



Review article

“Click” chemistry in polymeric scaffolds: Bioactive materials for tissue engineering

Yuan Zou^{a,1}, Lin Zhang^{b,1}, Lei Yang^a, Fang Zhu^a, Mingming Ding^a, Fei Lin^{c,*}, Zhao Wang^{c,*},
Yiwen Li^{a,*}

^a College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, China

^b Electronic Materials Research Laboratory, Key Laboratory of the Ministry of Education & International Center for Dielectric Research, Xi'an Jiaotong University, Xi'an 710049, China

^c Department of Polymer Science, College of Polymer Science and Polymer Engineering, The University of Akron, Akron, OH 44325-3909, United States

ARTICLE INFO

Keywords:

Polymeric scaffolds
“Click” chemistry
Tissue engineering
Fabrication
Drug controlled release

ABSTRACT

Polymeric scaffolds have attracted great interests in recent years, due to their fascination with a large variety of examples with promising utilization. Recently, extensive efforts have been devoted to the exploitation of robust and functional polymer-based biomaterial scaffolds with high efficiency. The recent entry of so-called “click” reactions that include kinds of selective and orthogonal reactions under mild conditions have generated real stimulus not only in preparing elegant bioactive materials of choice but also in making the leap to industrial scale build-up of multifunctional products. In this review paper, we account several kinds of polymeric scaffolds prepared/modified via “click” reactions, with emphasis on their synthetic/functionalized strategies, tunable properties, and biomedical applications.

1. Introduction

During the past decades, significant efforts have been made on the development of functional biomaterials for various biomedical purposes. By the definition from European Society for Biomaterials (ESB), a biomaterial describes a material that interfaces with a biological system to evaluate, treat, augment or replace any tissues, organs or functions of the body [1]. Among different types of biomaterials, polymeric biomaterials composed of organic compositions are particularly interesting due to their unique properties including controllable preparation, easy processing, and versatility [2]. Polymeric biomaterials have gained enormous impact, and have been widely used in tissue engineering [3–8], regenerative medicine [9], drug delivery [10–12], and gene therapy [13] in recent years. Notably, polymeric biomaterials are easily fabricated into various dimensional structures, such as 1D (i.e. fibers, tubes), [4,5] 2D (i.e. disks, plates, films) [6,7,14], and 3D (i.e. gels [3,15,16], microspheres [15], porous scaffolds [17]) structures to support the specifically desirable biological functions. These prepared polymeric scaffolds could provide space and biological motifs for metabolic reactions, as well as temporal or permanent mechanical support for surrounding cells and tissues [18].

Unlike other types of biomaterials, such as metals and ceramics,

polymers usually possess the unique strength of nearly unlimited pool of potential starting materials, implying that the chemical, physical and biological properties of the resulting biomaterials can be precisely controlled at the molecular level [2]. For example, polymer-biomolecule hybrids can be directly prepared via conjugating bioactive motifs onto polymer skeletons [4], or simultaneous copolymerizing of organic and biomolecular units in a controlled manner [19]. A large variety of bioactive motifs can be employed as the promising candidates to produce polymer-biomolecule hybrids, including peptides, drugs, genes, proteins, saccharides, vitamins, nucleosides, amino acids, and so on [2]. The molecular engineering of polymeric biomaterials with those bioactive motifs offers numerous opportunities to stimulate specific cellular responses at the molecular level, directing cell proliferation and differentiation, and extracellular matrix (ECM) production and organization [20]. Furthermore, comparing with conventional physical absorption and encapsulation methodologies, chemical bioconjugation method seems more versatile and controllable. However, the selection of suitable chemistry tools that can proceed under mild conditions with high efficiency remains a big challenge. More importantly, considering that biomolecules generally hold complex chemical and physical structures, the chemistries utilized should be considered as “orthogonal” to each other to avoid any side reactions when conjugating more

* Corresponding authors.

E-mail addresses: fl4@uakron.edu (F. Lin), zw11@uakron.edu (Z. Wang), ywli@scu.edu.cn (Y. Li).

¹ These authors contribute equally to this work.

than one kind of biomolecules onto polymeric backbone.

