule for the NHs. Micellar nano-assemblies composed of thermosensitive amphiphilic block polymers are formed spontaneously above their CMC. For these types of polymers, the

aqueous solubility properties depend on temperature, and micellar nanostructures are formed by self-assembly above their lower critical solution temperature (LCST) in aqueous media. For this purpose, poly N-isopropylacrylamide (pNIPAAm) block-copolymers were synthesized by free radical polymerization using a polyethylene glycol based macroinitiator. Upon dissolution in aqueous solvents and heating above the LCST, these polymers are able to form micelles. To stabilize these micelles, functional groups facilitating ?-? stacking interactions were introduced into the polymers. For this purpose, riboflavin as aromatic moiety was coupled to the polymer backbone by azide-alkyne âclick chemistryâ reaction. Two block-copolymers, methoxy poly(ethylene glycol)-b-(N-isopropylacrylamide)-co-(2-azidoethyl) methacrylate (mPEG-b-p (NIPAAm)-co-AzEMA) and the corresponding derivative methoxy poly (ethylene glycol)-b-(N-isopropylacrylamide)-co-(2-azidoethyl) methacrylate-riboflavin (mPEG-b-p(NIPAAm)-co-AzEMA-Rfv), were synthesized and compared in terms of physico-chemical properties, micelle size, drug retention and release. Micelles were formed by heating the polymeric aqueous solution from 0 to 50Â°C, and paclitaxel (PTX) was encapsulated by mixing a concentrated drug solution in ethanol with the polymer solution in phosphate buffer followed by heating. Three different feed PTX loadings (feed drug loading concentration of 5, 10 and 20%) were tested in micelles of both block-copolymers. Upon introduction of riboflavin in the polymeric backbone, a lower critical micelle temperature was obtained by (26Â°C for mPEG-b-p(NIPAAm)-co-AzEMA and 24Â° C for the corresponding riboflavin containing polymer, respectively). An average size of ~50 nm for the mPEG-b-p(NIPAAm)-co-AzEMA and ~90 nm for the mPEG-b-p(NIPAAm)-co-AzEMA-Rfv micelles in water was observed respectively for empty micelles, which increased with ~20 nm in phosphate buffered saline. Drug loading resulted in an increase of the micelle size by approximately 10-20 nm (from 60 to 75 nm for mPEG-b-p(NIPAAm)-co-AzEMA based micelles, and from 150 to 170 nm for mPEG-b-p(NIPAAm)-co-AzEMA-Rfv based ones). Increased encapsulation efficiency (EE%) and drug loading (DL%) were obtained for micelles containing riboflavin; almost 100% encapsulation of PTX was found for a feed PTX loading of 5% . Nevertheless, drug release resulted be faster for the Rfv-derivative backbone based micelle, likely due to their lower polymer density. Overall, this novel Rfv containing system is very promising in bringing advantages with regard to drug loading, warranting further investigations in tunability of drug release profiles for further application in stimuli-responsive anticancer therapy.

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