Chapter 16
Novel Biocompatible Hydrogels via Click Chemistry

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Abstract Currently, the designing and development of advanced hydrogel platforms is one of the important research areas due to their applications in the fabrication of functionalized materials useful in biomedical sciences. The rich literature reveals that most of these advanced materials are derived from the utilization of click reaction-based approaches through arranging the appropriate building blocks together in order to fabricate the desirable hydrogel architectures useful mainly in tissue engineering and drug delivery including the stem cell differentiation. Among the limitations of these materials, the non-degradability of synthetic polymers is responsible for the restricted usage in biomedical fields. Therefore, there is a constant demand to develop systematic methodologies for the synthesis of novel hydrogel materials to improve the degradability of the hydrogels by fine-tuning the functional groups and by incorporating more hydrophilicity for the ready hydrolysis.

Keywords Biocompatible polymers · Synthetic routes · Click chemistry · Cross-linking networks · Applications

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Abbreviations

PAA	Polyacryliccacid
PEO	Polyethylene oxide
PVA	Polyvinyl alcohol
DMSO	Dimethyl sulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
NHS	N-Hydroxysuccinimide
PBS	Phosphate-buffered saline
TEMPO	2,2,6,6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
AS	Ally starch
CS	Chitosan
St	Starch
CS–Fu	Furan–functionalized chitosan
CS–AMI	Themaleimide–functionalized chitosan
Py–SA	TOMFC
MES	2-(N-morpholino)ethanesulfonic acid
HA	Hyaluronic acid
HAAA	Hyaluronic acid-11-azido-3,6,9-trioxaundecan-1-amine
AA	11-Azido-3,6,9-trooxaundecan-1-amine
BH_3NH_3	Boranemonoammoniate
4-AC-TEMPO	4-Acetamido-2,2,6,6-tetramethylpiperidin-1-yl)oxyl
NaBr	Sodium bromide
NaClO	Sodium-hypochloride
$NaIO_4$	Sodium metaperiodate
CuCl	Copper(I) chloride
$CuSO_4$	Copper(II) sulfate
BMI	Bismaleimide
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
Cu(I)	Copper (I)-iodide
DNA	Deoxyribonucleic acid
$NaBH_4$	Sodium borohydride
CS-Fu-BMI	Chitosan-furfural-bismaleimide
PEG MA	Poly(ethylene glycol) methacrylate
PEG TMA	Trimethylamine polyethylene glycol
DMF	Dimethylformamide
AIBN	Azobisisobutyronitrile
DMAP	4-Dimethylaminopyridine
DCC	N,N′-Dicyclohexylcarbodiimide
P(NIPAAm-co-HEMA)	Poly(N-isopropylacrylamide-co-hydroxylethyl methacrylate)
HA/PEG	Hyaluronic acid/polyethylene glycol
ATRP	Atom transfer radical polymerization

PEG TMC	Polyethylene glycol-trimethylene carbonate
PHEA	Poly(2-hydroxyethyl acrylate)
HEP	1,4-Di(2-hydroxyethyl)piperazine
HDI	1,1-Diisocyanatoethane
DBTDL	Dibutyltindilaurate
FGE	Furfurylglycidyl ether
PAH	Polycyclic aromatic hydrocarbon

1 Introduction

The term "click chemistry" introduced by Sharpless in 2001 describes the high yield coupling of two molecules A and B which are versatile, stereospecific, simple to perform, and can be performed in easily removable or benign solvent systems [1, 2]. In addition to this, these reactions produce by-products that can be removed without any chromatographic technique. Also, these reactions found high significance in synthetic organic chemistry of therapeutic applications [3]. In general, the first step of mechanism embraces the activation of biomolecules (via compatible "click" functional groups) and the subsequent step involves the coupling of activated molecules to arrange a stable conjugate. The key advantages of this click chemistry are: (i) facile reactions in nature, (ii) excellent yields, (iii) easily separable by-products, (iv) stereospecificity, (v) the usage of environmentally benign solvents, (vi) to support both in vitro and in vivo of enzymatic activities with non-radioactive analysis, and (vii) high selective, etc. The "click" reactions are of several types including [4] (i) "one-pot" synthesis with thermodynamically feasible reactions, e.g. nucleophilic ring opening of aziridines and epoxides, (ii) reactions of carbonyl groups (non-aldol type), e.g. formation of heterocycles, (iii) formation of carbon–carbon multiple bonds, e.g. reactions from Michael addition and epoxides, and (iv) reactions through alkyne–azide cycloaddition. One of the best examples for a click reaction is the copper(I)-catalyzed 1,3-dipolar cycloaddition between azides and alkynes. Azide and alkyne groups are stable to aqueous solutions and have almost no reaction with biomolecules. This allows further achieving the target-guided synthesis and further activity-based protein profiling [5]. In addition to this, Bertozzi et al. established a route to the [3 + 2] azide–alkyne cycloaddition reaction due to the ring strain in the absence of copper(I) as this method avoids the cytotoxicity due to the presence of copper(I) [6].

In general, hydrogels are polymeric materials and exhibit the ability to swell and hold a significant fraction of water within the structure without dissolving in water. Due to this, hydrogels also possess a definite degree of flexibility similar to the natural tissues [7]. Consequently, these hydrogels function as delivery vehicles in cell transplantation efficiently in a controlled manner and tolerate the culture of stem cells under different environments. Due to their biocompatible nature, the research on the development of new hydrogel polymers has been gained high significance for their

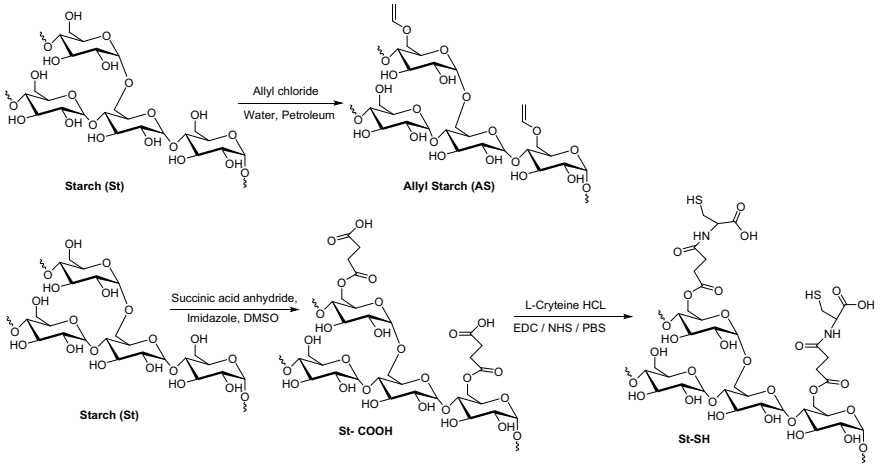
prospective applications in the construction of devices for drug delivery [8, 9], tissue engineering [10, 11], coating [12, 13], cell culture [14, 15] and so on. In view of this, click chemistry appears as a suitable alternative for the fabrication of chemically cross-linked and polysaccharide-based hydrogel materials [16]. Among all these click reactions reported, the Diels–Alder reaction has received huge demand as this reaction has been carried out under mild reaction conditions without any side products [17] and the biocompatibility of the material in the absence of catalysts or initiators [18]. Further, this reaction has been used in synthesis of chitosan-based hydrogels [19] and other types of polymeric hydrogels [20, 21]. The remarkable impact of click chemistry on extraordinary efficiency and reliability of these reactions which enabled rapid synthesis of hydrogel materials with appropriate network structures has been highlighted. Also, the peptide sequences of biomolecular building blocks have been incorporated efficiently through various click reactions either during or after the synthesis of hydrogels. Further, this has led to the fabrication of many stimuli responsive or "smart" hydrogels in recent years [22]. Natural polymers such as collagen, gelatin or hyaluronate and synthetic polymers (e.g. PAA, PEO and PVA) are the right choice to design the biocompatible hydrogel architectures having large surface area. As the entire polymer support of these hydrogels is exposed to aqueous solutions and enzymes, this can probably lead to quick hydrolysis [23]. Interestingly, these unique properties including great water absorption capacity and water preservation ability make hydrogels remarkable candidates in contact lenses, diapers and drug reservoirs [24].