“Click” chemistry, first introduced by Sharpless in 2001, has made extensive contributions to overcome those challenges in preparing biomolecule-polymer hybrids with well-defined structures, as a result, with controllable properties [21]. These reactions possess unique identities, namely rapid process in mild reaction conditions, high yielding, tolerance to various chemical groups, producing stable products, simple to perform and insensitive to moisture and oxygen. By introducing the “click” concept in constructing functional scaffold materials, one can significantly simplify the procedures of preparing well-defined and complex scaffold materials due to the wide scope, modularity, orthogonality and simplicity features of “click” reaction. There are several well-known reactions that comply with the “click” philosophy. For example, copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) is the reaction between azides and terminal alkynes. While the exact reaction mechanism has not been fully understood today, the active Cu(I) can interacting with alkyne to form copper acetylide specie intermediates offering the key step in this reaction [22]. In comparison, strain-promoted alkyne-azide cycloaddition (SPAAC) share the same [3 + 2] cycloaddition mechanism as the Huisgen 1,3-dipolar cycloaddition. The alkyne is destabilized due to the ring strain and electron-withdrawing groups [23]. Thiol-X reactions can be classified into two large groups. One is based on the radical-initiated coupling of a thiol group to an unsaturated carbon-carbon bonds, whereas another is based on the typical nucleophile-mediated Michael addition mechanism [24,25]. Diels-Alder (DA) reaction describes a conjugation addition reaction between diene and dienophile, which is thermodynamically favorable due to the formation of new σ -bonds [26]. Oxime ligation represents a kind of carbonyl-condensation reaction between ketone/aldehyde groups and nucleophiles forming stable imine, hydrazine or oxime-bond under mild acidic conditions [27].

In this review paper, we strive to offer a full account of current status and projected outlook on future progress of “click” chemistry in polymeric scaffold preparation and fabrication (Scheme 1). As such, we start by a brief overview of various polymeric scaffolds and a “click” toolbox. The first section will discuss natural and synthetic polymers, including their chemical structures, limitations in fabrication and applications for constructing bio-scaffolds like hydrogels, fibrous scaffolds, polymer films and so on. The next two sections highlight recent

significant progress in developing “clickable” building blocks, namely initiators, monomers, post-modifiers and cross-linkers, and fabrication strategies for architecture and functionalization constructions of polymer scaffold with the aid of “click” toolbox, focusing primarily on the synthetic and molecular engineering strategies. Finally, we suggest several avenues towards combinational multiple “click” chemistry in a dynamic manner that hinges on the ability to overcome the current limitations of in the static and non-responsive systems.

2. Polymers and polymeric scaffolds

2.1. Natural and synthetic polymers as bio-scaffolds

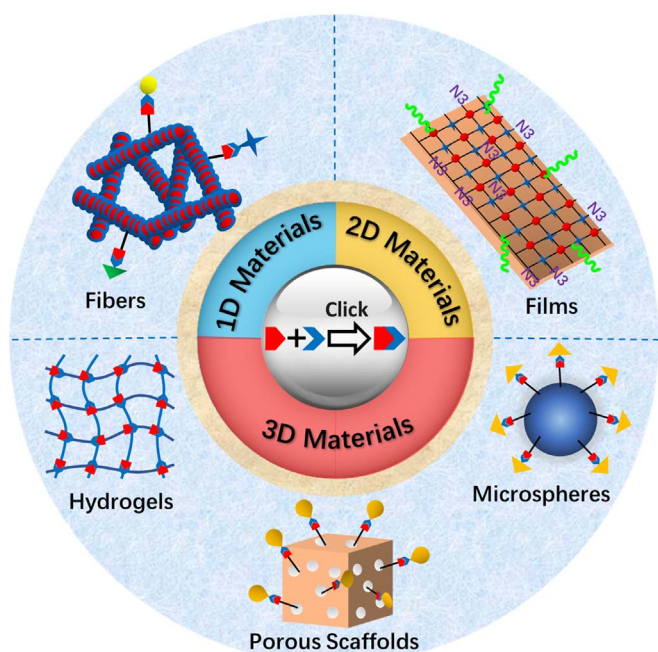
There are many resources of polymers as promising biomaterials, from natural biomass to synthetic polymers [28]. Most widely used natural and synthetic polymers are summarized in Table 1. Note that all the listed polymers show good biocompatibility both *in vitro* and *in vivo* studies. Natural polymers are usually formed during the growth cycles of all organisms, and generally available in large quantities [28]. The use of natural polymers for biomedical and healthcare-related applications dates back thousands of years [29]. The group includes polysaccharides such as starch and cellulose, proteins like enzymes and collagens, lipids and some polyesters produced from fermentation process [30]. Natural polymers advance in the inherent bioactivities that can promote cell adhesion, proliferation and tissue recovery. However, there are still some limitations, such as, difficult to purify, potential risk for disease transmission, batch to batch difference, and lack enough mechanical properties and thermal stability [2].

