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Kinetically stable metal ligand charge transfer complexes as crosslinks in nanogels/hydrogels: Physical properties and cytotoxicity

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ABSTRACT

A terpyridine end-functionalized 8-arm poly(ethylene glycol) was prepared using the reaction of a 4′-aminopentanoxy substituted terpyridine with a p-nitrophenyl chloroformate activated PEG-$(OH)_8$. Supramolecular complexation of the polymer terpyridine moieties by Fe^{2+} ions was investigated using NMR, UV–Vis and dynamic light scattering experiments. At low concentrations addition of Fe^{2+} ions to an aqueous solution of the polymer conjugate afforded nanogels with a single size distribution around 250 nm. At concentrations above 3 wt%, and at a 1:2 metal to ligand molar ratio, hydrogels were formed with increasing mechanical properties at increasing polymer concentrations. Using bovine chondrocytes, the biocompatibility and potential cytotoxicity of the polymer conjugate, nanogels and hydrogels were studied. The polymer conjugate with free ligands was toxic to the cells likely due to depletion of essential metal ions. When the terpyridine groups were complexed with Fe^{2+} ions, both nanogel suspensions and hydrogels showed no cytotoxicity in direct contact with chondrocytes. Indirect contact of gels with chondrocytes using transwells revealed the absence of toxic components by leaching. A Live-Dead assay on chondrocytes encapsulated in the hydrogels indicated that the hydrogels are cytocompatible, revealing the potential use of these materials for biomedical and pharmaceutical applications.

Statement of Significance

The binding between transition metal ions and ligands with multiple binding sites can be almost as strong as covalent bonds. This metal–ligand charge transfer (MLCT) complexation was used to crosslink water soluble polymers into hydrogels. This approach to novel materials may find applications in the biomedical and pharmaceutical fields. Transition metal ions are essential trace elements present in tissue but up to now no cytotoxicity data of free ligands are available. Data presented show that free ligands are toxic to cells likely by depletion of trace metal ions, whereas kinetically stable complexes are not cytotoxic even when embedded in hydrogels. These results provide fundamental issues to be considered in the design of hydrogels crosslinked through metal ligand complexation.

1. Introduction

Hydrogels, hydrophilic polymer networks that can retain large amounts of water, gained increasing interest in the past decades for their potential use in biomedical and pharmaceutical applications [1–4]. Due to their high water content, hydrogels do not evoke serious immune reactions and generally show good cytocompatibility. Hydrogels are commonly prepared by either chemical or physical cross-linking of polymer molecules. Chemical cross-linking generally affords mechanically stable polymer networks. Such hydrogels can be pre-formed before implantation or gelation can be performed in-situ by chemical or enzymatic reactions of polymer conjugates [1,2,5–10]. Physical cross-linking occurs through supramolecular interactions like dipole interactions, ionic or biomimetic interactions, hydrogen bonding, π–π stacking, stereocomplex formation or host–guest complexation [11,12]. As such physically cross-linked hydrogels do not require

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chemical reactions, conditions needed to form a gel generally can be mild. Moreover, the reversibility of these interactions provides material properties, like stimuli-responsiveness and self-healing, which are highly useful characteristics when applied in the biomedical and pharmaceutical fields.

The formation of host–guest complexes, as a method to induce physical cross-linking, can result in stable materials when multiple bases in a molecule donate electrons to a single Lewis acid. The thermodynamic stability of complexes not only depends on the multiple bonding interactions present, known as the chelate effect, but also on the preorganization of the ligands prior to complex formation [13,14]. In recent years it was shown that, although these interactions can be relatively strong, and depend on the transition metal ion used, the kinetic stability of the metal coordination bond can vary widely. The kinetic properties of coordination complexes are particularly of relevance for their possible exploitation, for example, as dynamic cross-links in hydrogels. Such stimuli-responsive gels are nowadays termed metallo supramolecular polymer gels (MSPGs) [15].

Several strategies may be applied to construct polymer gels using metal–ligand coordination, as recently reviewed by the group of Gohy [16]. The complexation of two 2,2′:6′,2″-terpyridine ligands with a single transition metal ion, as a method to induce cross-linking, was frequently studied to prepare MSPGs [17,18]. In particular, the use of multi-armed poly(ethylene glycol)s end-functionalized with terpyridine groups provides a versatile method to prepare hydrogels by cross-linking through transition metal ion complexation. Kimura et al. [19] showed that a terpyridine end-functionalized 4-arm PEG formed a hydrogel at a relatively high concentration of 20 wt% upon complexation with Fe^{2+} ions in water. This gel could be dissociated by the addition of ammonium hydroxide, as shown by the disappearance of the characteristic purple color of Fe^{2+}-terpyridine complexes. When a terpyridine functionalized 3-arm PEG was complexed with Co^{2+} ions, oxidation to Co^{3+} by aeration led to the formation of a hydrogel. Reduction–oxidation cycles revealed the reversibility of the system [20]. A more elaborate study on 4-arm PEG end-functionalized with terpyridine groups through click chemistry was performed by Rossow and Seiffert [21–23]. They studied the network mechanical properties of gels formed by complex formation with Zn^{2+}, Co^{2+} and Mn^{2+} ions. The complexation of Co^{2+} ions leads to network heterogeneity, which appears absent in the case of gels formed with Zn^{2+} ions. When complexed with Ru^{2+} ions, terpyridine end-functionalized 4- and 8-arm PEG afforded hydrogels. Rheological measurements revealed that the sol–gel transition occurred much more quickly in the 8-arm PEG than in the 4-arm PEG, but that the number of efficient cross-linking points participating in the gel network is almost the same in both systems [24]. Recently, multi-responsive supramolecular cross-linked polyglycerol hydrogels by hydrogen bonding and metal complexation were developed. The responsiveness allows degelling by a change in pH to reverse the cross-linking by hydrogen bonding. Metal chelators can be used to reverse the cross-linking by metal complexation. This class of hydrogels is regarded promising for use in life science studies [25]. In a recent study it was shown that 8-arm poly(ethylene glycol) partially functionalized with terpyridine groups formed hydrogels with the transition metal ions Ni^{2+}, Fe^{2+}, Co^{2+} and Zn^{2+} at concentrations above 5 wt%. The Ni^{2+} and Fe^{2+} complexes appeared kinetically stable and showed minor changes in mechanical properties at temperatures between 5 and 60 °C, whereas the Co^{2+} and Zn^{2+} complexes revealed a low kinetic stability. Interestingly, the reversible sol to gel transition temperature of the Zn^{2+} complexes was independent of polymer content [26].