2 Synthesis of Biocompatible Hydrogels via Click Chemistry

Li et al. reported the synthesis of thiolene-based hydrogel material as shown in Scheme 1 [25]. The analysis revealed that the hydrogel displays adjustable swelling capacity and good mechanical properties. The degradation was due to the combination of both diffusion and surface erosion.

A chemically cross-linked hydrogel derived from chitosan was successfully synthesized through Diels–Alder reaction [26]. Further, chitosan derivatives for example furan-modified chitosan (Cs–Fu) resulted from the reaction of furfural and free amino groups of chitosan. The maleimide–functionalized chitosan (Cs–AMI) was prepared from the reaction of a maleimide-modified aminoacid with the amino groups of chitosan (Scheme 2). These hydrogel materials were found to be pH-sensitive, biocompatible and anti-bacterial.

Lueckgen and the co-workers fabricated a flexible and degradable cross-linked alginate-based polymer with tunable material properties by introducing either tetrazine or norbornene functional groups for cross-linking (Scheme 3) [27]. The degradation behaviour, swelling ability and the cell compatibility were assessed to determine the in vivo functionality of the materials. Further, the biomaterial was



Scheme 1 The chemical structure of AS, St-SH and schematic illustration of thiolene click hydrogel

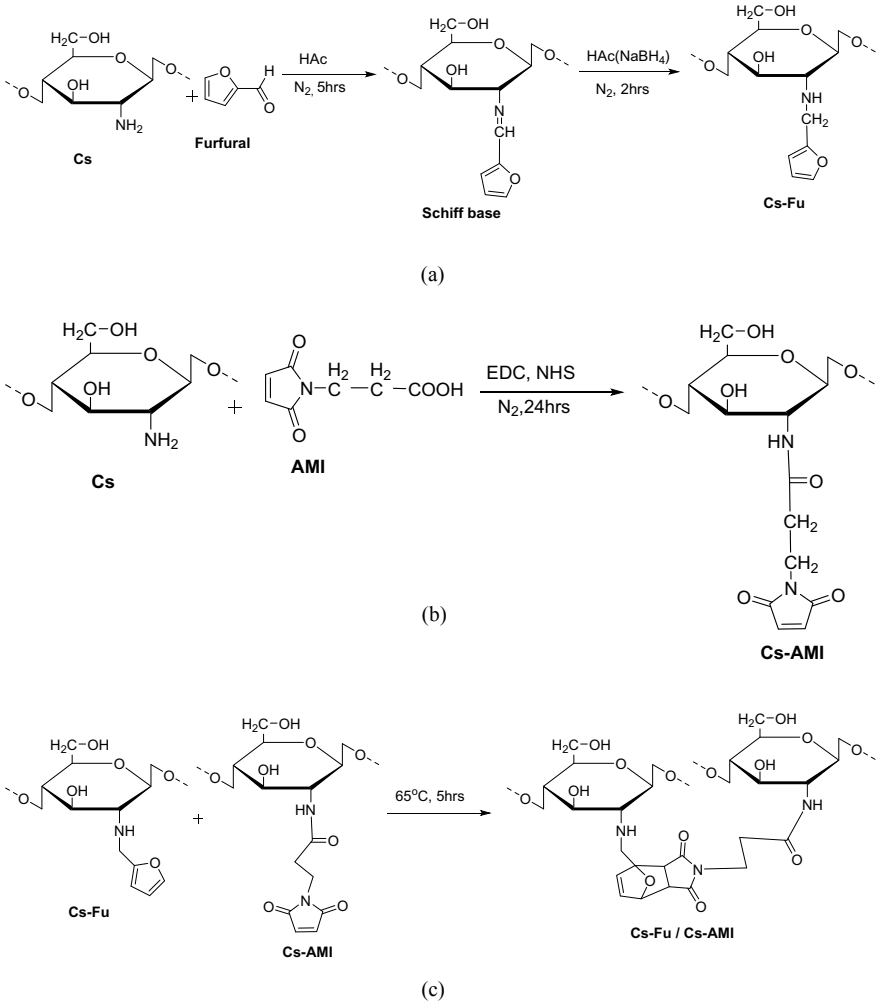
implanted internally into the mice, and further, the degradation and cytocompatibility were determined via histological staining.

Alginate-based hydrogels have been fabricated by introducing micro-fibrillated cellulose oxidized by TEMPO into the in situ polymerization of pyrrole to build PPy/SA/TOMFC conductive hydrogels [28]. Interestingly, the incorporation of TOMFC resulted in the significant improvement of structural integrity, enhanced electrical conductivity and mechanical properties of the composite hydrogels. The preparation of Cu(I)) catalyzed water-soluble polysaccharide derivatives bearing side chains endowed with either azide or alkyne terminal functionality was carried out by mixing together in aqueous solution through a 1,3-dipolar cycloaddition reaction [29] as shown in Scheme 4.

Hyaluronic acid was effectively modified structurally through chemical reactions like oxidation/reductive amination and cross-linking via click chemistry (Scheme 5) [30]. The combination of 4-acetamido-TEMPO/sodium hypochlorite/NaBr was found as good alternative towards the modifications of the C-6 of hyaluronic acid. These modified hydrogel materials were found as biocompatible.

