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Feature Article

Reversible disulphide formation in polymer networks: A versatile functional group from synthesis to applications

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ABSTRACT

A substantial effort has been made in the last few decades to develop responsive materials that produce a selective answer to well-defined environmental stimuli. In our present review, we focus on the chemistry of thiol–disulphide equilibrium and its incorporation into polymer-based soft materials. Because several papers and extensive reviews have focused on reduction-sensitive drug delivery and gene transfer, we would like to especially emphasise the importance of disulphide formation and the exploitation of reversible thiol–disulphide interconversion in synthesis and its applications. We report the most important synthetic strategies that utilise disulphide formation. However, a major portion of our overview will concentrate on taking advantage of the thiol–disulphide exchange and the reversibility of this reaction in a wide range of applications, such as advanced drug delivery vehicles, bioartificial implants and self-healing and shape-imprinting polymers. In certain cases, the reversibility is only proven and used in one cycle, but in some cases, the process is practically reversible, at least in the time range of the dedicated application. The reversibility of the reaction is an important requirement for the long-term use of these polymers as implant materials; therefore, aside from better understanding the redox processes in living cells, the future direction of this research can lead to the improvement of reversible responses.

Contents

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1. Introduction

In recent years, responsive materials, including smart polymer hydrogels, are one of the central aspects of materials science. This can be verified by the increasing number of original research articles and reviews related to their synthesis and possible applications [1–4]. The beauty of this research field is its strong interdisciplinarity; this research requires knowledge of physics, materials science, chemistry, applied biochemistry and cell biology.

Hydrogels are three-dimensional hydrophilic polymer networks that swell but do not dissolve due to the presence of chemical or physical cross-links, and they contain a considerable amount of aqueous fluid. Hydrogels possess several properties that make them biocompatible, such as structural similarities to the body tissues, low friction surfaces and low interfacial tension with the surrounding biological tissues [5,6]. Responsive hydrogels are able to produce a pre-determined response to the alteration of certain environmental stimuli, such as temperature, pH, light (visible or UV), electric or magnetic fields, ionic strength, solvent composition, redox potential or enzymatic conditions, at a desired point and time [7–17]. Due to these attractive physical characteristics and their variable composition, responsive hydrogels are potentially beneficial materials in biotechnology, and they are actively studied. The environmental responsivity can be exploited in the continuously developing field of targeted drug and gene delivery vehicles, injectable implants, sensors and actuators as well as in modern self-healing materials. We emphasise that utilising pH-sensitivity for targeted drug delivery in the gastrointestinal tract has been well-known for several years and has led to widespread applications of intestine-solvent coatings and delivery vehicles [18], while other types of stimuli (e.g., redox effects) have not yet been exploited in such a wide range in the biomedical field. The main reason for this may be the uncertainty of knowledge on redox gradients in the human body, and the utilisation of redox differences can be more difficult because of the larger variability among patients with the same health state. The key issues of the redox processes in the body and the biological function of glutathione are briefly summarised later in this review. In their work, Meng and coworkers [19] elegantly summarised the advantageous properties of the thiol–disulphide exchange reaction. This exchange reaction is readily reversible without significant heat effects. The disulphide bonds possess excellent stability in circulation and in extracellular physiological conditions, but in a reductive environment, such as cytoplasm, they can undergo rapid cleavage. The time scale of the exchange reaction can be adjusted from minutes to hours.

The first part of this review focuses on incorporating thiol or disulphide functional groups into polymer networks. We report thiol and disulphide groups in our review because of their high biological relevancy, but we must mention that there are several other functional groups that can render redox-responsive properties into a polymer chain (e.g., chelated metal cations [20,21] and ferrocenyl-type groups [22]).

The second part of this review will focus on the applications of the proposed networks. In this field, most of the publications focus on bioreducible polymers and bioconjugates mainly for biomedical applications [23]. A significantly smaller number of studies are concerned with the utilisation of thiol oxidation and building polymer networks with reversible cross-linking, which is the main focus of the present review. Disulphide bond formation can be utilised in chemical sol–gel synthesis, which allows for an effective and homogenous encapsulation of drugs. This type of entrapment can stabilise the highly ordered structure of proteins and maintain the biological functions of living cells in the polymer matrix. The formation of the polymer matrix by thiol oxidation can be advantageous in the case of injectable implants or simply can be a key step in synthesis strategies. Reversible thiol–disulphide exchange can be triggered to initiate controllable changes in the material properties (e.g., in the modulus of a hydrogel) for several cycles without degradation.

In this review, we discuss the synthetic methods of thiol-modified polymers, which can produce sol-to-gel transitions through the oxidation of thiol groups, followed by recent developments and applications of the thiol–disulphide exchange reaction in diverse areas. This review highlights and emphasises the role of molecular structure in the determination of the macroscopic behaviour of hydrogels.

2. Synthesis of thiol–disulphide containing polymer structures

The chemically reversible cross-linking of natural and synthetic polymer chains has been of considerable interest for decades. The reversible cross-links are usually incorporated into the polymer chains via reductively cleavable disulphide groups. The most frequent reagents to incorporate thiol or disulphide functional groups, as well as the common reducing and oxidising agents in thiol–disulphide chemistry, are listed in Fig. 1.

Two basic synthetic routes are utilised to prepare disulphide cross-linked hydrogels: the application of disulphide-containing molecules to incorporate disulphide groups into the polymer network and the modification of the polymer chains with thiol-containing side chains followed by chemical cross-linking (i.e., the oxidation of thiol groups to establish intermolecular linkages). Both methods have their own applications; each of them is discussed here along with the most relevant characterisation methods for analysing the prepared polymers.

2.1. Incorporation of disulphide bonds directly into polymeric networks

A widely used method for the synthesis of reduction sensitive polymers and hydrogels is the building of disulphide linkages directly between the polymer chains as described in detail in a recent review by Hennink et al. [19]. Two examples are presented here to demonstrate the importance of thiol–disulphide interconversion during the synthesis and application of hydrogels with reversible redox-responses. Disulphide groups can be introduced into a polymeric network simultaneously with the radical polymerisation of the pre-cursor monomers. Ravi et al. [24]

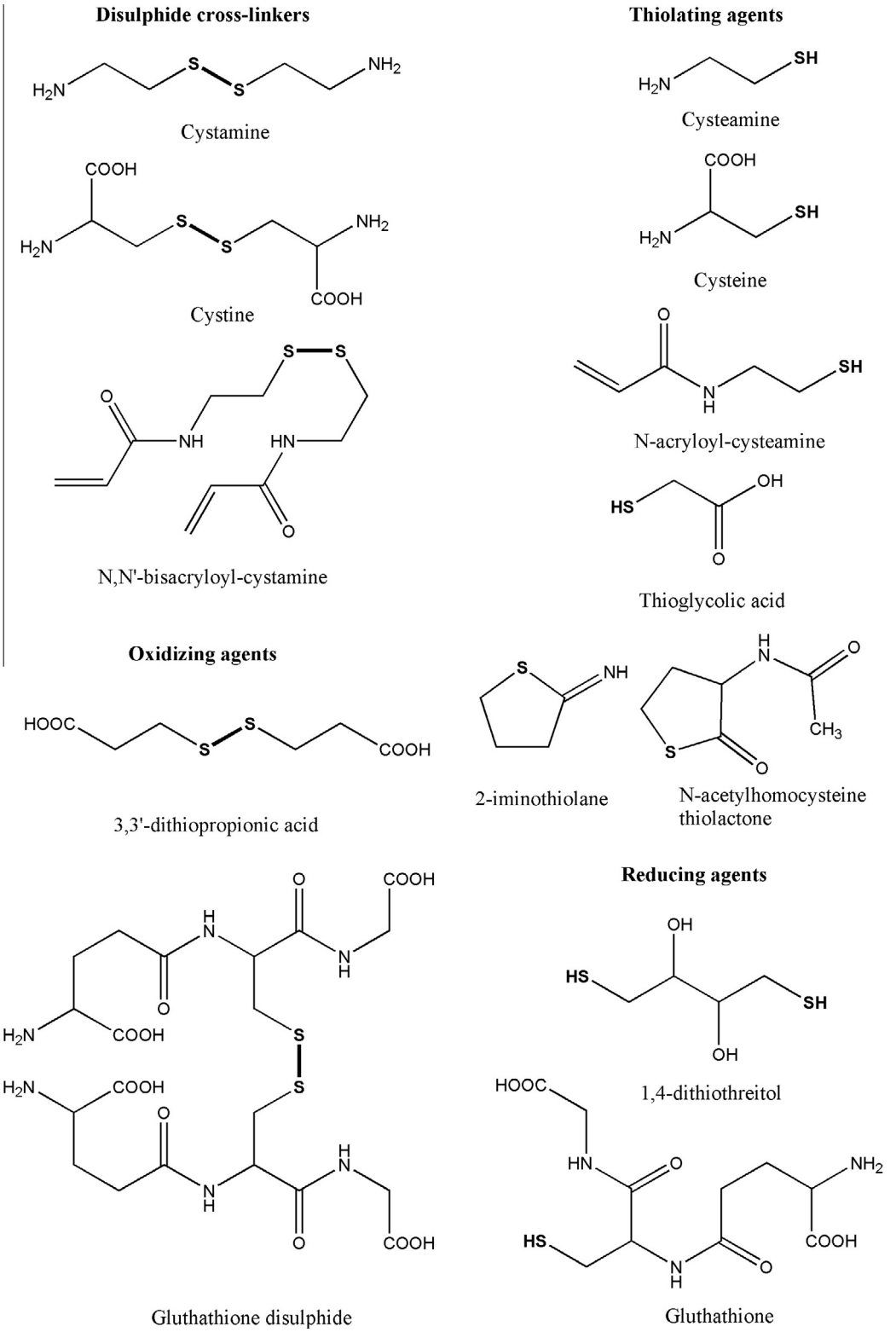

Fig. 1. Thiol and disulphide compounds used in synthetic pathways and redox reactions.

synthesised disulphide-containing acrylamide hydrogels by the free radical polymerisation of *N,N′*-bis-acryloylcystamine with acrylamide in aqueous ethanol (Fig. 2).