Given that these shortcomings, synthetic polymers have been rapidly developed as biomaterials during last several years. They could possess well-controlled macromolecular structures and uniformities, addressing several critical requirements in tissue engineering applications [31]. When used as scaffolds in tissue engineering, synthetic biopolymers are required to exhibit proper mechanical properties to support the growth of new tissues without inflammation or immune reactions in the body [20]. So far, a large scope of polymers has been successfully employed in this field, from traditional polyolefins like polyethylene (PE), polypropylene (PP) and polymethyl methacrylate (PMMA), to degradable polymers such as poly(lactic acid) (PLA), poly(glycolide), polypeptides, polyurethanes (PU), polycarbonates (PC) and so on. Among them, poly (lactic acid) (PLA) and poly(glycolide) have been widely used as scaffold materials in human since 1970s [32,33]. Other kinds of biodegradable polymers that don't produce toxic degradation products have also be regarded as safe and promising candidates in tissue engineering [2]. Bioactivities of synthetic polymers could be improved via blending them with natural polymers or coupling them with functional biomolecules [2].

2.2. Biopolymeric scaffolds in tissue engineering

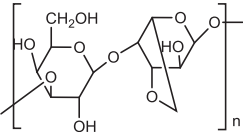
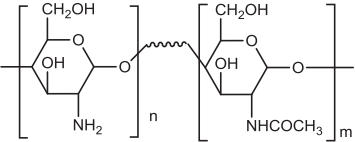
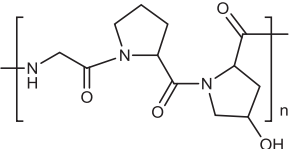
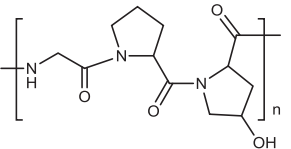
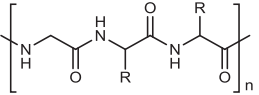
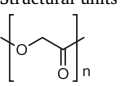
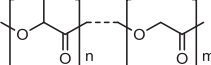
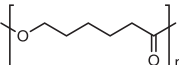
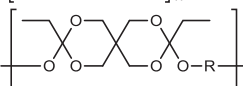
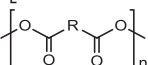
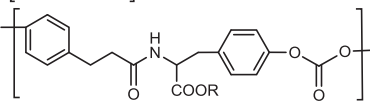
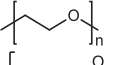
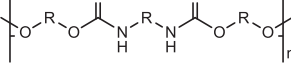
Biopolymers are processed into constructs with defined shapes and architectures for biomedical applications, including large implants such as bone screws and small ones like sutures and drug delivery devices [2]. For tissue engineering purpose, several types of polymeric scaffolds are non-trivial, such as hydrogels, fibrous scaffolds, polymer films and others. Those biopolymeric architectures are mimicking natural ECM. For drug delivery purpose, other different forms of polymeric scaffolds can also be fabricated into 3D porous matrix, microsphere, etc., which could achieve high drug loading and efficiency to specific sites [46].

In general, hydrogels, fibrous scaffolds, and polymer films are three kinds of typical scaffolds in tissue engineering [47]. They have distinct dimensions and microstructures, and are used for various biomedical applications. Although synthetic polymers greatly expand the scope of bioactive materials and overcome several limitations of natural polymers, they still suffer from the lack of surface functionality that mimics the natural systems. More importantly, the active and dynamic nature



Scheme 1. Overview on “click” fabrication of 1D, 2D, and 3D polymeric scaffolds.

Table 1
Several kinds of natural and synthetic polymers for bioscaffolds and related applications.