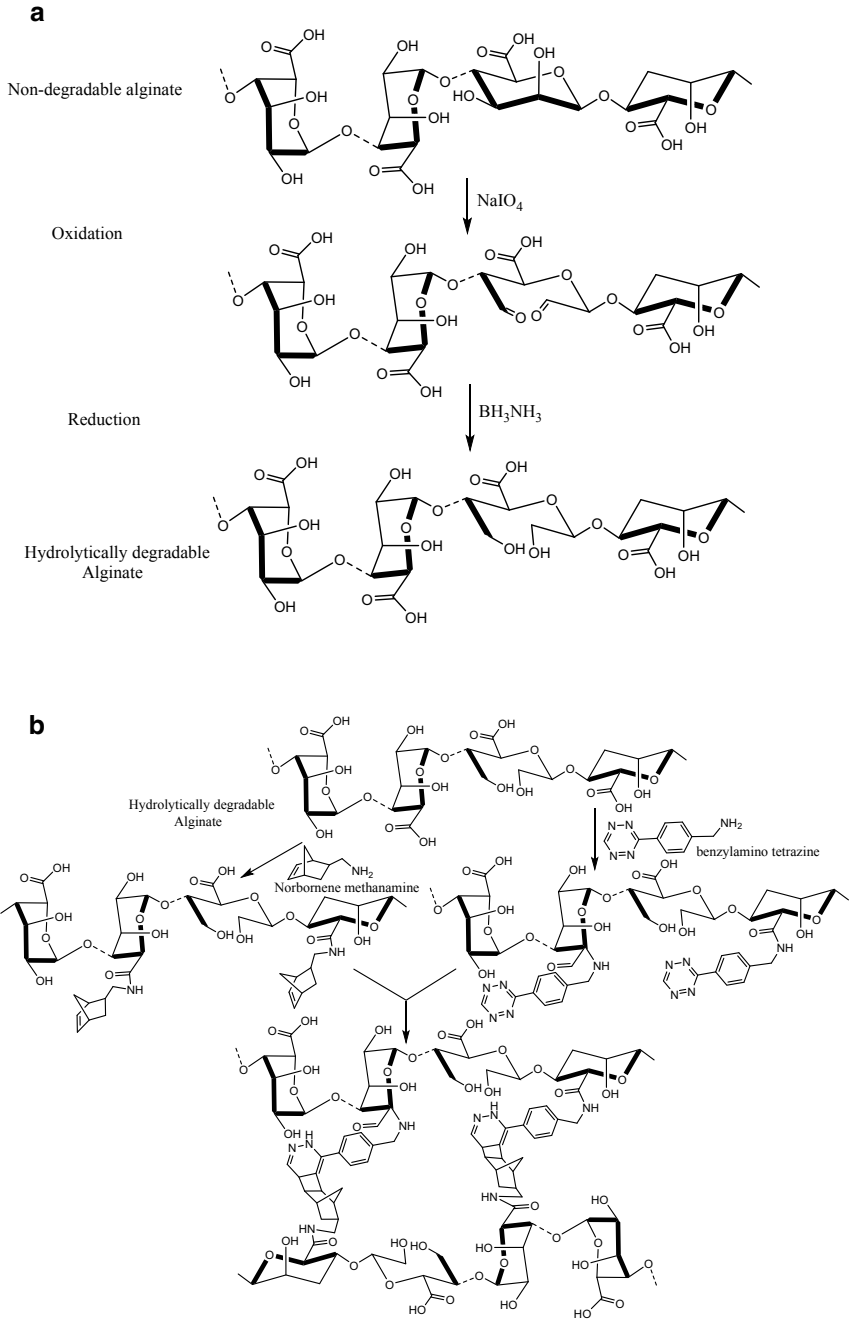
Starch-based hydrogels were prepared by cross-linking through Diels–Alder reactions between furan-modified starch and bismaleimide as given in Scheme 6. The conducting properties of these materials were remarkably improved by graphene layers as active nanofillers [31]. The effect of increasing the furan/maleimide ratio on the structural, morphological, rheological and swelling properties of hydrogels was evaluated. The pore size decreases up on increasing the cross-linker content and this leads to an effective network structure. As the presence of bismaleimide imparts the hydrophilic character, graphene nanosheets produce nanocomposite hydrogel with better rheologic al properties, electrical conductivity and antimicrobial activity.

The azide-alkyne cycloaddition (CuAAC) click reaction catalyzed by Cu(I) yields cross-linked functional polymer chains having a sieving gel which was useful for

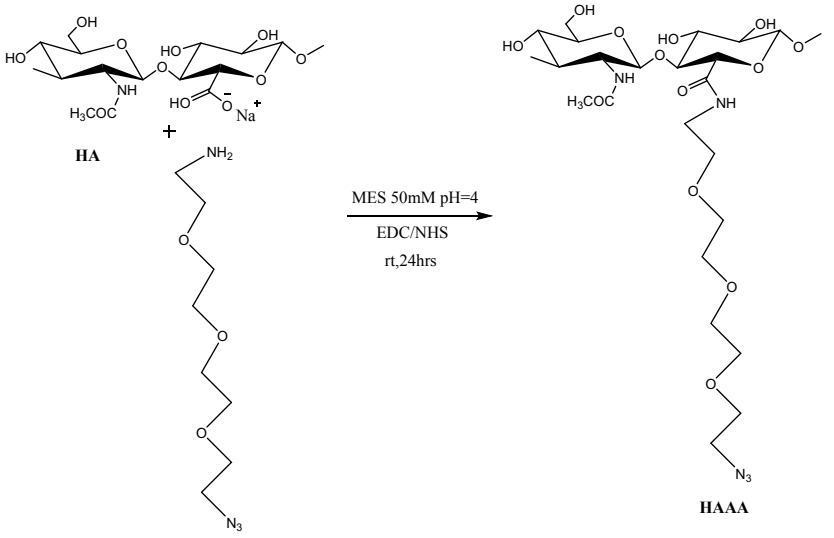


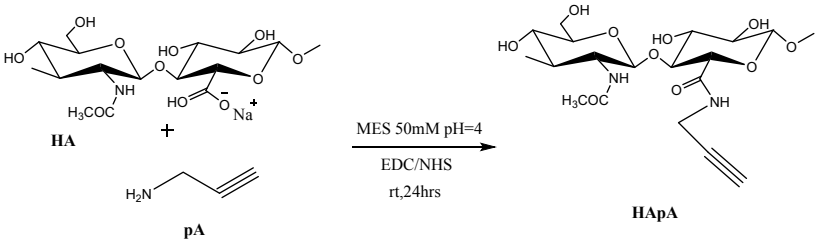
Scheme 2 **a** Synthesis of Cs–Fu through the reaction of chitosan and furfural; **b** synthesis of Cs–AMI through the reaction of chitosan and AMI modifier; **c** Diels–Alder reaction between furan–functionalized chitosan (Cs–Fu) and maleimide–functionalized chitosan (Cs–AMI)

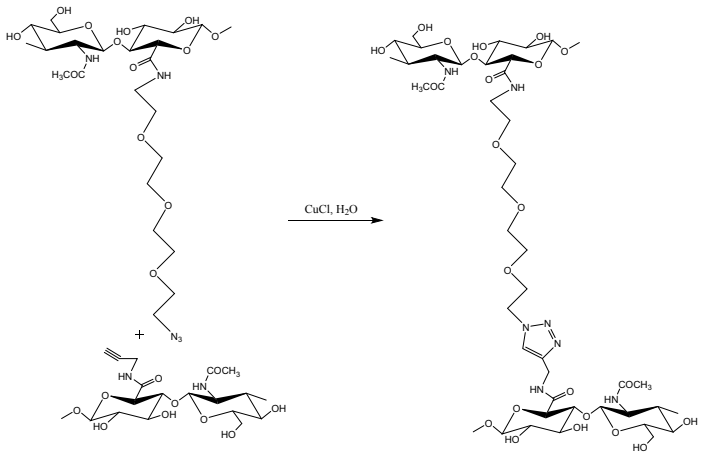
DNA electrophoresis [32]. The competence of this reaction offers hydrogels with near-ideal linkage connectivity with improved physical properties under mild conditions. The sieving environment was formed by reacting two polymers holding reactive functional groups like poly(dimethylacrylamide) with an alkyne moiety in the presence of poly(ethylene glycol) functionalized bis-azideazido groups at both ends. In addition to this, the Diels–Alder reaction (Scheme 7) was employed in the fabrication of stimuli–responsive chitosan-based cross-linked hydrogels for biomedical applications by reacting furan-modified chitosan (Cs–Fu) with polyetheramine derived