The hydrogel was liquefied by a reducing agent. The liquefied polymer was used as injectable material while in situ oxidation by the air caused the re-gelation of the polymer and proved its feasibility for its application as a gellable implant. Optical properties and stress–strain behaviour were examined for their application as refilling material in ocular cavities. The initial cross-linking step facilitated the removal of non-toxic monomers; thus, dialysis was not necessary. The oxidation-induced re-gelation possessed advantageous features for bio-related uses; only the heat of disulphide cross-linking developed during the endocapsular gelation process, instead of the heat of polymerisation, which prohibits the application of a number of in situ forming networks in vivo because of the damage it causes to the surrounding tissues at elevated temperatures. The monomer-free gelation also enhances the biocompatibility of the proposed polymer.

Hisano et al. [25] synthesised a similar redox-sensitive network by the co-polymerisation of *N,N′*-bis-acryloylcystamine with acrylamide in water to obtain disulphide-containing poly(acrylamide). The aqueous solution of the polymer was liquefied by adding a strong reducing agent (dithiothreitol) and allowing it to be oxidised by the air. Oxidation also proceeded in the presence of low-molecular-weight disulphides (e.g., cystamine, 2-hydroxyethyl disulphide, 3,3′-dithiodipropionic acid and glutathione disulphide). The authors demonstrated the reversibility of the process through the reliquefaction of the hydrogels by reducing agents (either glutathione or L-cysteine).

The benefit of these synthetic methods was the easy removal of non-reacted monomers after the first cross-linking step, which reduced the cytotoxicity of the re-gelled networks. This is an essential requirement for the polymers produced from potentially harmful pre-cursor agents. Disulphide cross-linked polymers can also be synthesised from natural and biocompatible polymers in the same manner; however, in these cases, the modification of polymers with thiol groups proved to be a more beneficial method as discussed in Section 2.2.

2.2. Synthesis of thiolated polymers

A general approach for introducing thiol groups into polymer chains is demonstrated here through the work of Bernkop-Schnürch et al. [26–29]. They reported the preparation of several different polymers, modified with thiol-containing agents, for biomedical applications, especially for mucoadhesive delivery devices [30,31]. Material properties and adhesiveness are strongly influenced by thiol content, which can be controlled during the synthesis [28]. The principles of the synthetic route are shown in Fig. 3.

As a general method, a polycarboxylic acid type compound is activated to prepare more reactive functional groups. Carboxylic acid moieties are activated by a water-soluble carbodiimide, i.e., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), at a controlled pH. In the second step, amide groups are formed between the repeating units of the polymer and an amine type, small-molecular weight reagent, such as cysteine, to yield thiol-modified polyacid. Modified polymers are usually purified by dialysis and lyophilised. This synthetic strategy is generally useful for the preparation of any type of thiol-grafted polymers (also referred to as thiomers) from poly(carboxylic acid)s through amide bond formation.

Possible synthetic routes for immobilising thiol groups on polymer chains are extended in the case of polymers that have a more reactive functional group in their repeating units. Chitosan is a polysaccharide-based natural polymer with one primary amine group per repeating unit. Amine groups can be attached to carboxylic acids (e.g., thioglycolic acid) in the same way as mentioned previously (Fig. 4a) [27]. Amines can also be modified without a mediating agent like carbodiimide to yield thiolated polymers (Fig. 4b). More interestingly, this polymer has a polycationic character because the precursor material is a polyamine, which may generate an exciting class of thiomers with a potential use in the delivery of negatively charged macromolecules, such as gene sequences.

Numerous examples of thiomers can be found in the literature. Gelatine and collagen are frequently used as precursors of thiolated polymers [32,33]. Notably, the thiolating agent has a strong influence on the concentration of thiol groups on the modified polymer. For example, in the case of gelatine, use of the Traut reagent led to a significantly larger degree of modification than the use of *N*-acetylhomocysteine thiolactone (Fig. 5).

Similar to cysteine-containing polypeptides, thiol-modified polymers have a well-known ability to form intra- and intermolecular disulphide cross-links, which can be exploited at different levels of application. In this manner,

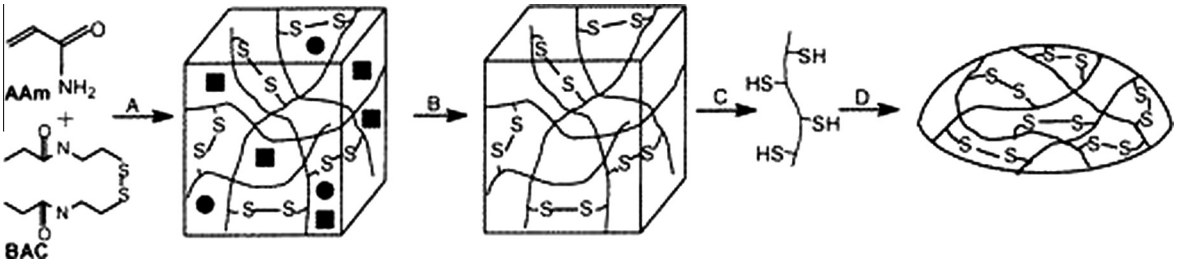

Fig. 2. (a) Radical polymerisation of acrylamide (AAm) and *N,N′*-bis(acryloylcystamine) (BAC); (b) Swelling, washing, and removal of unreacted AAm (black squares) and BAC (black circles); (c) Liquefaction using dithiothreitol (DTT); (d) Re-gelation using 3,3′-dithiodipropionic acid (DTDP) at pH 7 (reprinted with permission from Ref. [24] 2005, American Chemical Society).

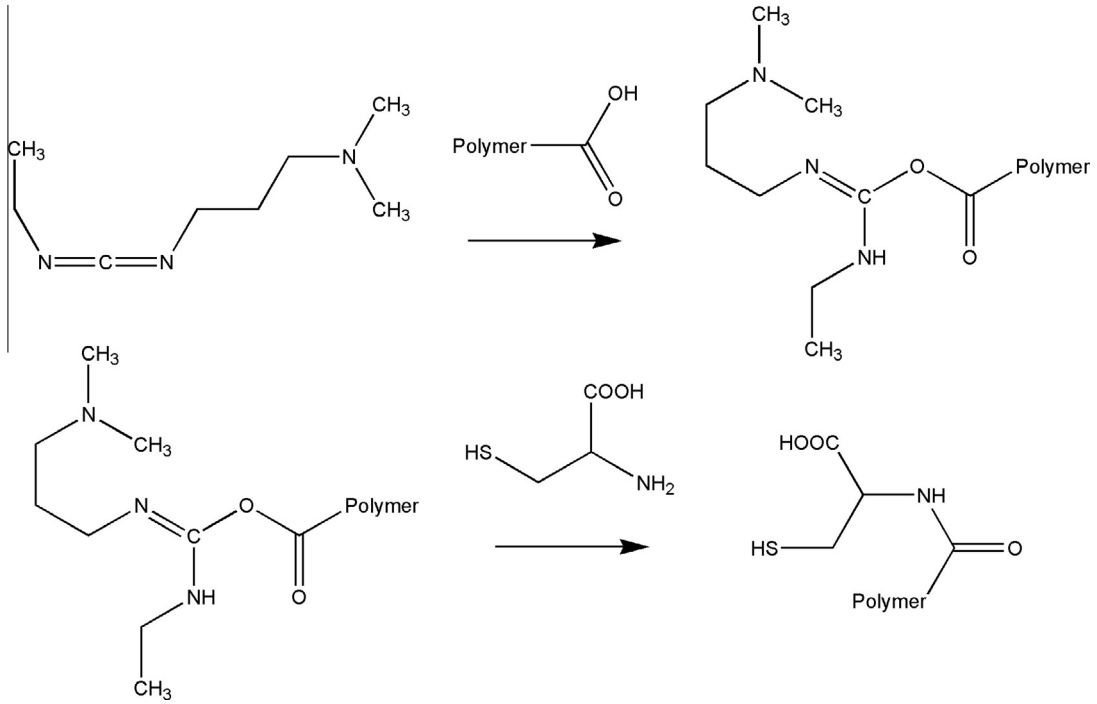

Fig. 3. Synthetic pathway for the covalent attachment of L-cysteine to polycarbophil and carboxymethylcellulose mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [28].

the thiolated polymers reported in this chapter can be readily converted into disulphide cross-linked hydrogels. The in situ formed cross-links can stabilise the structure as reported by Marschütz and co-workers [30]. A cysteine-modified poly(acrylic acid) of high molecular weight could form disulphide cross-links, which improved the structural cohesiveness of the gel, while the remaining thiol groups in the network ensured excellent mucoadhesive characteristics. The rheological characteristics of the hydrogel could be controlled with the initial molar ratio of thiol groups introduced into the polymer backbone. In another application-oriented work, a thiolated chitosan was synthesised by the same group [34]. A high degree of thiolation was used; thus, the polymer could form a hydrogel within 30 min under physiological conditions.

Gyarmati and co-workers [35] synthesised thiol-modified poly(aspartic acid) with reversible sensitivity to environmental redox potential. The particular benefit of the synthesis route is that polysuccinimide, a highly reactive polymer, was used in the initial step of the thiolation; thus, the degree of modification could be easily controlled by composition, and the reaction could be performed under mild conditions. The characterisation of the sol–gel–sol process of the synthesised poly(aspartic acid)s—including rheological measurements, Raman microscopy and Ellman's assay—proved its reversibility in more cycles. In addition to their possible use as injectable materials, the authors proposed reduction induced targeted drug delivery as a potential application.

The great advantage of the formation of disulphides from the thiolated form over the direct incorporation of disulphides is that the modification of polymers can be precisely adjusted during synthesis (polymer concentration, thiol-to-polymer molar ratio and pH), and the composition of the polymer (most interestingly, the grafting density of thiols) can be determined quantitatively. Therefore, in the second (oxidation) step, a polymer with a well-defined structure is converted into a gel, which increases the reproducibility of the gelation process.

2.3. Characterisation methods for the qualitative and quantitative determination of thiol and disulphide groups

Different analytical methods were developed to quantify the concentration of thiol and/or disulphide groups in polymers and polymer networks. The sulphur content can be determined through an elemental analysis of the polymers [33]. However, the number of thiol groups or disulphide groups is of higher importance than the total number of sulphur atoms.