Natural polymers	Chemical structures	Biomedical applications	Ref
Agarose		Cell encapsulation and proliferation	[34]
Chitosan		Encapsulate hepatocytes, orthopedic scaffold	[35]
Collagen		Wound healing, promote blood coagulation, scaffold for cells	[35,36]
Gelatin		Support cells for orthopedic applications	[37]
Silk		Cell attachment, bone growth	[38]
Synthetic polymers	Structural units	Biomedical Applications	Ref
Poly (glycolic acid)		Heart valve engineering, support for muscle and endothelial cell growth	[39]
Poly(D,L-lactic acid-co-glycolic acid)		Regenerate an extracellular matrix	[40]
Poly(ε-caprolactone)		Support of cell viability	[41]
Polyorthoester		Bone reconstruction	[40]
Polyanhydride		Weight-bearing orthopedic	[42]
Polycarbonate(tyrosine derived)		Bone scaffolds	[43]
Poly(ethylene glycol)		Forming scaffolds copolymerized with other polymers	[44]
Polyurethane		Support cell attachment	[45]

of biological environment requires the adequate design in a spatial and temporal control manner, which is highly relied on safe, precise and efficient chemistry tools [21,48]. Traditional chemical modifications, like many non-“click” methods, usually have limitations in the precisely control over the bulk and surface properties due to their low conjugation efficiency in mild conditions. To fabricate these scaffolds with designed bulk and surface properties, robust and orthogonal chemical tools are highly demanded in this scenario. “Click” reactions fulfill the criteria that can be rationally employed to synthesize and fabricate these scaffolds with desirable properties and functions [49]. Synthesis and modification of these typical scaffolds will be discussed in this section.

2.2.1. Hydrogels

Hydrogels are defined as 3D cross-linked networks which can absorb a large amount of water without dissolving [50]. They are particularly useful in supporting the growth of new cells and tissues due to their rubber elasticity, high water content, and solute transportation [51]. Hydrogels can be prepared by nature polymers (*i.e.* collagen, gelatin or hyaluronate) and synthetic polymers like polyacrylic acid (PAA), polyethylene oxide (PEO) and polyvinyl alcohol (PVA) after extensively swelling with water [50]. They are not only structural like the ECM but also diffuse into hydrophilic nutrients and metabolites rapidly as well. Note that for natural hydrogels, the entire polymer backbone is exposed to aqueous conditions with water-soluble

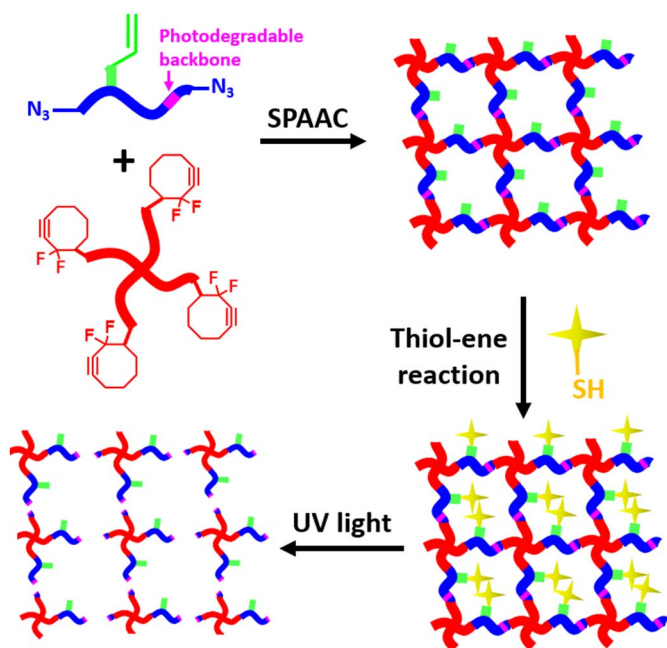


Fig. 1. Synthesis, photocoupling, and photodegradation for tuning chemical and physical properties of click-based hydrogels [3].

enzymes, probably leading to rapid hydrolysis [52,53].