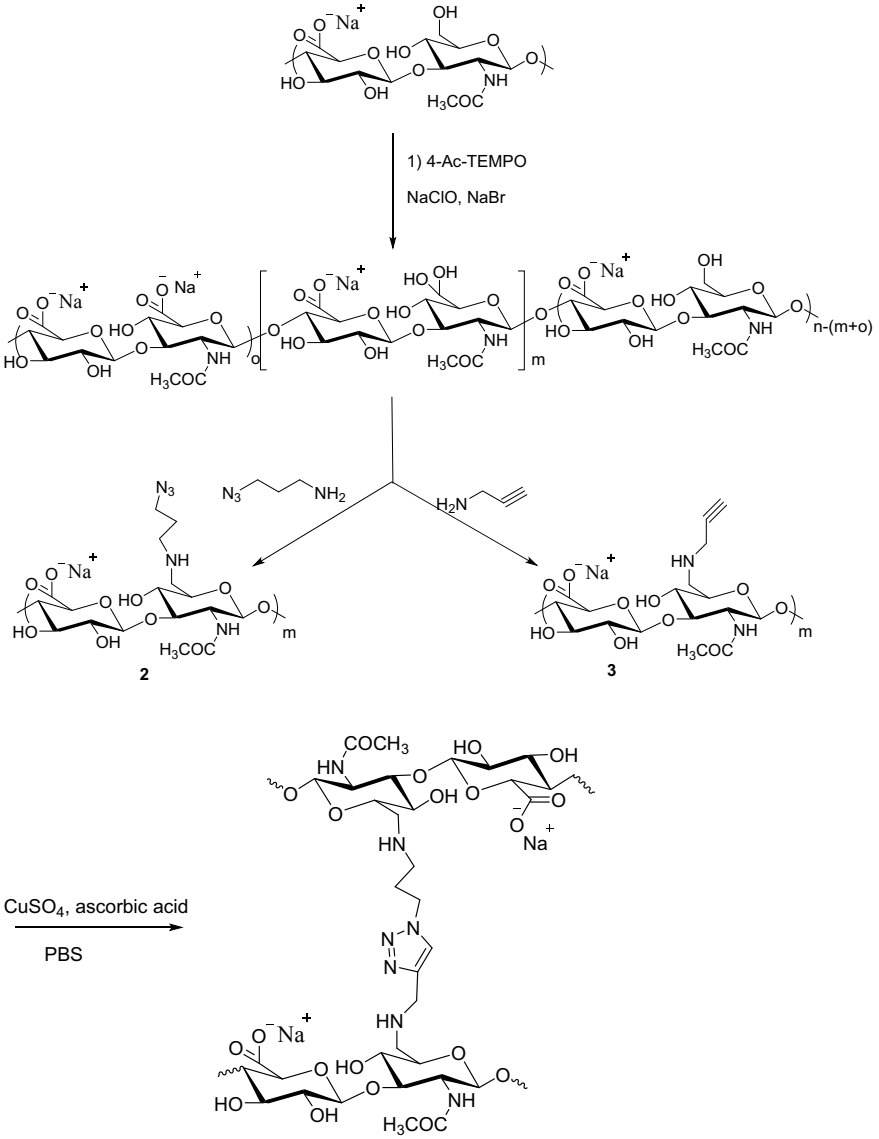
Scheme 3 Synthesis of hydrolytically degradable click cross-linked alginate gels. Oxidation of non-degradable base material by sodium periodate and then reduction through ammonia borane (**a**). Dashed boxes represent reacting groups. Spontaneous covalent cross-linking by Diels–Alder reactions (**b**). Fine-tuning the properties



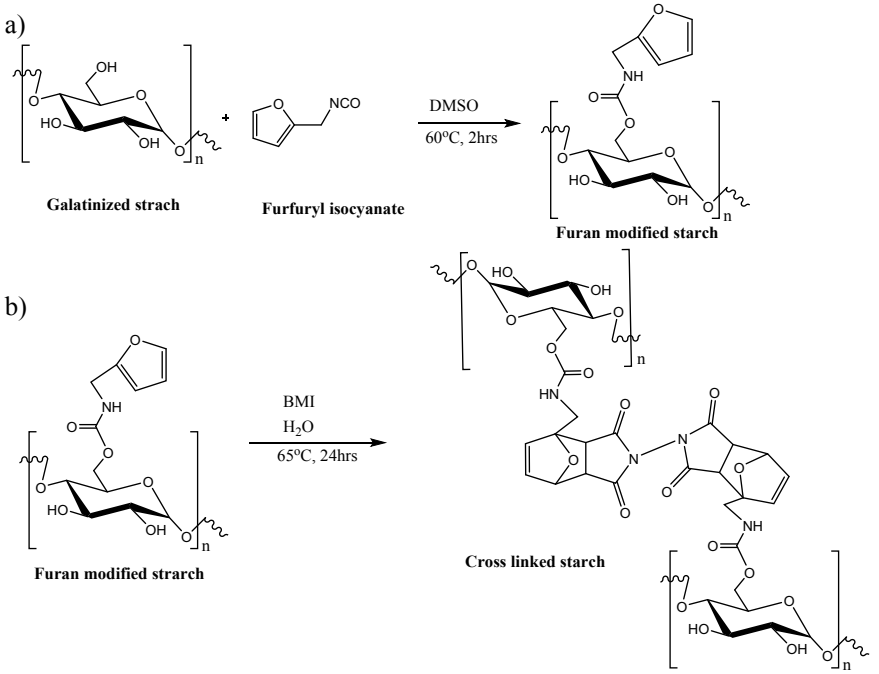




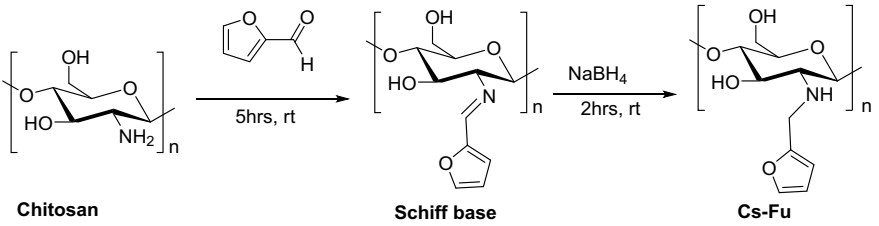
Scheme 4 Preparation of polysaccharide derivatives bearing side chains functionalized with either azides or alkynes



Scheme 5 Structural modification by xidation/reductive amination and cross-linking reactions via click chemistry



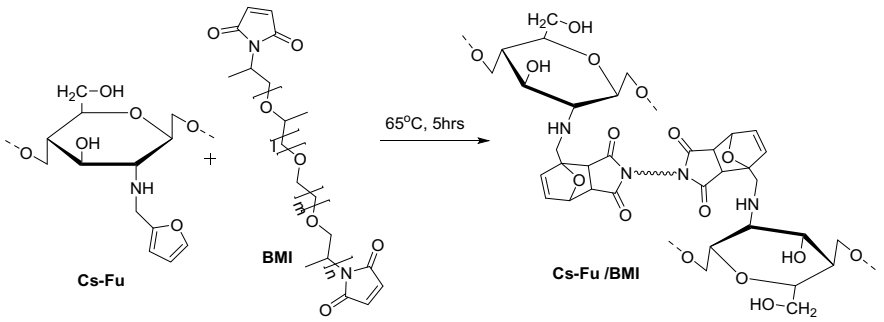
Scheme 6 Preparation of starch-based cross-linked hydrogels through Diels–Alder reactions



Scheme 7 Synthesis of Cs–Fu through the reaction of chitosan and furfural

from bismaleimide (Scheme 8) [33]. Both the final storage modulus and the sol–gel transition value for the different formulations were almost similar and close to 40 min and 400 Pa, respectively. Studies on the influence of the quantity and the behaviour of the cross-linker in the properties of these polymers were investigated by varying the furan to maleimide ratio.