Most papers focus on the determination of immobilised thiols [17]. Ellman's assay is widely used because of its simplicity and reliable results. Ellman's reagent is converted into its reduced form in the presence of thiols and can be measured directly by UV–Vis spectrometry. However, a wide range of derivatisation agents is available for quantifying thiols, mostly in aqueous medium (for details, please see Table 1). Most of these reagents are also used in high-performance liquid chromatography for pre-column and post-column derivatisation to analyse mixtures of thiols (e.g., endogenous compounds). The released products of reagents can be detected spectrophotometrically, but in the case of ortho-phtal-dialdehyde, monobromobimane and *N*-(1-pyrenyl)maleimide or diethylamino-3-(4′-malei-

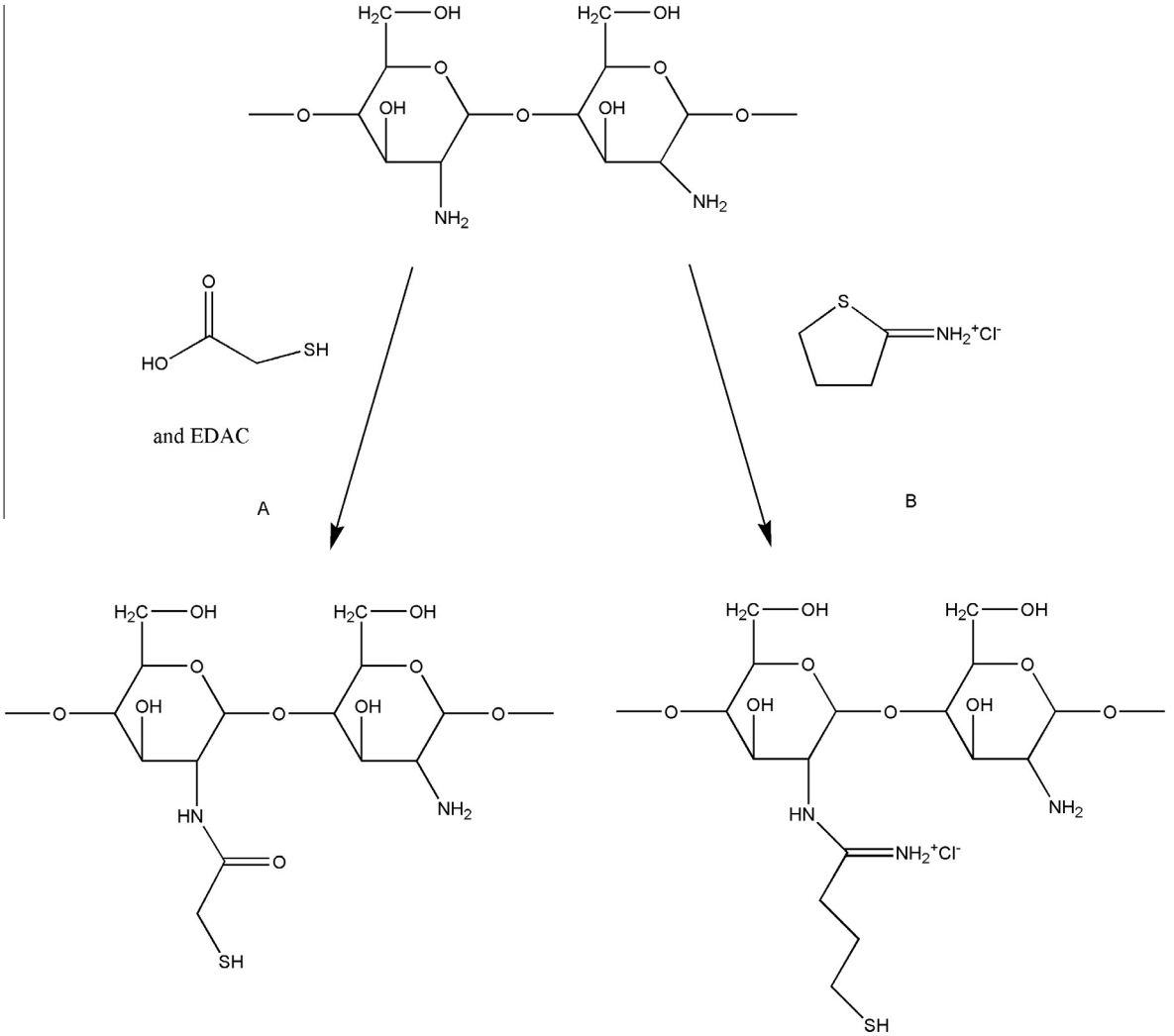

Fig. 4. Synthetic pathways for thiolating chitosans with (a) thioglycolic acid mediated by a carbodiimide and (b) 2-iminothiolane [27].

midophenyl)-4-methylcoumarin an increased selectivity can be achieved by using laser induced fluorescence. We highlight the work of Hansen et al. for 4,4′-dithiodipyridine, which is applicable to detect thiols in the picomolar range, and, as opposed to other reagents, it can be used in acidic medium, which prevents thiols from ambient molecular oxidation during the measurement. It is worth to note that indirect methods can also be accurate in certain cases (e.g., the degree of thiolation of gelatine polymers was determined by measuring the remaining amine side groups of gelatine [17]).

When thiol groups are converted into disulphides in the presence of an oxidising agent (chemical reagent or air), the decrease in thiol groups can be continuously followed by Ellman's assay [26]. Raman spectroscopy is an effective method for detecting thiols and disulphides at the same time (Fig. 6), and the method has the great advantage that no special sample preparation is needed prior to the measurement. Aliyar et al. [24] investigated the liquefied and re-gelled state of a disulphide-containing, acrylamide-based polymer by Raman measurements.

The decrease in the number of thiol groups is caused by the formation of intra- and intermolecular disulphide bonds. Intermolecular disulphide linkages affect the viscoelastic properties of the thiolated polymers [42]. As the number of thiol groups decreases and these groups are converted into disulphides, the storage modulus increases significantly, which proves the in situ cross-linking process. The authors also proved by frequency-sweep measurements that the initial, physically entangled network becomes a chemically cross-linked polymer during disulphide formation. Chemical and viscoelastic changes can be followed simultaneously, as shown in Fig. 7 [42]. Vlierberghe et al. [32] used rheometry to characterise the oxidation-induced increase in the storage modulus of thiolated gelation.

2.4. Effect of reaction conditions on the formation and cleavage of disulphide linkages

The kinetics of thiol–disulphide reactions is an essential issue during applications. The gelation time of the

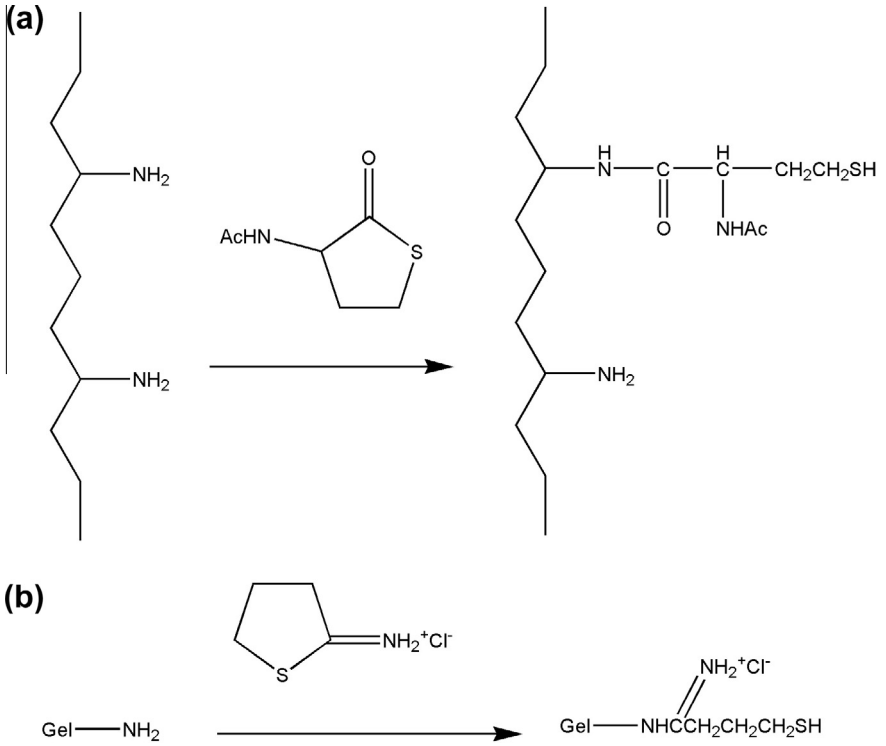

Fig. 5. Synthesis scheme for the preparation of thiolated gelatine using (a) *N*-acetylhomocysteine thiolactone and (b) Traut's reagent [32].

Table 1
Commonly used reagents for quantification of thiols.

Reagent	Solvent, pH	Detection	Refs.
5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent)	Water, pH = 7.5–8.5	Absorbance	[36]
Ortho-phtal-dialdehyde (OPA)	Water, pH = 7.5–10.5	Absorbance/fluorescence	[37,38]
Monobromobimane (MB)	Water, pH = 8.0–8.5	Absorbance/fluorescence	[37]
N-(1-Pyrenyl)maleimide (NPM)	Acetonitrile	Absorbance/fluorescence	[39]
4,4′-Dithiodipyridine (DPS)	Water, pH = 2.5–7.0	Absorbance	[40]
Diethylamino-3-(4′-maleimidophenyl)-4-methylcoumarin (CPM)	DMSO/water, pH = 7.4	Fluorescence	[41]

injectable materials must be controlled from seconds to minutes. The reduction induced delivery of genes and proteins also demands knowledge of the factors that affect the rate of reduction.