Several strategies are employed to produce hydrogel networks via connecting functional polymers or polymeric segments. The formation of junctions between each polymer segments are vital in manipulating gel's mechanical strength and stability, which has been realized via formation of covalent and non-covalent bond with different strength and density [54] [55]. Physical hydrogels are formed by the transient crosslinking between polymer chains via various kinds of physical

interactions [55]. However, the weak physical cross-linking usually leads to low mechanical strength. Nevertheless, their dynamic self-assembly/disassembly feature can be employed to generate self-healing properties. A typical example is the mixture of clay and a dendritic molecular binder to prepare high-water-content self-healing hydrogels by Aida and coworkers [56]. In contrast, chemical hydrogels generally possess networks created by crosslinked covalent bonds, and their mechanical properties can be easily tuned via controlling the cross-linking density. For example, Anseth and coworkers reported the fabrication of smart degradable hydrogel by sequentially performed CuAAC and thiol-ene reactions, whose functionality and architecture can be independently controlled by light [3]. As shown in Fig. 1, the backbone of resulting hydrogels can be degraded along with the photocleavage of included nitrobenzyl ether moieties under ultraviolet (UV) light casting. In this way, chemical and physical properties of such hydrogels are rigorously tuned. Additionally, they found > 95% of human mesenchymal stem cells (hMSCs) remain viable after SPAAC encapsulation, thiol-ene coupling, and network photodegradation, showing good biocompatibility of the hydrogel *in vitro* [3].

Interestingly, several unique properties, like high water absorption and preservation, make hydrogels serving as good candidates in diapers, contact lenses, drug reservoirs, etc. [57] Considering their large surface area, biocompatibility and water absorption ability, hydrogels are considered as promising scaffolds for cell encapsulation and tissue regeneration [58].

2.2.2. Fibrous scaffolds

Fibrous scaffolds, such as nanofibers, are 1D materials with nanoscale diameters in width [59]. These nanofibers possess large surface area that are easy to immobilize various bioactive ligands and molecules [60]. In addition, the fibrous morphology mimics the fibrillar structure of the native ECM, enhancing the cellular attachment, proliferation and migration [61,62]. Three general approaches have been widely used to fabricate nanofiber meshes: electrospinning, self-

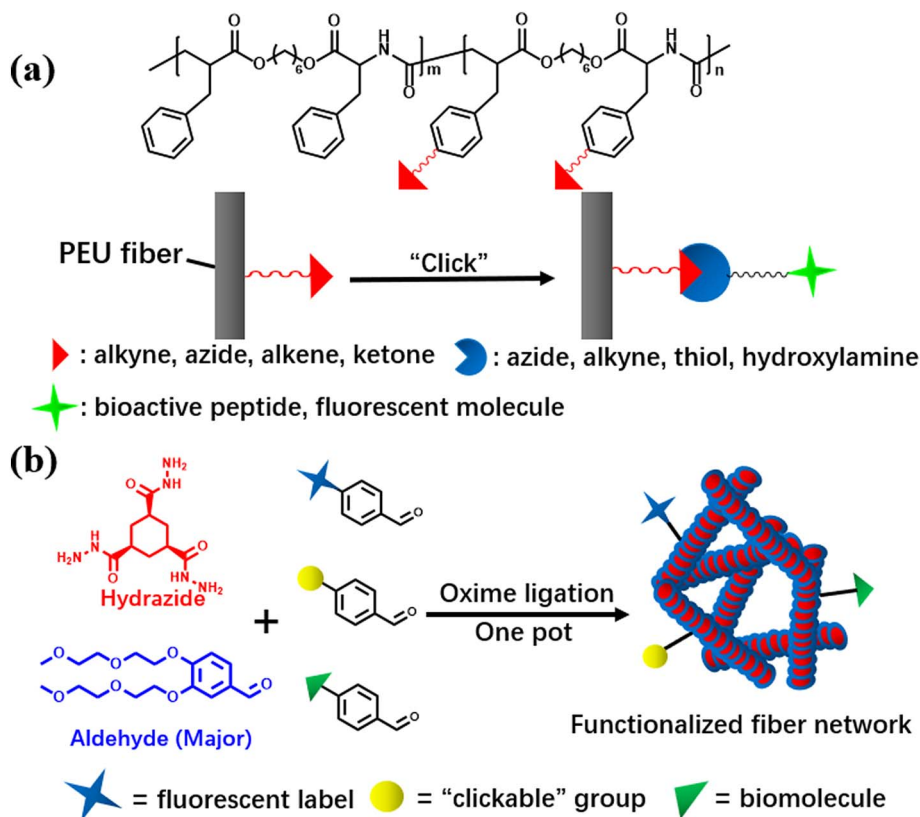


Fig. 2. (a) Synthesis and modification of PEU-based nanofibers [4], and (b) Preparation of biofunctional fiber network via self-assembly of small molecular gelators [5].