Several strategies were used to produce 3D-hydrogel networks by joining functional polymers or polymeric fragments for various applications including tissue engineering [34]. The development of junctions between each polymer segments is important in altering the stability and mechanical strength of these gels. This was recognized via formation of covalent and non-covalent bonds with different

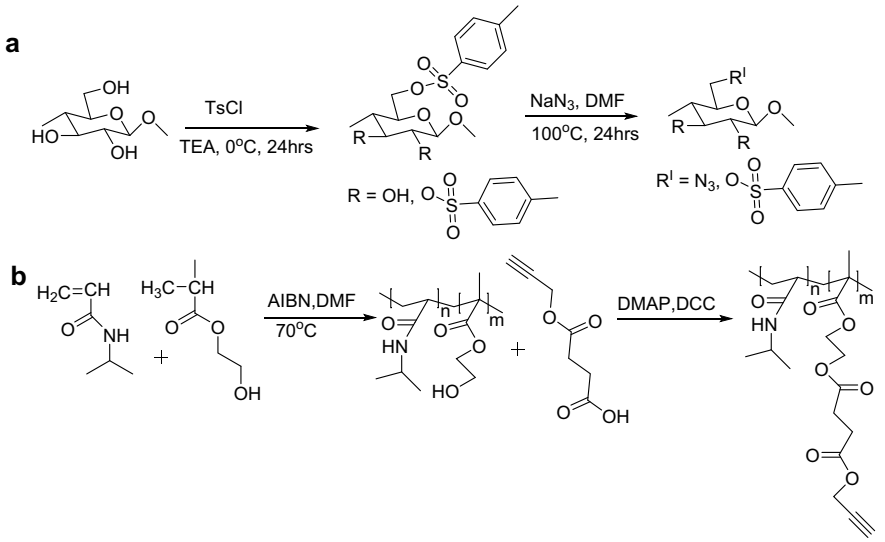


Scheme 8 Hydrogel formation through DA reaction between Cs–Fu and BMI

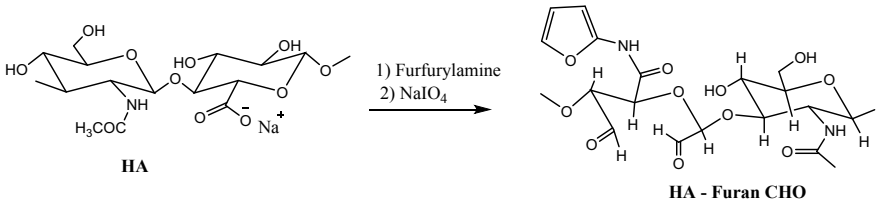
strengths and density [35, 36]. Even though the physical hydrogels were formed by the transient cross-linking between polymer chains through various kinds of physical interactions [36], these weak physical cross-linking generally provides to low mechanical strength. Nevertheless, these interactions play a vital role in the generation of self-healing properties via dynamic self-assembly/disassembly features. A typical example for this is a combination of clay and the dendritic molecular binder to fabricate self-healing hydrogels as reported by Aida and co-workers [37]. In contrast, chemical hydrogels usually hold networks produced by cross-linked covalent bonds, and their mechanical properties can be controlled with the cross-linking density. In this connection, Anseth et al. reported the fabrication of photo-controlled degradable hydrogel by sequentially performed CuAAC and thiolene reactions with variable architecture and functionality [38].

In general, the Michael-type thiolene "click" reaction was reported by carrying out under mild conditions which are similar to human physiological conditions [39]. The gelation of PEG–MA and PEG–TMA was carried out to prepare two biodegradable and biocompatible PEG hydrogel derivatives with multienes or multithiols by polycondensation employing scandium trifluoromethane sulfonate ($Sc(OTf)_3$) as a chemo selective catalyst and further the influence of concentration and pH values was evaluated [39]. Zhang and co-workers have reported a series of thermosensitive hydrogels derived from the chemoselective cross-linking reaction between two different types of polymer backbones with cellulose modified by azide and alkyne-modified poly(N-isopropylacrylamide-co-hydroxylethyl methacrylate) P(NIPAAm-co-HEMA) in the presence of Cu(I) catalyst [40]. Also, alkyne-modified P(NIPAAm-co-HEMA) and azide-modified cellulose were produced to investigate the formation of in situ hydrogel through "click" chemistry by Zhou and co-workers [41]. The synthesis routes of the two polymers were given in Scheme 9.

A double cross-linked network was designed and further prepared by Diels–Alder click reaction, followed by the incorporation of acylhydrazone bond (Schemes 10 and 11). As the Diels–Alder reaction preserved the structural integrity and mechanical strength of hydrogel under physiological environment, the flexible covalent acylhydrazone bond leads to the development of hydrogel's self-healing property and



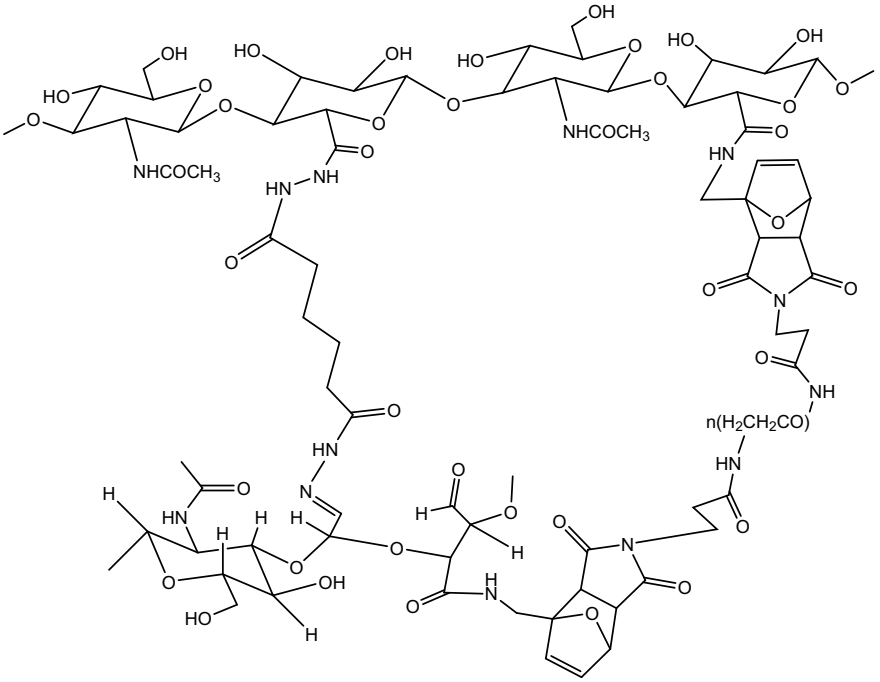
Scheme 9 The synthesis of the azide-modified cellulose (**a**) and alkyne-modified P(NIPAAm-co-HEMA) (**b**)



Scheme 10 The preparation of HA-furan-CHO

controlled the on–off switch of network cross-link density. At the same time, the aldehyde groups present in hydrogel further support the integration of hydrogel based on the formation of imine from the aldehyde–amine Schiff-base reaction [42].