Reduction of disulphides is usually realised in a thiol–disulphide exchange reaction since thiol-containing reducing agents are preferred in applications to mimic biological conditions (e.g., glutathione mediated drug delivery). A significant number of oxidation processes also involve thiol–disulphide exchange (e.g., using dithiopropionic acid as an oxidising agent). The general reaction scheme is summarised by the following equation:

$$2\text{R-SH} + \text{R}'\text{-SS-R}' \rightleftharpoons \text{R-SS-R} + 2\text{R}'\text{-SH} \quad (1)$$

The detailed mechanism is shown in the following equation:

$$\begin{aligned} &\text{R-SH} + \text{H}_2\text{O} \rightleftharpoons \text{R-S}^- + \text{H}_3\text{O}^+ \\ &\text{R-S}^- + \text{R}'\text{-SS-R}' \rightleftharpoons \text{R-SS-R}' + \text{R}'\text{-S}^- \\ &\text{R-SS-R}' + \text{R-S}^- \rightleftharpoons \text{R-S-S-R} + \text{R}'\text{-S}^- \\ &\text{R}'\text{-S}^- + \text{H}_3\text{O}^+ \rightleftharpoons \text{R}'\text{-SH} + \text{H}_2\text{O} \end{aligned} \quad (2)$$

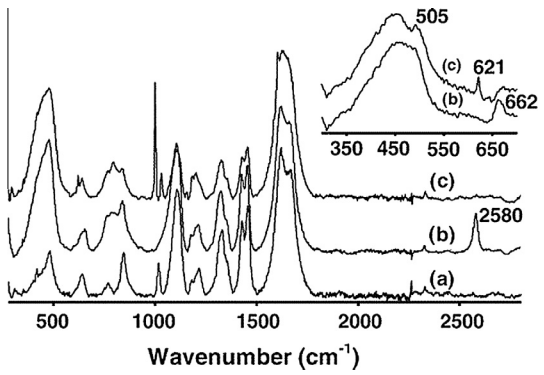

Fig. 6. Raman spectra of (a) polyacrylamide polymer, (b) disulphide-containing polymer in liquefied and (c) re-gelled state. The characteristic peaks of thiols (662, 2580 cm^{-1}) and disulphides (505, 621 cm^{-1}) can be easily distinguished (reprinted with permission from Ref. [24] 2005, American Chemical Society).

The key component in the exchange reaction is the deprotonated form of the thiol compound [43]. The concentration of thiolate anions is determined by the pH of

the medium; therefore, the environmental pH plays a decisive role in the reaction rate of thiol–disulphide reactions. The pH-values under the pK_a of the thiol impede the exchange reaction while increasing the pH, whereas alkaline pH especially accelerates it.

A number of reactive oxidising agents are generated in the different pathological states of cells, especially under oxidative stress [44]. Hydrogen peroxide, superoxide, hypochlorous acid and other oxyradicals are the most common species in human cells. Almost all these reagents can react only with the thiolate anion (except hypochlorous acid); therefore, disulphide formation is strongly affected by the pH under physiological conditions. The oxidation of thiol by air also has a strong pH-dependence. The key step of the oxidation is the formation of a thyil radical in a one-electron transfer [45].

$$\begin{aligned} &\text{R-SH} + \text{H}_2\text{O} \rightleftharpoons \text{R-S}^- + \text{H}_3\text{O}^+ \\ &\text{R-S}^- + \text{O}_2 \rightleftharpoons \text{R-S}^{\cdot} + \text{O}_2^- \\ &2\text{R-S}^{\cdot} \rightarrow \text{R-S-S-R} \end{aligned} \tag{3}$$

In several cases, the mechanism involves a two electron transfer type step with the generation of sulphenic acid in the intermediate step. The possible mechanism is generally determined by the oxidising agent; however, the pH-dependent character is observed in almost every case; therefore, we discuss the effect of pH independently from the exact mechanism of disulphide formation. As it will be shown, the role of the oxidising agent is also essential because the reaction kinetics and selectivity are not only determined by the pK_a value of the reactants but the mechanism of oxidation also effects the reaction rate and the ratio of side reactions to disulphide formation. The majority of papers concentrate on the formation of disulphides from thiols, however, we emphasise that the over-oxidation of disulphides to sulphenic or sulphonic acid moieties can also be exploited to extend the multi-responsive properties of a polymer network with oxidation-induced degradation [46].

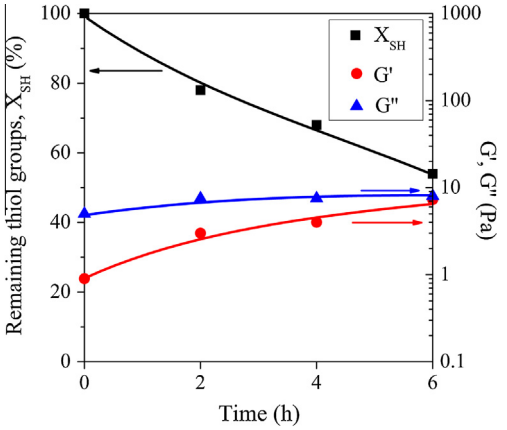

Fig. 7. Correlation between the increase of the storage modulus G′ (red circles) and loss modulus G″ (blue triangles), and the decrease of free thiol groups (black squares) of chitosan–thioglycolic acid polymers at pH 5.5. The increase in the storage modulus is caused by disulphide formation (adapted with permission from Ref. [42] 2003, Elsevier). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

An excellent example of the influence of pH is reported by Bernkop-Schnürch et al. [26] for a cysteamine-conjugated polymer. The reaction rate of disulphide formation from thiol side chains had a strong pH-dependence. Disulphide formation was practically inhibited in acidic medium, while an increased reaction rate of oxidation to the disulphide was observed at physiological pH (pH = 7.4). This phenomenon is especially important for the development of biomedical devices because in situ gelling and mucoadhesive characteristics have to be optimal at the prevalent pH values of membranes in the oral cavities, in the gastrointestinal tract and in the vagina. This pH-dependent oxidation is reported for several polymers, including cysteine- and cysteamine-modified carboxymethylcellulose and polycarbophil [26], thioglycolic acid-modified chitosan and 2-iminothiolane-modified chitosan [27].

Liu et al. [47] exploited the pH-dependent kinetics of the reaction to synthesise an in situ gelling system with controllable properties in a biological process. A core–shell branched polymer was prepared from *N,N′*-bis(acryloylcystamine) and 1-(2-aminoethyl)piperazine, and the polymer molecules could form a disulphide cross-linked core via intermolecular thiol–disulphide exchange reactions. This reaction could be switched on or off by adjusting the pH to basic or acidic values, respectively. Hydrogel properties, including elastic modulus and viscosity, could be tailored by reaction time, and the reaction could be re-started at any time by modulating the environmental pH. The living polymerisation process can be exploited further to prepare micro/nano-hydrogels, fibres and capsules with precisely controlled properties.

Apart from the environmental pH, the type of oxidising agent also strongly affects the rate of disulphide formation. The control of gelation time was investigated in the work of Hisano et al. [25]. The oxidising agent had a well-defined influence on the gelation time: oxidation in the air took from 6 to 24 h, depending on the pH of the solution, while the application of chemical oxidising agents decreased the gelation time to seconds. Well-defined differences exist between the chemical oxidising agents as well; the use of 3,3′-dithiodipropionic acid led to a gelation time of 100–1000 s, while cystamine shortened the time of gelation to less than 10 s. As demonstrated here, the pH and type of oxidising agent play a decisive role in the gelation process.

We also would like to draw attention to the fact that the type of reducing agent can modify the response of a hydrogel. Pan et al. [48] showed that using glutathione instead of dithiothreitol can drastically change the release profile of encapsulated doxorubicin. In most cases, papers report reducing and oxidising agents that can be applied under mild conditions, like dithiothreitol, glutathione and solid phase reductants [49] or dilute solutions of hydrogen peroxide and DMSO [50]. Most of these reagents are well-known and are widely used in protein chemistry [51–53].

We would like to emphasise that real biomedical applications or clinical trials usually involve oxygen or air as the oxidising agent instead of other potentially harmful chemicals. However, the rate of disulphide formation is unsatis-

factory in several cases, as shown by the group of Tirelli [41,54]. They exposed nanoparticles with cysteine thiols to air for 48 h, and even after disulphide formation, the nanoparticles still contained a considerable number of free thiols, up to 22% of their initial number [41]. The Tirelli group recognised that air induced disulphide formation can be enhanced by increasing the concentration of free radicals. The disulphide content could be increased to 90% by applying ammonium persulfate and tetramethylethylenediamine. Free radicals are also generated by all aerobic cells and are known to participate in a wide variety of biological and biochemical processes [55]. Fenton-type reactions (i.e., the formation of hydroxyl and ferryl radicals from peroxides and iron(II) compounds or complexes) play a crucial role in the physiological and pathological processes in biological sites. Therefore, oxidation-induced gelation catalysed by Fenton-type reagents can be advantageous in injectable materials with in situ gelation. Goessl et al. [54] synthesised a thiol-modified, branched PEG-based polymer, and its reaction with oxygen was accelerated by the use of a Fenton-based catalyst. This material showed an accelerated gelation under physiological conditions. Considering real clinical needs, disulphide formation upon storage is also an important issue. There are only a few examples of methods that can minimise the oxidation of thiols prior to the use of the polymers. The same paper by Goessl et al. [54] reported a synthetic route to avoid disulphide formation. A PEG-based allyl-functionalised star polymer was synthesised, and protected thiols were introduced as polymer end-groups by the thiol-ene type addition of thioacetic acid to the allyl groups. Protective groups could be readily and quantitatively removed by using sodium hydroxide, and the following oxidation step could be initiated with a polymer solution using a precisely known thiol content. The same method of Tirelli et al. was utilised to prepare oxidation sensitive polymeric nanoparticles with controllable free thiol content [46]. Because disulphide formation, even in the case of solid polymers, may be not negligible, special care should be taken when handling polymers with active thiol content, and a thiol protective strategy should be taken into consideration to provide precise control over the cross-linking and gelation processes.

In addition to the thiol content, the rate of oxidation is determined by the polymer concentration and molecular weight. This latter factor plays an essential role in the kinetics of disulphide formation. For example, the thiolated chitosan, with a medium molecular weight (20 kDa) did not form a hydrogel, even with a high thiol content, but rapid gel formation occurred with a lower thiol content when the molecular weight was larger (300 kDa), as reported by Wua et al. [56]. Swelling and mechanical characteristics could be easily controlled and tuned to in vitro applications for protein delivery and cell encapsulation.

