pH Responsive Injectable Hydrogels by Hierarchical Self-organization of a Triblock Copolypeptide

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Herein we report an injectable biocompatible/biodegradable hydrogel formed by hierarchical self-assembly of a triblock copolypeptide composed of poly(L-glutamic acid) (PGA) central block, end-capped by two hydrophobic poly(L-alanine) blocks (PAla), ($PAla_5$-PGA_{11}-$PAla_5$). The copolypeptide under investigation was designed as to have the poly-L-alanine blocks, providing the hydrophobic driving force for self-assembly, flanking at both ends a pH-responsive poly(L-glutamic acid) block, in analogy to classical triblock copolymer gelators. More importantly, we anticipated that this system might exhibit novel self-assembly behavior due to the well-known peptide chain conformation secondary structures (β-sheet, α-helix, random coil) that can be tuned by manipulating conditions, such as pH, ionic strength and temperature.

This system had shown a pH-responsive transition from short tapes to spherical assemblies by increasing pH, as a result of deprotonation of PGA block and conformational change from α-helix to random coil. At physiological pH (7.4) and ionic strength (0.15M NaCl), self-assembly of PAla end-chain blocks through H-bonding led to the formation of twisted tape fibrils. Upon increasing concentration, the triblock copolypeptide chains underwent hierarchical growth of fibrils into giant supermolecular tapes that could be disrupted and rearranged into a supermolecular network of twisted fibers upon temperature treatment. Self-supporting opaque hydrogels were obtained when the concentration was above 4.5wt%, characterized by high elasticity ($G' \sim 10^6$ Pa) and fast recovery from shear thinning.

Furthermore, benzaldehyde end-capped triblock copolypeptide (Bz-PAla-PGA-PAla-Bz) was designed to provide a pH-responsive dynamic character arising from the imine bond. This chemical modification endows the systems with pH-sensitivity within a narrow pH window relevant for *in vivo* applications.

The sol-gel transition was reversible upon caping/decaping of the benzoic moiety triggered by a slight change of pH, in addition to a significant reduction of the gelation temperature. Such novel dually responsive, dynamic covalent bond based, end-capped triblock copolypeptide formulation was investigated as matrix for encapsulation of the pentablock vesicular nanocarriers charged with a hydrophilic probe, calcein, in their aqueous lumen. The release of calcein from the composite was examined at pH 7.4 and 6.5 relevant for *in vivo* applications. As expected, the polymersome/polypeptide sol, prepared at physiological pH, showed significantly faster release profile relatively to the composite hydrogel at pH 6.5. Additionally, both formulations (i.e., sol and hydrogel), showed a significantly improved drug retention as compared to the bare polymersome nanocarriers. Such a composite polymersome hydrogel formulation encloses the benefits of biocompatibility, dual pH and thermo responsiveness of the hydrogel matrix, together with the robustness and tunability of the polymersome nanocarrier, with potential as controlled drug delivery systems.