HA/PEG hydrogels formed by Diels–Alder reaction showed with short gelation times and appropriate mechanical properties [43]. Unlike traditional Diels–Alder hydrogels, the series of HA/PEG hydrogels, i.e. DS1 (1:1), DS1 (3:1) and DS2 (1:1) exhibited the required gelation times for cell encapsulation, survival and proliferation. Among these hydrogels, DS1 (3:1) was effective with fatigue resistance and high elasticity even after 2000 loading cycles. Studies on the use of propargyl acrylamide (PAm) as a comonomer along with acrylamide (AAm) and N,N′-methylene bisacrylamide (BAAm) as cross-linkers in photoinitiated polymerization were carried out [44]. Hydrogels with clickable acetylene groups can be prepared photochemically in a single step to achieve the selectivity by generation of free radicals towards acrylic function of PAm. Based on the acetylene functionality, the molecules



Scheme 11 The scheme of double cross-linked processes

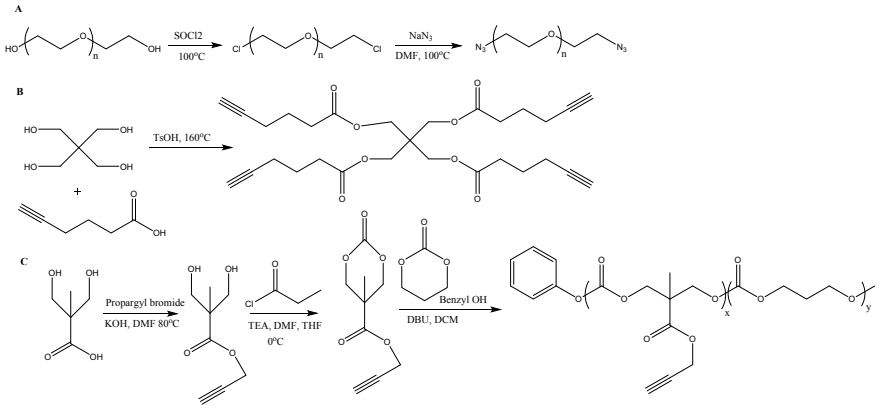
possessing azide groups can be conjugated onto hydrogel easily. The preparation of distinct sliding-graft semi-IPN of PEG and poly(2-hydroxyethyl methacrylate) (s-IPNPEG/R-CD-sg-PHEMAs)with grafted linear poly-2-hydroxyethyl methacrylate (PHEMA) on the grids of PEG linkages was possible via simultaneous CuAAC and ATRP [45] to achieve the biocompatible hydrogels having very good physical and mechanical properties. Further, the reaction of azide-terminated PEGs having 4-arms with dialkyne flanked peptides in the presence of CuBr/L-ascorbic acid/DMF yielded the hydrogels of the required template [46].

A click reaction was carried out to prepare zwitterionic antifouling hydrogels such as poly(2-hydroxyethyl methacrylate-co-glycidyl methacrylate) (poly(HEMA-co-GMA)) by implanting amino acids onto polymer chains through ring opening reaction (i.e. primary amino groups of amino acid and epoxy groups of polymer chains) in weakly alkaline aqueous solution. Further, the protonation of secondary amino groups and deprotonation of carboxyl groups at pH 7 were carried out [47, 48]. This zwitterionic structure possesses protonated secondary amino cations (PSA, $-NH_2^+-$) and deprotonated carboxyl anions (DPC, –COO–). Recently, thiol-ene "click" reactions (Michael addition type) between electron-deficient enes and thiols have been widely used over the well-known traditional polymer networks and they do not require light irradiation or a metal catalyst [49]. In this connection, a

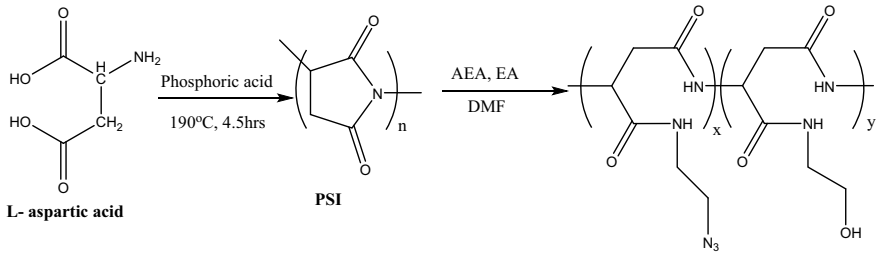
series of polyethylene glycol (PEG)-based derivatives containing multiple "click-able" groups by the polycondensation of dihydroxyloligo (ethylene glycol) with maleic anhydride/thiolmalic were prepared for biomedical applications [50, 51].

A series of PEG-TMC networks were prepared by Huisgen's 1,3-dipolar cycloaddition of azides with alkynes catalyzed by Cu(I) to yield completely hydrophilic PEG hydrogels, as well as PEG-poly(TMC) (PTMC) hydrogels with amphiphilic behaviour [52] (Scheme 12).

A facile preparation of poly(ethylene glycol) (PEG)-cyclodextrin containing hydrogels by radical thiol-ene reaction was reported using the hydrophilic matrix of the type alkene end-functionalized poly(ethylene glycol)s and thiol functionalized β-cyclodextrin as multifunctional cross-linker [53]. Two bis-alkyne reagents (isopropargyl succinate and bis-propargyl hexane urethane) were employed as cross-linkers to fabricate the click gels containing degradable ester or urethane groups based on azido-functional PHEA (PHEA-N3) and di-alkyne cross-linkers as shown in Scheme 13 [54].



Scheme 12 Preparation of hydrophilic PEG hydrogels by Huisgen's 1,3-dipolar cycloaddition of azides with alkynes