We must emphasise that the thiol–disulphide interconversion is not only induced by redox reagents but the disulphide exchange reactions can also be performed by photoirradiation. Otsuka and co-workers [57] prepared a disulphide-containing polyester with a narrow molecular size distribution in this way. After UV photoirradiation, the size distribution of the polyester broadened gradually with increasing irradiation time. Interestingly, when polymer fractions with two different molecular weights were prepared and irradiated in the same medium, a monomodal polymer was obtained. The authors proved with experiments using small-molecular weight components that the transformation into the broad and later monomodal size distribution resulted from the photoinitiated disulphide exchange reactions. This method can be applied to control the molecular weight after polymerisation in a bulk film and to hybridise the polymers with disulphide groups.

In conclusion, there are many methods to control disulphide formation; all material properties (molecular weight of the polymer and thiolation degree) and reaction parameters (polymer concentration, pH, oxidising and reducing agents) can be at our service to adjust the hydrogel characteristics depending on the proposed applications.

3. Application of thiolated polymers and disulphide cross-linked hydrogels

The disulphide formation of thiol groups and the reduction of disulphides to thiols are utilised in a number of synthetic strategies, as described previously. Furthermore, thiol–disulphide interconversion is a key step in many biological reactions, such as protein folding or glutathione-mediated redox processes. Redox potentials between the extra- and intracellular interior show a defined difference and also vary significantly in certain pathological states of cells and tissues. The exploitation of these characteristics combined with the benefits of hydrogels, including biocompatibility, controllable properties and high drug loading capacity, leads to various applications, from bioartificial use to the development of targeted drug delivery platforms. Redox-responsive behaviour can also contribute positively to the development of self-healing materials, as well as molecular and shape imprinting networks because thiol–disulphide interconversion can be precisely controlled by the reaction conditions.

3.1. Injectable polymers with a sol–gel transition

Injectable hydrogels are formed from pre-cursor polymer solutions by chemical or physical cross-linking. Externally induced sol–gel transition has received increased attention in injectable pharmaceutical applications and tissue engineering, as discussed recently in several reviews [58–60]. The main advantage of this class of materials is that only minimal surgery is needed to insert the material with the encapsulated drug or gene into the body. The precursor polymer solution is administered by a minimally invasive injection, and gelation occurs in vivo.

Temperature and pH-gradients are utilised to induce sol–gel transitions in most cases [59,60]. Responsive polymers, which are sensitive to both triggers, can be particularly beneficial because clogging can be avoided during injection due to the temperature itself—if the temperature elevates for any reason, it cannot induce gel formation. The degradation rate and release kinetics can be fine-tuned accordingly to different applications. Although the exploitation of redox-sensitivity is mentioned only in a few

papers [19,22,34,54,61], it may have an emerging potential in the field, considering recent trends. Several articles report hydrogels that can be chemically cross-linked, and the formed hydrogels usually have advantageous mechanical properties over the physically cross-linked gels [62]. A general drawback of this method is that the cross-linking reaction might be harmful in the case of in vivo applications. A polymer network with chemical cross-links can be an ideal candidate only in those cases when the use of a real, small-molecule cross-linking agent can be avoided. One particular requirement is that the heat of gelation should be sufficiently small so as not to damage the surrounding tissues or organs. Polymers with thiol moieties fulfil these requirements when an oxidising agent is applied to induce gelation [24].

Because hyaluronic acid is a major component of the extracellular matrix, hyaluronic acid-based polymers should be ideal candidates for injectable materials. A general work was published by Liu et al. [63] that provides an overview of the bio-related issues independent of the proposed application. At a first glance, short- and long-term implants and artificial tissues are the most promising uses of these polymers in the macroscopic size range. However, these types of applications have several requirements regarding stability, biodegradability, biocompatibility, and cell adsorption characteristics. In the referenced work, a highly thiolated hyaluronic acid polymer was oxidised by air and further disulphide linkages were built by adding a dilute solution of hydrogen peroxide [64,65]. Stability and degradation were investigated in vitro by adding bovine testicular hyaluronidase and in vivo by implanting small discs into rats. The films were stable for 1 week in a buffer solution not containing the hydrolysing enzyme. In the presence of the hyaluronidase enzyme, degradation was significantly accelerated, which proved that the intramolecular disulphide linkages and thiolation of the polymer do not hinder access to cleavable sites, ensuring the biodegradability of the films. Biodegradation was also confirmed by slow degradation in rats. Degradation was always slower when the polymer film was stabilised by a larger number of disulphides (i.e., the degradation rate could be controlled). The cytocompatibility of the films was investigated by growing fibroblast cells for 6 days in wells containing the films. The polymer was not cytotoxic to the cells, but coating the films with poly D-lysine was necessary to improve cell attachment. The films proved to be an excellent medium for cell growth. The lack of additional cross-linkers was especially important because the use of small-molecular weight, possibly toxic, cross-linkers necessitates the careful removal of the excess cross-linkers for biomedical uses, and this often requires difficult purification steps. The in vivo tissue reactions were characterised by different methods following the subcutaneous implantation of hyaluronan films into rats, including infiltration of polymorphonucleocytes, infiltration of lymphocytes and the degree of vascularisation. Further investigations were made on films implanted into the peritoneal cavity of rats. These results suggested that the implants caused a very mild tissue reaction, and the reactions could be considered a typical foreign body reaction; therefore, the films can be further investigated in clinical applications. As shown, there is a need for very diverse assays to prove the possibility of biological applications. The results of several papers must be handled with care because they report the synthetic pathway, in vitro experiments and only the preliminary biological tests in most cases. The perspective of these studies for practical applications is usually difficult to predict, but the concepts are valuable and may contribute to the development of the field.

Anumolu et al. [66] developed a disulphide cross-linked hydrogel from 8-arm-poly(ethylene glycol) by oxidation with hydrogen-peroxide. The hydrogel could be reversibly dissolved in a reducing medium containing glutathione (Fig. 8). The main objective was to apply the in situ gelling system as an implantable drug delivery vehicle in dermal wound healing, and the gel should be easily removed by reduction after the healing process.

The authors induced dermal wounds by the topical application of nitrogen mustard (NM) on mice. The precursor polymer with doxycycline along with a proper amount of hydrogen peroxide were injected into the wound, and further additives such as glycerine, poly(vinyl-pyrrolidone) and poly(ethylene glycol) were applied to enhance the retention of the hydrogel. Skin biopsies were taken to determine the efficiency of wound healing, and samples from untreated mice and mice treated with a hydrogel containing placebo were applied as controls. Preliminary drug release experiments showed sustained release of encapsulated doxycycline for 10 days. Skin healing was followed over 240 h. The in vivo wound healing efficacy was high in the case of doxycycline hydrogels compared to the NM-exposed, untreated skin and the skin treated with placebo hydrogels (Fig. 9).

An even more complex challenge is to design hydrogels for utilisation as artificial organs. Hisano and co-workers [25] prepared a reversible disulphide-containing polyacrylamide hydrogel that shows promise as a bioartificial pancreas in future applications. The developed polymer network met several requirements essential for successful application: the hydrogel and the oxidising agent did not damage the islets, and the islets could fulfil their function in the matrix, i.e., they responded to the elevated glucose level with insulin secretion (Fig. 10).

The applied oxidising agent strongly affected the function of the islets (e.g., cystamine deteriorated the islets). However, by applying the proper oxidising agent, the authors could prepare a hydrogel that impeded the aggregation of encapsulated islets and could be easily liquefied when there was a need for refilling the bioartificial pancreas. A relatively high concentration of polymer can be prepared, which prevents the permeation of immune components such as antibodies. Despite the lack of in vivo experiments at the present state of the work, the proposed hydrogel could be an excellent candidate for artificial organs.

We can conclude that the controllable thiol–disulphide reaction has led, and surely will continue to lead, to an incredibly wide range of applications in injectable implants, cell growing media and tissue engineering. And, hopefully, the potential of this scientific field will be exploited in the next decades in the service of human health.

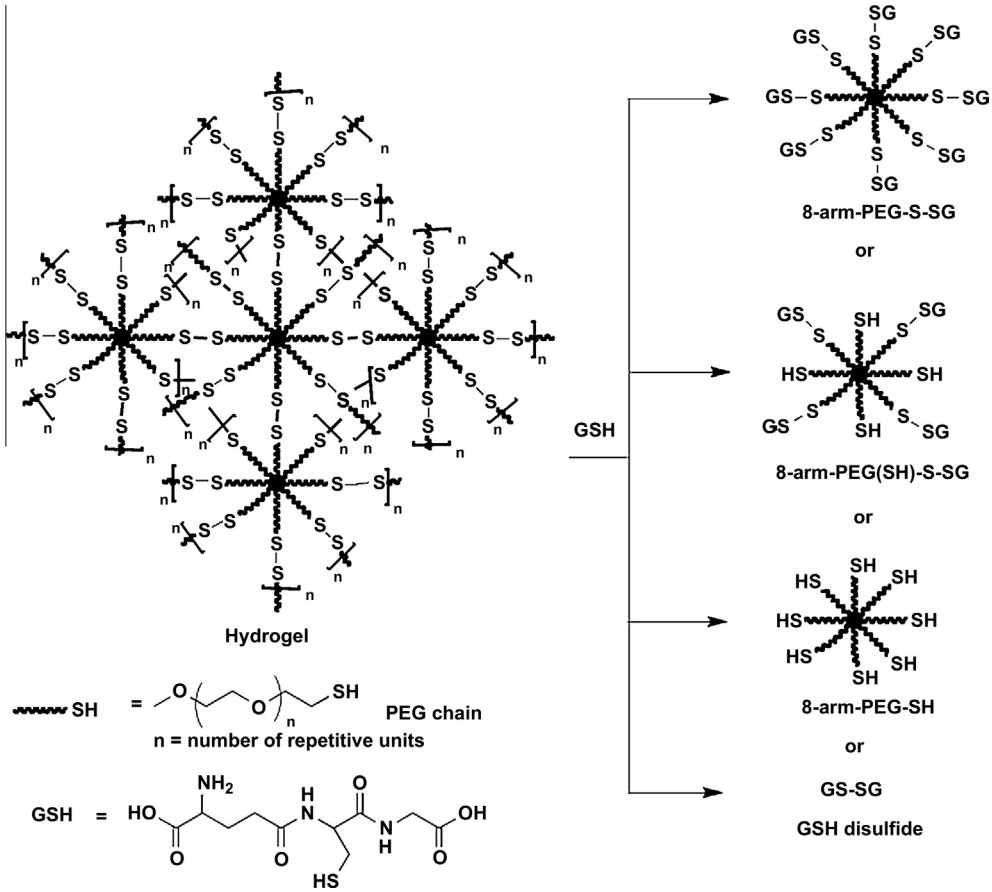

Fig. 8. Glutathione-induced dissolution of hydrogel into 8-arm moieties (reprinted with permission from Ref. [66] 2011, Elsevier).