Polymeric hydrogels based on terpyridine end-functionalized PEGs may have potential in biomedical and pharmaceutical applications as in-situ forming materials. Up to now the biological evaluation of this class of hydrogels has not been investigated. It may be envisaged that released metal ions may invoke a toxic effect or that decomplexed ligands will complex essential trace metal ions from the tissue. In this study, research was focused on a terpyridine end-functionalized 8-arm PEG either in the uncomplexed and complexed state with Fe^{2+} ions and the potential toxic effects were investigated in direct or indirect contact tests using chondrocytes. Nanogels and hydrogels were physically characterized and biologically evaluated through proliferation, viability and cytotoxicity assays of chondrocytes.

2. Materials and methods

2.1. Materials

Hydroxyl terminated 8-arm poly(ethylene glycol) (PEG-$(OH)_8$, $M_{n,NMR}$ = 21,400 g/mol) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. 4-arm PEG acrylate (PEG-DA, Mw = 20 kDa) was obtained from Laysan Bio (Arab, AL, USA). 4′-Chloro-2,2′:6′,2″-terpyridine (99%), 5-amino-1-pentanol (95%), pyridine (99.8%), lithium chloride (LiCl, ⩾98%), potassium hydroxide (KOH, ⩾85%), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959), Iron(II) chloride (anhydrous, 99.99%) and anhydrous dimethyl sulfoxide (DMSO, ⩾99.9%) were obtained from Aldrich-Sigma (Zwijndrecht, The Netherlands). *p*-Nitrophenyl chloroformate (PNC) was purchased from Fluka (Zwijndrecht, The Netherlands) and was purified by sublimation under vacuum at 90 °C. LiCl was dried at 105 °C in a vacuum oven before use. All solvents were from Biosolve (Valkenswaard, The Netherlands). All chemicals and solvents were used as received.

2.2. Synthesis

5-(2,2′:6′,2″-Terpyridine-4′-yloxy)-pentyl-1-amine (TPA): To a stirred suspension of powdered KOH (0.505 g, 9.02 mmol) in DMSO (50 mL), 5-amino-1-pentanol (1.67 g, 16.2 mmol) was added drop wise at 40 °C. After 20 min 4′-chloro-2,2′:6′,2″-terpyridine was added (1.00 g, 3.73 mmol) and the mixture was stirred for 2 h at 40 °C and then poured into deionized water (500 mL). The product was filtered, washed with deionized water and subsequently the crude product was recrystallized from hot ethanol. The pale yellow solid was dried under vacuum (yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52–1.57 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.87 (t, 2H, $OCH_2CH_2CH_2CH_2$), 2.73 (t, 2H, CH_2NH_2), 4.23 (t, 2H, OCH_2CH_2), 7.32 (m, 2H, terpyridine), 7.84 (dd, 2H, terpyridine), 8.03 (s, 2H, terpyridine), 8.60 (d, 2H, terpyridine), 8.69 (d, 2H, terpyridine). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 23.4 ($CH_2CH_2\underline{C}H_2CH_2CH_2$), 28.9 ($CH_2\underline{C}H_2CH_2CH_2CH_2$), 33.6 ($CH_2CH_2CH_2\ \underline{C}H_2CH_2$), 42.2 ($CH_2CH_2CH_2CH_2\underline{C}H_2NH_2$), 68.1 ($O\underline{C}H_2CH_2CH_2CH_2\ CH_2$), 107.4, 121.3, 123.8, 136.8, 149.0, 156.2, 157.1, 167.3 (terpyridine).

PEG-$(PNC)_5$-OH_3: To a solution of PEG-$(OH)_8$ (2.00 g, 0.093 mmol) and lithium chloride (1.00 g, 23.8 mmol) in 100 mL of DMF were added *p*-nitrophenol chloroformate (0.804 g, 4.00 mmol) and pyridine (0.978 g, 12.4 mmol) at 0 °C. The polymer solution was stirred at 0 °C for 1 h and then poured into 500 mL of cold ethanol/diethyl ether (4/1, v/v). The precipitate was filtered and washed with cold ethanol and diethyl ether, and then dried in a vacuum oven, yielding PEG-$(PNC)_5$-$(OH)_3$ as a white solid (yield 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.63 (PEG protons), 4.34 (m, OCH_2CH_2OCO), 7.37–7.40 and 8.26–8.28 (m, aromatic protons).

PEG-$(TPA)_5$-$(OH)_3$: To a solution of PEG-$(PNC)_5$-$(OH)_3$ (2.00 g, 0.092 mmol) in 20 mL of dichloromethane was added 5-(2,2′:6′,2″-terpyridine-4′-yloxy)-pentyl-1-amine (0.272 g, 0.814 mmol) at