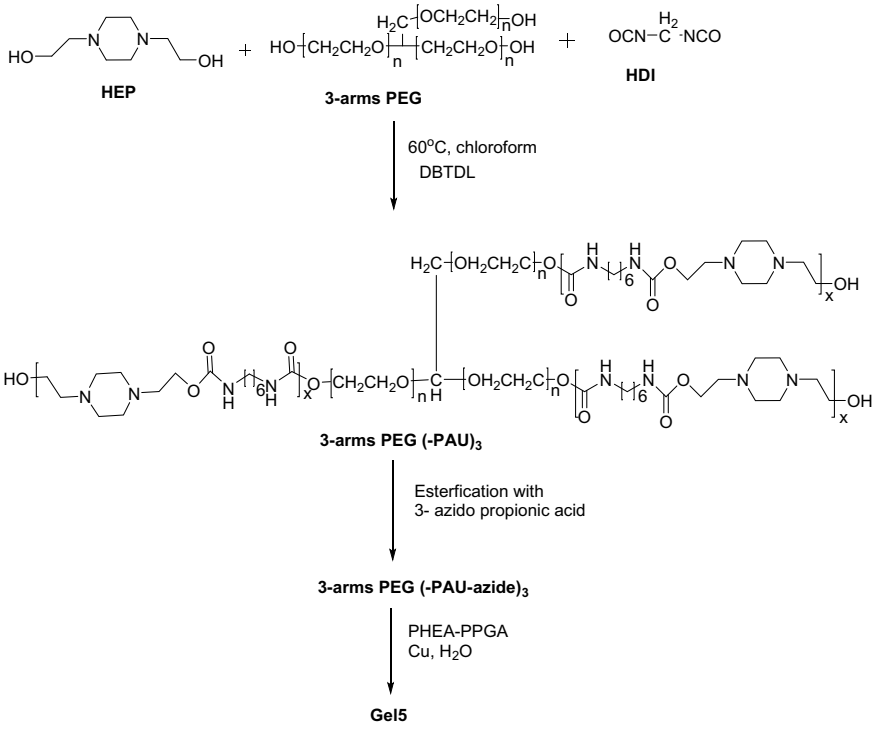


Scheme 13 Synthesis of PHEA-N_3

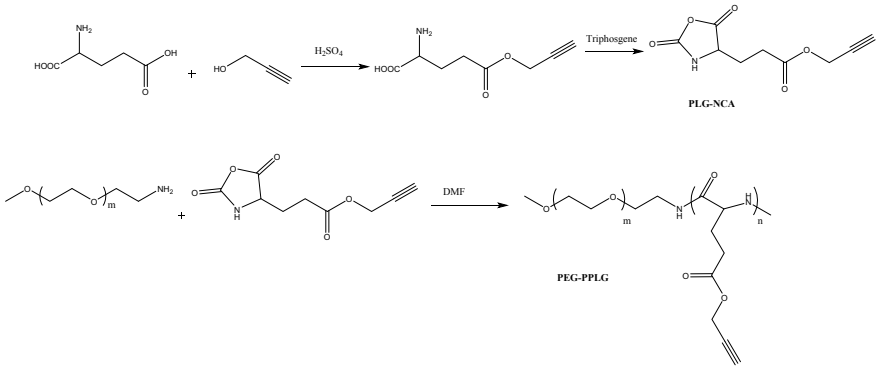
A series of click hydrogels were obtained by the reactions of functionalized alkyne groups with azido-terminal cross-linkers of the types 3-arms poly(ethylene glycol) (PEG) and 3-arms poly(ethylene glycol)-poly(amino urethane) (PEG-$(PAU)_3$) with 3-azido-1-propionic acid (APrA). Then the "click" hydrogels can be obtained with cross-linkers (Schemes 14 and 15) [55].

As showed in Scheme 15, poly(ethylene glycol)-block-poly(γ-propargyl-L-glutamate) (PEG-PPLG) with pendent alkynyl groups derived by click chemistry displayed good cytocompatibility in vitro and acceptable in vivo biocompatibility [56]. A bactericidal poly(ethylene glycol)-based (PEG) hydrogel was synthesized and utilized as a layer with covalently attached antimicrobial peptides (AMP) stabilized against proteolytic degradation [57]. New hydrogels were developed based on furan-modified gelatine using bismaleimide cross-linker [58]. The furan groups were grafted on to gelatin by the reaction of epoxy-amine with furfuryl glycidyl ether, and then further cross-linked with Jeffamine®-based bismaleimides (Schemes 16 and 17). Attempts were also made to prepare the hydrogels with polyampholyte based on dextran with cryoprotective properties for tissue engineering applications [59].

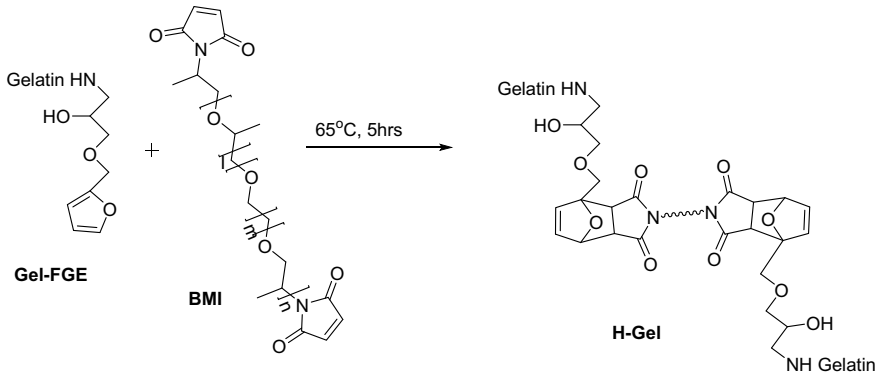
A facile synthesis of PAH from PMA with quantitative conversion of carboxylates to carbonyl hydrazides was carried out using various hydrazide based click reactions



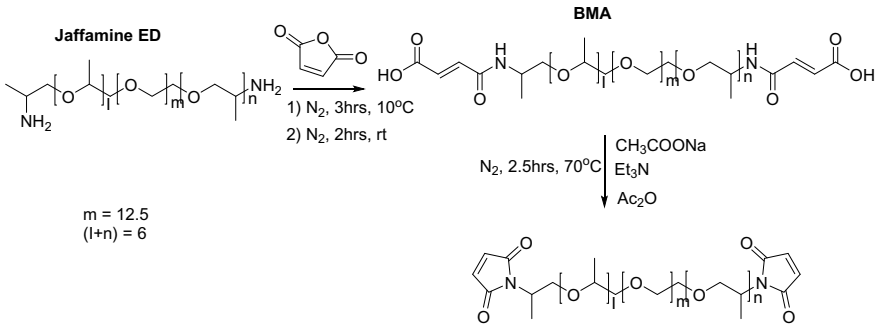
Scheme 14 Synthesis of 3-arms based poly(ethylene glycol)-poly(amino urethane)



Scheme 15 Synthesis of poly(ethylene glycol)-block-poly(γ-propargyl-L-glutamate) (PEG-PPLG) with pendent alkynyl groups



Scheme 16 Synthesis of furan-grafted gelatin by the reaction of epoxy-amine with furfuryl glycidyl ether



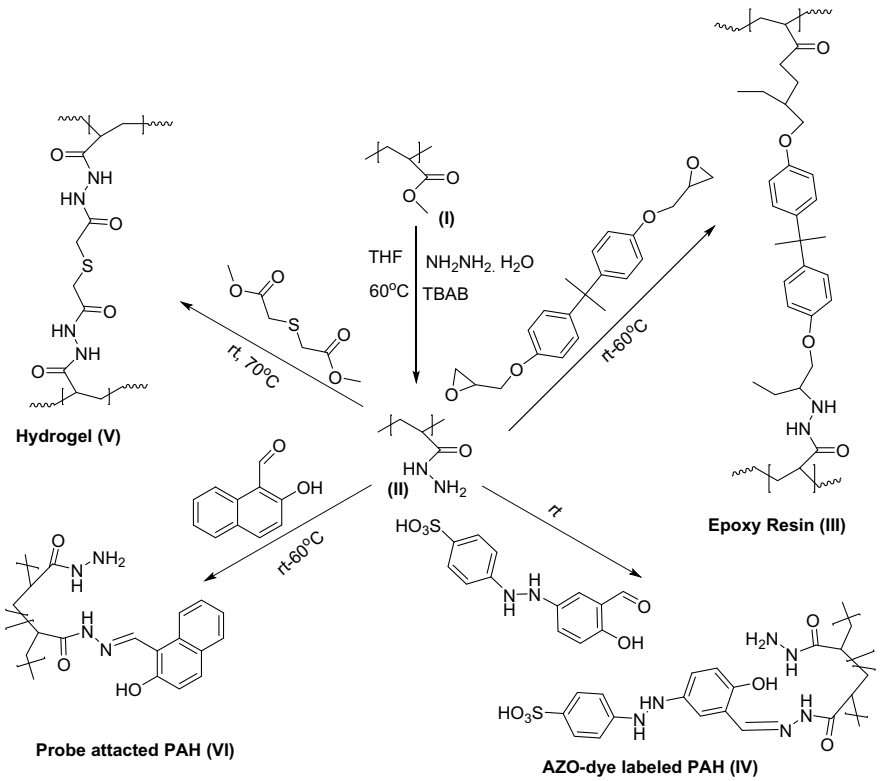
Scheme 17 Preparation of cross-linked Jeffamine-based bismaleimides

to produce a range of useful materials like pH sensors, stimuli responsive hydrogels, ion exchange epoxy resins, and polymer–dye conjugates as outlined in Scheme 18 [60].