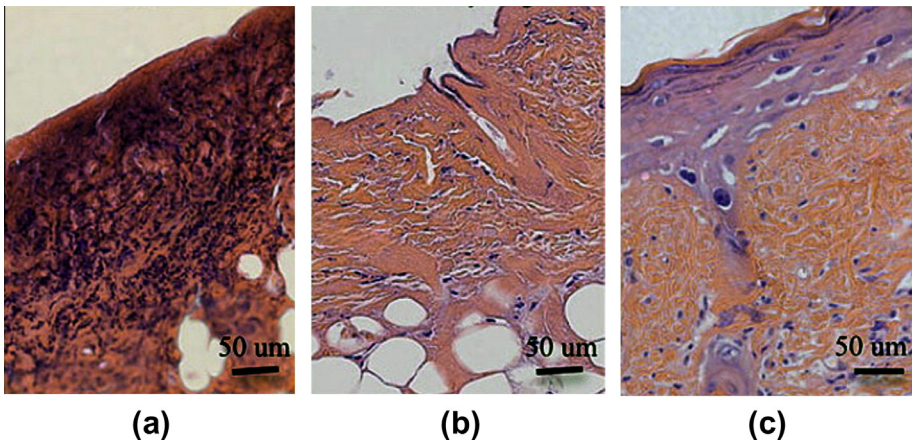

Fig. 9. Histology of mouse skin. (a) A 168 h post-exposure to NM caused extensive necrosis of the skin. (b) Placebo hydrogels provided some protection. (c) A significant improvement in the skin was found in the case of post-treatment with doxycycline-containing hydrogels (reprinted with permission from Ref. [66] 2011, Elsevier).

3.2. Targeted drug and gene delivery

The utilisation of thiol or disulphide containing polymer networks in targeted delivery vehicles also appears to have great potential. We refer here to the thorough work of Schafer et al. [67] to explain the basic principles of redox-mediated applications. The authors clarified the term "redox state" earlier as being the ratio of a certain

redox couple in the biological fluid or cell (e.g., NAD+ and NADH), but it should be defined as a real electrochemical potential that can be quantified, similar to the classical Nernst-equation. Each process in the cells during oxidative or reductive stress can be explained by the redox potentials of the cell compartments. The most important redox couples in biological platforms are NAD+/NADH, NADP+/NADPH, the thioredoxin system and glutathione (oxidised-reduced). Presently, we have the most detailed information on the role of glutathione. It is clear that glutathione plays an essential role in the control of cell life through the folding of proteins. Basically, the concentration of reduced glutathione differs significantly in the extra- and intracellular area, and the concentration changes during the lifetime of the cell. Typically, the intracellular concentration is approximately 10 mM, while the concentration is approximately 0.002 mM in the extracellular compartments [8]. The role of several other oxidising species (e.g., hydrogen peroxide, peroxynitrite and hypohalogenous acid) should also be considered [44]; however, the most abundant redox mediator, glutathione, is targeted for biomedical uses. In cancer cells, proliferation becomes the dominating process, which leads to a strongly reducing environment. This phenomenon is discussed in a large number of related publications [19,23], although the redox mechanisms are not known precisely in biological systems. We do not go into more details in redox biochemistry in this article, but we are certain that vast knowledge will accumulate in this area in the next few decades that may lead to an extended use of redox-sensitive hydrogels in targeted delivery. Presently, a great number of papers report the results of in vitro cell tests for the prepared responsive hydrogels due to a general interest in biomedical applications. A significantly smaller number of publications discuss detailed in vivo investigations. We present the most important studies in this area here because there is an increasing demand to discuss material characteristics from this aspect for the selection of biomaterials with the genuine potential for applications in human health care.

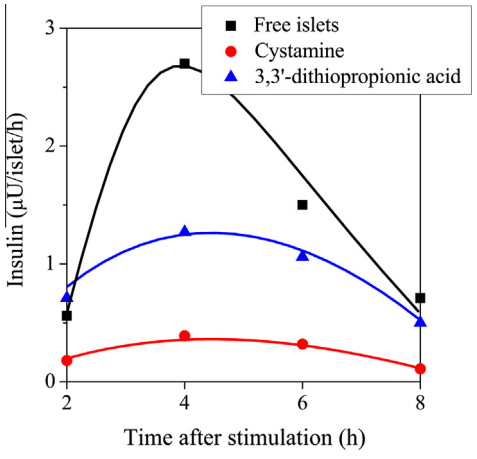

Fig. 10. Increase in insulin release of disulphide-containing polyacrylamide gels in response to glucose stimulation. Burst release occurred in the case of free islets (black squares), while insulin release was sustained from hydrogels oxidised by 3,3′-dithiopropionic acid. Cystamine damaged the islets, resulting in the poor release of insulin (reprinted with permission from Ref. [25] 1998, Wiley).

Moore et al. [68] characterised the swelling kinetics of 2-hydroxyethyl methacrylate (HEMA) hydrogels cross-linked with *N,N′*-cystamine-bis(acrylamide). In this case, the cross-linker possessed redox-sensitive properties, while intrinsic cross-linking points were proposed to arise from chain transfer processes during polymerisation as permanent cross-links. Swelling characteristics depended strongly on polymer composition, environmental pH and the concentration of the reducing agent, leading to potential applications in drug delivery as well as for biosensors. Bromberg and co-workers [69] reported poly(acrylic acid) microgels covalently bonded to pluronic polyether copolymers with an effective permanent cross-linker and a disulphide cross-linker. The swelling kinetics were determined in both directions: an increasing swelling ratio was observed when reducing agents were applied, while the gels shrunk in oxidising media containing hypochlorite. We would like to emphasise the "kinetic selectivity" observed by the authors, whereas significantly faster swelling occurred in the presence of a strong reducing agent, tris(2-carboxyethyl)-phosphine (TCEP), compared to dithiothreitol. This phenomenon can be of great importance in the controlled delivery of drug molecules and site-specific answer of hydrogels. Gaulding et al. [70] prepared poly(*N*-isopropyl methacrylamide)-based hydrogels with dual-crosslinks by applying a nondegradable cross-linker and *N,N′*-bis(acryoyl)cystamine. The swelling degree could be changed by adding a reducing agent to the solution. The most exciting part of their work was the synthesis of the so-called "gel of microgels". The reduced, double cross-linked particles created interparticle cross-links after oxidation when the concentration of particles was large enough, resulting in a bulk hydrogel with elastic properties (elastic modulus was above 1 kPa, which is comparable to conventional disulphide cross-linked bulk hydrogels). The particles could be released again in reducing medium. This system is suitable for the design of a delivery vehicle for the sustained release of drug molecules via the delivery of microgel particles.

Kakizawa and co-workers [71] contributed to the field with a polyion complex micelle stabilised with a disulphide bond. Poly(aspartic acid) was used a polyanion and thiol-modified poly(ethylene glycol)-block-poly-(L-lysine) was used as a polycationic component. After the spontaneous association of the components, the core of the micelle was stabilised by aerial oxidation of the thiol side chains into disulphides. Stabilisation occurred through disulphide formation as proven by light scattering measurements. The cleavage of disulphide bonds by a reducing agent led to the complete dissociation of the micelles and to the release of poly(aspartic acid). Their system can be considered to be the model of a delivery vehicle for negatively charged DNA chains. The proposed vehicle can dissociate under physiological conditions due to the increased concentration of glutathione in the intracellular compartments. Transport through the cellular membranes can be improved by introducing ligands, which enhance receptor-mediated endocytosis.

After introducing the principles for the utilisation of disulphide-containing hydrogels as delivery vehicles, we report a number of promising in vivo results and potential applications in the biomedical area. Wang et al. [72] synthesised chitosan-*N*-acetyl-L-cysteine nanoparticles to develop a nasal insulin delivery device. Nanoparticles were prepared via the ionic gelation of thiol-modified chitosan with tripolyphosphate; thus, cross-links did not form primarily by disulphide bridges. The main role of the incorporated thiol groups was to improve mucoadhesiveness because the interaction was strongly improved by the thiol groups, and thiolation also enhanced the solubility of chitosan as well as the encapsulation efficacy of insulin. Thiol groups can form disulphide linkages with the cysteine residues of the mucus layer and prolong the delivery process of the drug. Reduced cytotoxicity is a basic advantage of thiolated chitosan as opposed to chitosan-4-thiobutyl-amidine, which also has considerably high pharmacological efficacy. A very simple approach could be the introduction of a large number of thiol groups into the polymer to achieve unique mucoadhesiveness. However, according to the authors, thiol groups have an optimal concentration, above which the mucoadhesiveness is reduced. This can be explained by the thiol–disulphide exchange reaction involved at high thiol concentrations. Thiols formed disulphides in aqueous medium by air oxidation, and disulphide bridges caused a rigid structure. The rigidity and the reduced number of thiol groups caused a weaker interaction between the nanoparticles and the mucus layer. The results suggested that precise control over the concentration of thiol groups was needed to produce nanoparticles with good adhesive characteristics. In this case, in vivo experiments showed prolonged release of insulin, resulting in a significantly decreased blood glucose level in the rats, while orally administered insulin did not affect the glucose level.

Hahn et al. [73] prepared injectable hyaluronic acid microhydrogels for a controlled release formulation of erythropoietin (EPO), a red blood cell stimulating drug molecule. The microhydrogels were synthesised from thiol-modified hyaluronic acid by oxidation with sodium tetrathionate. Oxidation occurred in 30 min compared to the 1 day reaction time of air oxidation. Spray drying was used to prepare microparticles in the presence of Tween 20 as a surfactant. The particle size was approximately 2–3 μm. In vitro measurements showed a burst release of EPO for 3 days and a sustained release for another 9 days. In vivo measurements in rats showed that the EPO formulation resulted in an elevated plasma concentration, and an efficient concentration of EPO could be maintained for up to 7 days. The drug molecule did not lose its biological activity, and the formulation did not show any adverse effects during the animal experiments. These results demonstrated that microhydrogels are promising injectable systems for the controlled administration of various drug molecules.

Apart from particle-based targeting, gene delivery with reduction-sensitive micellar polymeric vehicles has been a rapidly developing area in recent years [19]. Polycationic devices are widely used for the encapsulation of negatively charged DNA sequences. Polyethylenimines (PEI) have particularly advantageous features for these types of applications because they have a strong DNA compaction capacity, intrinsic endosomolytic activity and relatively high transfection efficacy. However, gene delivery efficiency can be enhanced by increasing the molecular weight, which also increases cytotoxicity. To overcome this problem, Peng et al. [74] reported the synthesis of a disulphide cross-linked PEI, which had a compact structure in the oxidised state, but that structure could be reduced by introducing glutathione into the intracellular milieu, leading to the release of the encapsulated genes inside the cells. Low molecular weight PEIs were treated with methylthiirane to incorporate thiol moieties, which were oxidised to disulphides by DMSO. The hydrogel could form a very stable complex with the plasmid DNA due to its polycationic nature. These so-called polyplexes (i.e., complex compounds of the polycationic and polyanionic species) were formed in the nano-size range. Cytotoxicity tests showed the reduced toxic effects of these polyplexes compared to the non-modified PEI of the same molecular weight, which could be explained by the biodegradability of cleavable disulphide cross-links. These polyplexes produced a higher gene transfection efficiency than the polyplexes of the non-modified PEIs during in vitro experiments on cells. The authors investigated the effect of DMSO on oxidation and observed that the reaction could also be performed by the air, but this reaction was significantly slower than the reactions involving chemical oxidising agents. The transfection efficiency of polyplexes synthesised by air oxidation was low, but the efficacy could be gradually increased with reaction time; therefore, air oxidation could be used as a precise control of disulphide formation. Consequently, disulphide cross-linked PEIs can be utilised as an advantageous alternative to high molecular weight PEIs.

As a final example of gene delivery solutions, we refer to the work of Mok and Park [75]. They used the so-called KALA peptide to prepare polyplexes. The KALA peptide is a synthetic molecule of 30 amino acids with cysteine residues at both terminal ends, and it could be organised to form polyplexes by the oxidation of thiol groups to disulphide bridges with DMSO. According to gel electrophoresis, the self-cross-linking process connected 10 monomers to each other on average. The polyplexes formed nanoscale complexes with small interfering RNA molecules by means of electrostatic interactions. The cytotoxicity was much smaller than that of conventional PEIs, while the gene transfection efficiency was similar. A great advantage of these networks is that they show unique gene silencing efficiencies, which can lead to numerous applications in nucleic acid based therapeutics. We would like to emphasise that the chosen examples were used to demonstrate the utilisation of the reversible thiol–disulphide exchange. We recommend the work of Kim et al. [23] for further examples of bioreducible polymers, which can be applied in gene delivery.

We can conclude that, according to literature data, the application of redox-responsive polymeric matrices as delivery vehicles has just begun to evolve in recent years. A number of exciting applications of thiol–disulphide containing polymers has been reported in this field; however, the benefits of degradable, easily cleavable and re-

formable disulphide bridges will certainly be exploited to a greater extent in the future.

3.3. Self-healing materials

In this section, we review an important advanced application of thiol–disulphide interconversion, which is not primarily related to biomedical uses, but it may also find applications in regenerative medicine. A general review of self-healing materials, including their importance, mechanisms, theory and future challenges, is published by Wool [76]. The main interest in this class of materials arises from their potentially enhanced lifespan and prolonged use, which results from the ability to recover after mechanical and/or thermally induced damage. The molecular mechanism of self-healing usually involves a transition process (e.g., a transition from hard to soft matter and vice versa around the glass transition temperature of the polymers). Here, we focus on reversible interactions that contribute to self-healing features. One approach for preparing self-healing materials is based on the synthesis of supramolecular structures with non-covalent host–guest interactions (e.g., the reversible complexation of a poly(acrylic acid) that possesses ferrocene as a guest polymer with β-CD modified poly(acrylic acid) as a host polymer) [77,78]. Another possible solution is the reversible formation and cleavage of covalent bonds in the structure. These types of self-healing materials can be more stable, but bonds must be able to cleave under mild conditions. Acylhydrazone and disulphide groups are two well-known dynamic bonds that might be suitable for building self-healing materials. Deng et al. [79] reported the preparation of an environmental adaptive poly(ethylene oxide) hydrogel with both acylhydrazone and disulphide bonds (Fig. 11a). The self-healing features at acidic and neutral pH values are not discussed here because these features were independent of the disulphide linkages. It is worth mentioning, however, that the hydrogel can choose self-healing routes to adapt to the environmental pH. Thiol–disulphide exchange became dominant under mild alkaline conditions. Disulphide linkages were cleaved by the reducing agent (dithiothreitol) and re-formed by hydrogen peroxide, indicating a reversible redox-induced sol–gel transition. To demonstrate their adaptive characteristics, the gels were cut into three pieces, the pieces were held together for 24 h in alkaline medium (pH = 9) and a uniform plate formed (Fig. 11b). Tensile tests showed that the breaking stress and strain of the samples reached more than 50% of the original strength and elongation values at the break. Self-healing cycles were repeated, and the mechanical characteristics of the material were similar to those after the first cycle; thus, the process proved to be reversible. Authors suggested that this type of material has potential applications in organ repair and stimuli-responsive drug delivery.

Yoon et al. [61] synthesised self-healing polymer films based on reversible cross-linking through thiol-disulphide exchange reactions and deposited them onto silicon wafers. According to the earlier studies of Matyjaszewski and co-authors [80,81], they studied multiarm star polymers containing thiol groups at the periphery of the branches, which were characterised by a low intrinsic viscosity. SS arm cross-linked, star branched polymers were synthesised by atom transfer radical polymerisation (ATRP), and the subsequent cleavage under reducing conditions to form thiol functionalised THF soluble star polymers is summarised in Fig. 12a and b. The solutions were deposited on silicon wafers and were oxidatively cross-linked. The self-healing process can be efficiently followed by atomic force microscopy (AFM) and optical microscopy (Fig. 12c). AFM was used to induce damage on the surface of the cross-linked polymer on the micrometer and nano-

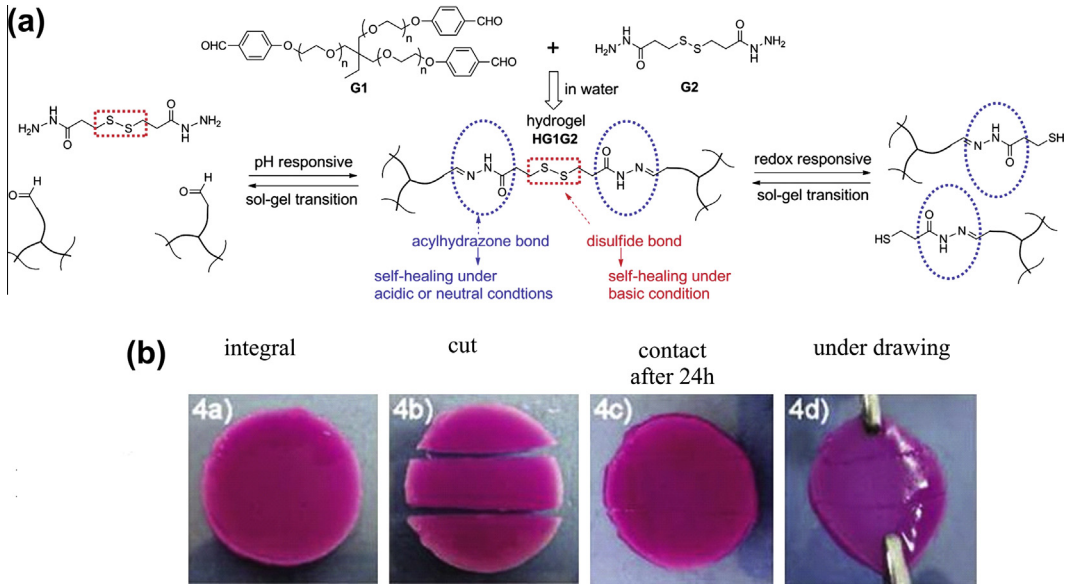

Fig. 11. (a) Strategy for constructing a dynamic hydrogel with an environmental adaptive self-healing ability and dual responsive sol–gel transitions based on acylhydrazone and disulphide chemistry. (b) Self-healing properties of HG1G2 (10 wt%) self-healable hydrogel at pH 9 (reprinted with permission from Ref. [79] 2012, American Chemical Society).