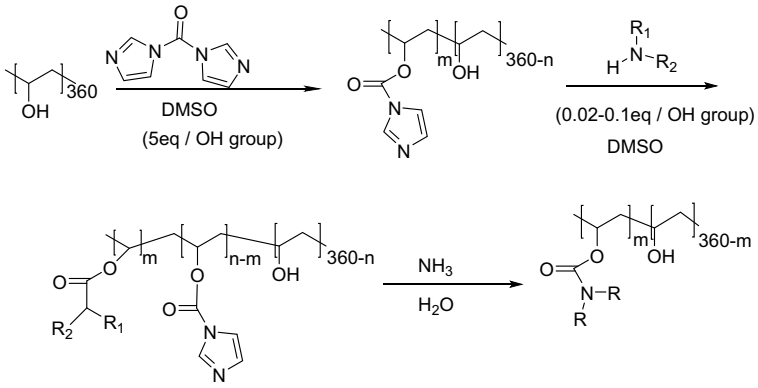
Ossipov and Hilborn investigated a click reaction by grafting the azide and alkyne pendant groups onto poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) in the formation of hydrogel in order to derive structure–property relationships as shown in Scheme 19 [61]. The first approach describes the telechelic PEG-diazide as a cross-linker for the PVA functionalized with alkyne groups, whereas the second approach deals with the functionalization of two PVA components with azide and alkyne groups.

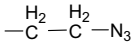
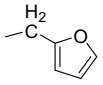
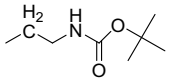
PEG-based hydrogels were synthesized in well-defined networks with significantly improved mechanical properties and the selectivity of the azide/acetylene coupling reaction allows the incorporation of functional groups into the hydrogel architectures as described in Scheme 20 [62].

Interestingly, the single-walled carbon nanotubes (SWNTs) were fused into hydrogel networks to encourage the electron transport leading to the formation of



Scheme 18 Synthesis of functionalized materials from hydrazide based click reactions



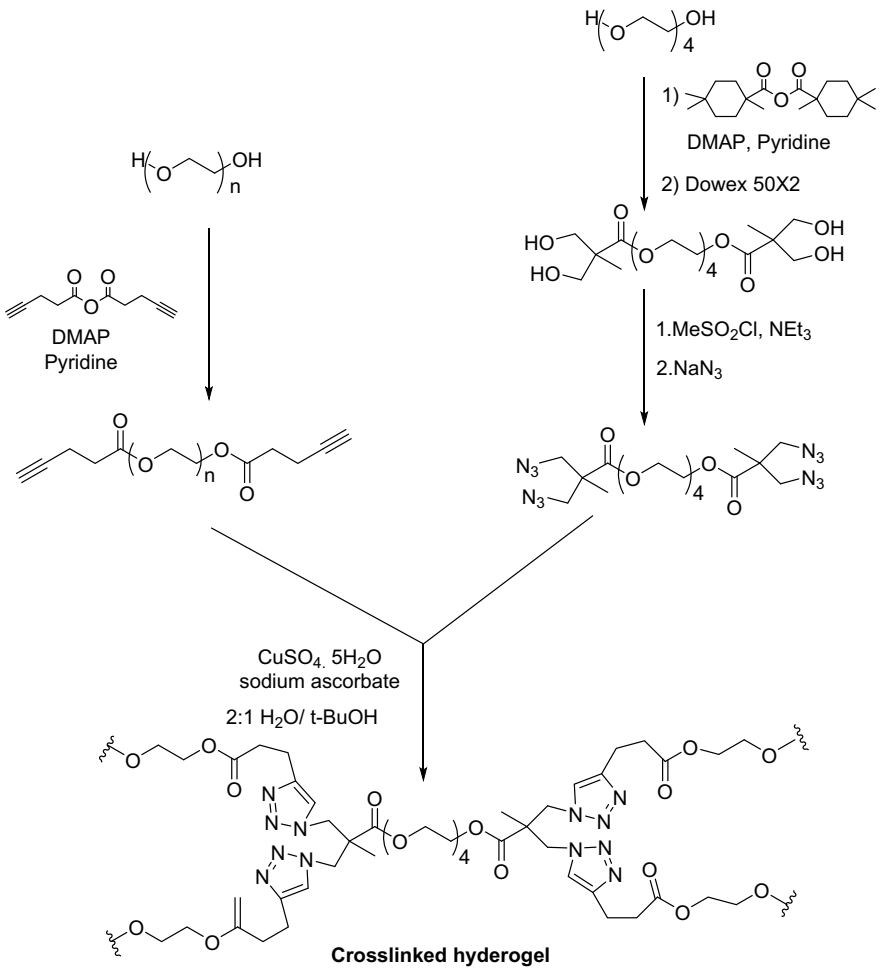
Compounds	R_1	R_2
1	H	
2	Me	
3	H	
4	H	
5	H	

Scheme 19 Synthesis of grafted azide and alkyne based PEG and PVA hydrogels

well-dispersed co-networks of electroactive polymers [63]. The fabrication of PEG–CMC hydrogel was carried out via thiol-ene photo polymerization using thiol groups anchored CMC and by norbornene immobilized tetra-arm poly(ethylene glycol) (PEG4NB). The properties of PEG–CMC hydrogel materials allow these materials towards a pH-responsive drug release carriers (Fig. 1) [64].

3 Conclusion

Recently, there has been a tremendous development in the growth of functional hydrogel platforms due to their applications in the fabrication of advanced materials and in biomedical sciences. Certainly, this has largely originated from the utilization of click reaction-based approaches toward the construction and functionalization of these hydrogel materials. The impact of these chemical reactions to arrange the



Scheme 20 Construction of hydrogel materials based on click chemistry

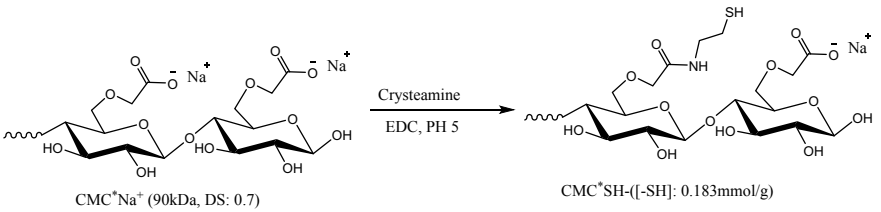


Fig. 1 Synthesis of CMC-SH materials

building blocks together has been established in the design of numerous hydrogel materials within the past few years. The literature witnesses the reports on a wide range of materials based on HA and their potential claims in tissue engineering and drug delivery. In addition to this, HA-based hydrogels can also exhibit biological activity to cells up on interaction with biomaterials, as evident in cellular behaviour and stem cell differentiation. Similar to HA-based materials, a significant progress has been observed for thiolene hydrogels towards the controlled delivery of therapeutics. However, there are some challenges regarding the broad clinical translation of thiolene hydrogels that the retaining of bioactivity of cargomolecules when they are exposed to the hydrogel environment during formation or degradation. Maintaining the controlled drug cargo release is another challenge. One of the other challenges of these hydrogels is the non-degradability of synthetic polymers which is responsible for the restricted usage in biomedical fields. Therefore, there is a constant demand to develop systematic methodologies for the synthesis of novel hydrogel materials to improve the degradability of the hydrogels by fine-tuning the functional groups and by incorporating more hydrophilicity for the ready hydrolysis.

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