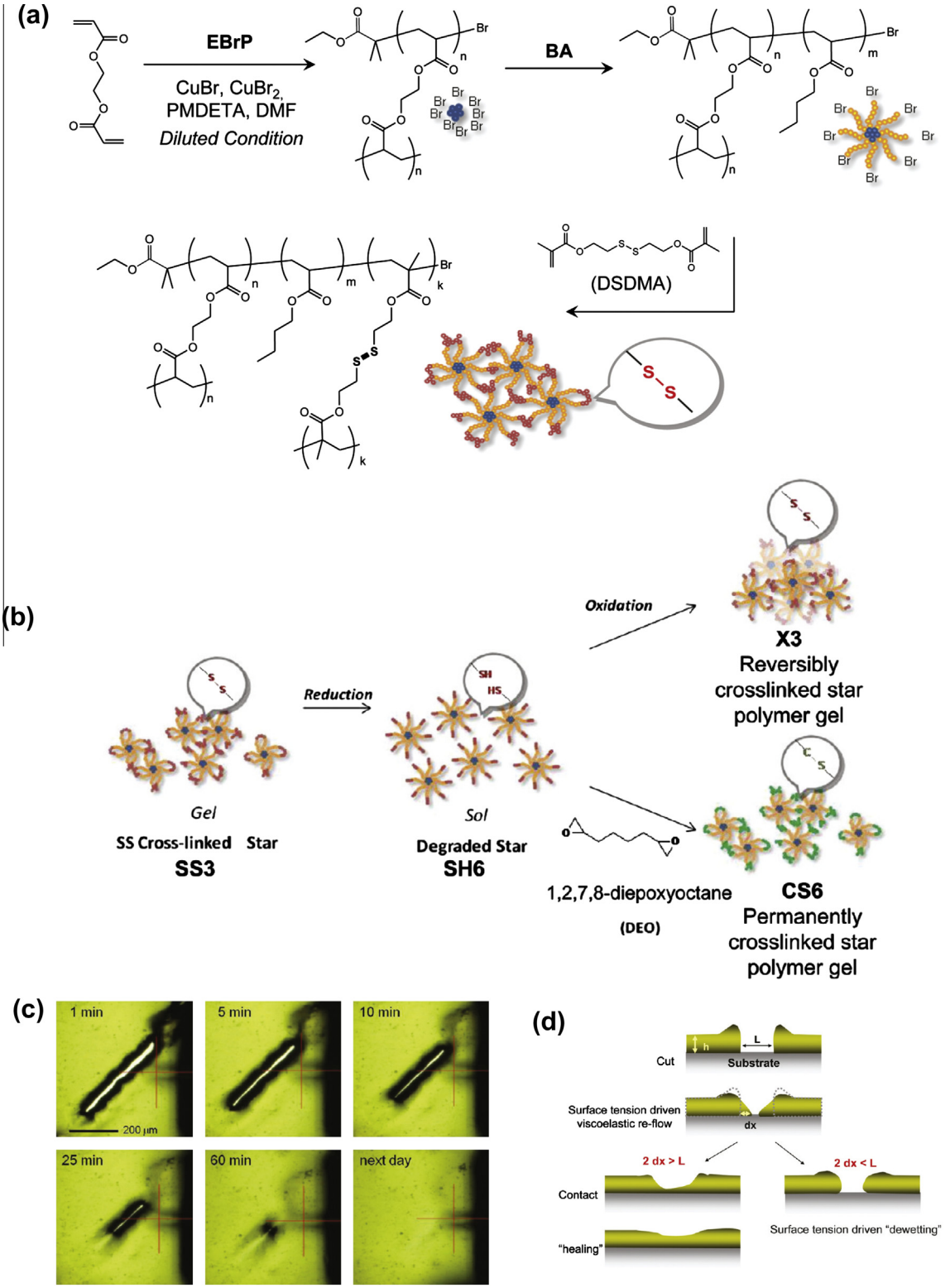

Fig. 12. (a) Synthesis of SS-functionalised star polymers (SS) from cross-linked poly(n-butyl acrylate) cores with consecutive chain extension by bis(2-methacryloyloxyethyl disulphide) (DSDMA). (b) Reduction and oxidation of SH/SS-functionalised star polymers and preparation of permanently cross-linked star polymers as a control. (c) Optical microscope images of the self-healing response of a coating for a penetrating cut. The experiment was conducted under ambient conditions (film thickness > 15 μm). (d) Proposed self-healing process. When 2 dx (the initial flowing-back distance) is larger than L (the cut width), self-healing occurs (reprinted with permission from Ref. [61] 2012, American Chemical Society).

meter scale and to investigate the self-healing kinetics. The kinetics of self-healing depended on the width and depth of the cut, and the authors also proposed a mechanism for the self-healing process (Fig. 12d). The chemical mechanism of the self-healing process involved thiol–disulphide, disulphide-disulphide exchange reactions and thiol to disulphide oxidation, which was confirmed by Raman microscopy. An irreversible C–S bond was created in the control sample after the reduction of disulphide bonds to thiols and after the same AFM tests were performed. In this case, only viscoelastic relaxation occurred, but real self-healing could not be observed, which demonstrated the determining role of the thiol–disulphide groups in the healing process. We mention here the recent work of Matyjaszewski et al. [82] in which they described the theoretical background of the self-healing process in cross-linked nanogel particles. They concluded that there is an optimal concentration of labile bonds required to develop strong, tough materials that are also capable of self-repair.

Canadell and co-workers [83] utilised disulphide linkages to prepare reversibly cross-linked poly(ethylene glycol-block-propylene glycol-block-ethylene glycol) type copolymers with self-healing abilities. The characterisation of self-healing was similar to instances previously described. Canadell and co-workers found that the recovery of strength after self-healing is determined by the disulphide concentration of the samples. They proposed the application of self-healing coatings based on polymers with low glass transition temperatures, which is essential to re-arrange the disulphide bonds among the mobile polymer chains.

3.4. Shape and molecular imprinting

In the case of shape and molecular imprinting, the structure has a memory of a defined molecular arrangement, which was realised as a macroscopic shape memory in the first case or as a memory of the structure of the binding sites in the latter case. Reversible disulphide bonds could render memory to polymer networks. In this case, the basic question is whether the same thiols establish the disulphide linkage during reduction–oxidation cycles or if a new disulphide linkage is established.

Greytak et al. [84] synthesised polyacrylamide based gels with cleavable, and a small amount of a permanent cross-linker. The disulphide linkages of the gels were cut by dithiothreitol, and a soft gel was obtained because of the low cross-linking density in the reduced state. The gel was distorted to form a new shape, which could be stabilised through disulphide linkages that were re-formed by oxidation with sodium bromate. This effect is similar to the rearrangement of the protein disulphides in hair to create a permanent wave. The authors proved that the shape imprinting ability could be improved with increasing thiol content and increasing distortion from the original shape. The shape-imprinting behaviour can be very beneficial in cases where the desired shape of the gel should be formed after the polymerisation process.

We now refer to another important work by Hiratani et al. [85] on molecular imprinting. In this case, a divalent-ion-imprinted gel was synthesised by the radical polymerisation of *N*-isopropylacrylamide in the presence of lead methacrylate. A polymer network was obtained after gelation with two breakable cross-linkers: the disulphide linkage and the complex binding sites (i.e., the carboxylic groups connected via metal cations). Lead was removed by washing, and the calcium-imprinting ability was determined. The initial gel showed a weaker ability to imprint calcium ions both in the reduced and re-oxidised state compared to the original gel. In the reduced state, the swelling ratio was larger, and the change in the distance between the carboxylic groups led to weaker complexation. After oxidation, the thiol groups could perhaps not re-form disulphide bridges with their initial thiol pair, but random re-arrangement occurred. This might have caused network frustration, which could result in weaker imprinting [86]. However, the authors developed a "post-imprinting" method with the re-formation of disulphide bridges in the presence of calcium ions, which were washed out after the re-oxidation process. In this way, a better molecular imprinting ability was achieved compared to that of the original gel, which could be explained by the effective encoding of target-binding sites into the network. These imprinted gels may have effective uses in binding well-defined target molecules in molecular recognition and analytics.

4. Conclusions and outlook

The utilisation of disulphide formation and thiol–disulphide exchange reactions is an attractive way to synthesise redox-responsive platforms and exploit them in biomedical fields and advanced applications in self-healing areas. A wide variety of polymers and redox-sensitive agents exist for the design of polymeric matrices to dedicated applications. A great number of challenges have already been addressed. Polymers from synthetic and natural sources were endowed with redox-sensitive properties in a controllable manner, and reliable analytical methods were developed for their characterisation. The responsive behaviour of the networks was analysed in detail, and the kinetics of their response to reductive effects have been investigated. A large number of fascinating biomedical applications have been described recently – e.g., artificial organs or targeted delivery vehicles for regenerative medicine. Mucoadhesive implants showed significant progress in recent years because several requirements of these applications can be met by the introduced systems. Disulphide formation represents a unique alternative to thermoresponsive injectable materials because stable chemical cross-links are established instead of physically entangled networks with poor mechanical properties, and the use of harmful small-molecule cross-linkers can also be avoided. The initial steps in redox-sensitive gene-therapy also yielded a great number of promising results. In addition to biomedical areas, thiol–disulphide conversion can be exploited in advanced materials science, where molecular re-organisation is potentially useful. A new generation of self-healing materials is based on the reversible nature of the disulphide linkages.

Nevertheless, a large number of future challenges can be identified if we consider every aspect of the research.

Controlled polymerisation techniques should be employed to a greater extent to achieve better control in the preparation of redox-sensitive networks and properties related to biocompatibility and biodegradability. Nature communicates using complex interactions, and the designed multiresponsive polymer networks must respond to a majority of these stimuli. Multiresponsivity is usually limited, in most cases, to two triggers: pH and temperature. However, in combination with additional environmental effects, (e.g., redox potential, enzymatic conditions) might lead to a more precise, selective and timed response. This is a synergistic phenomenon that helps us to uncover the true dynamic behaviour of biological systems. In general, even more bioinspired approaches are necessary to enhance the applications of redox-sensitive networks; for example, the utilisation of self-healing materials in bio-related fields can be improved by mimicking the peptide folding-refolding mechanism of amino acid based polymers. Polymer-cellular interfacial interactions and the life-cycle of the designed materials, including reversible behaviour and long-term stability, should also be considered as essential issues for further applications. These questions lead us to the need for human trials, which are the next step towards determining the potential impact of the proposed systems on the therapeutic field. At this point, the majority of the studies can contribute to the field with in vitro cell tests and in vivo animal experiments. Continued progress could be made with the development of scaled-up industrial processes and extensive clinical tests to achieve commercial exploitation, which can serve human health care with better therapeutic results and patient compliance. A large number of future perspectives can be discussed, and efficient communication is needed between the scientists, engineers and medical professionals to further improve these materials. However, considering the incredible progress that could have been witnessed recently, we can predict that the launching of new research directions will lead to extensive progress towards future medical applications in humans.

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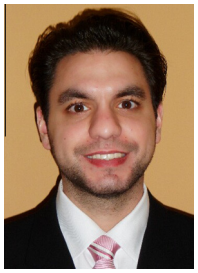

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