assembly and phase separation [63]. Among them, electrospinning is the most popular one because of large-scale productions, facile preparation process, and controllable 1D morphology [64]. It requires a suitable voltage between the tip of a capillary tube and a metal screen as the collector. When the electric field is strong enough to overcome the surface tension, the charged jet will eject to the collector. During the process, the solution exposes into atmosphere at room temperature meanwhile the solvent evaporates. The obtained nanofibers can be finally collected on the collector in a large scale [65]. For example, poly (ester urea) (PEU) nanofibers that contain several kinds of pendant “clickable” groups, were developed by Becker's group using electrospinning technology (Fig. 2a) [4]. Post-polymerization functionalization of PEU fibers with peptides and fluorescent probes via different “click” reactions enables a number of biofunctionalities on the fiber surface with interesting properties. Additionally, small molecular self-assembly approach is another emerging approach to generate functional nanofiber materials in a “bottom-up” fashion. In this method, molecular gelator has been widely used to build up supramolecular fibrous nanostructures [5]. For example, Eelkema and coworkers reported the use of self-assembled hydrazine gelator molecules and other molecules with “clickable” groups to prepare fiber networks. The resulting nanofiber structure can be further modified by “click” reactions to introduce fluorescent labels and protein tags (Fig. 2b) [5].

2.2.3. Polymer films

Polymer films, another kind of interesting 2D scaffolds, are widely used for tissue engineering, as a result of their good mechanical properties and readily available surface engineering methodologies [66]. It is a kind of important coating biomaterials tethered with various functional groups that could tune the cell-scaffold interactions [67]. Bioactive polymer films are usually produced by multiple methods, such as layer-by-layer (LBL) assembly [6], *in-situ* polymerization on the surface [68], chemical-vapor-deposited (CVD) coating [7], and others [69].

In general, the application of scaffolds in tissue engineering requires highly stable coating conditions during the fabrication process, and the “click” approach enables the covalent cross-linking via high efficient and modular reactions under mild conditions. One of the interesting examples is the LBL assembly of PAA by using “click” chemistry

(Fig. 3a) [6]. It was demonstrated the film thickness and morphology can be finely controlled via adjusting the assembly conditions, and the swelling properties could also be manipulated through the control over the density of “clickable” moieties. Moreover, using “clickable” monomers in CVD copolymerization fabrication could also offer the further conjugation of bioactive moieties onto prepared polymer thin films with multiple “clickable” groups. Orthogonal interface properties were obtained via CuAAC and thiol-maleimide “click” reactions for protein adsorption and cell attachment (Fig. 3b) [7].

2.2.4. Other scaffolds

Besides those three types of scaffolds discussed above, there are still many other kinds of polymeric scaffolds constructs used in tissue engineering, such as porous scaffolds and microspheres. Considering their excellent biocompatibility and suitable mechanical properties, they also play an important role in improving the regeneration and repair behaviors of tissues [15,17]. Particularly, porous scaffolds possess large surface area that allows cell attachment and growth. The pore size and porosity significantly affect cell growth by interfering the cell penetration, as well as nutrient and waste transportation [70]. To date, fiber bonding, solvent casting, gas foaming, and phase separation methods has been built to fabricate porous scaffolds with large and interconnected pore structures [71]. For instance, Vittorio and coworkers employed the “click” chemistry to prepare porous gels serving as drug-release materials and regeneration scaffolds [72]. Hyaluronic acid (HA) derivatives with either azide and alkyne groups were prepared in the first step, respectively. Then those two functionalized derivatives were mixed into aqueous solution together, resulting in a fast gelation after CuAAC reaction at room temperature. Cells could be distributed homogeneously and adhered smoothly to the surface of the inner pores of these click-gels. Caldwell and coworkers prepared degradable porous scaffolds using emulsion template by thiol-Michael “click” reaction [73]. Multifunctional thiols and acrylates were photopolymerized in water-in-oil high internal phase emulsions (HIPEs). The Young's modulus of the resulting porous scaffolds was related to the ratio of the acrylate monomers. 19% mass of the scaffolds degraded over 15 weeks under cell culture conditions. And cells were grown mainly on the surface of the scaffolds.

Additionally, microsphere scaffolds are usually fabricated by

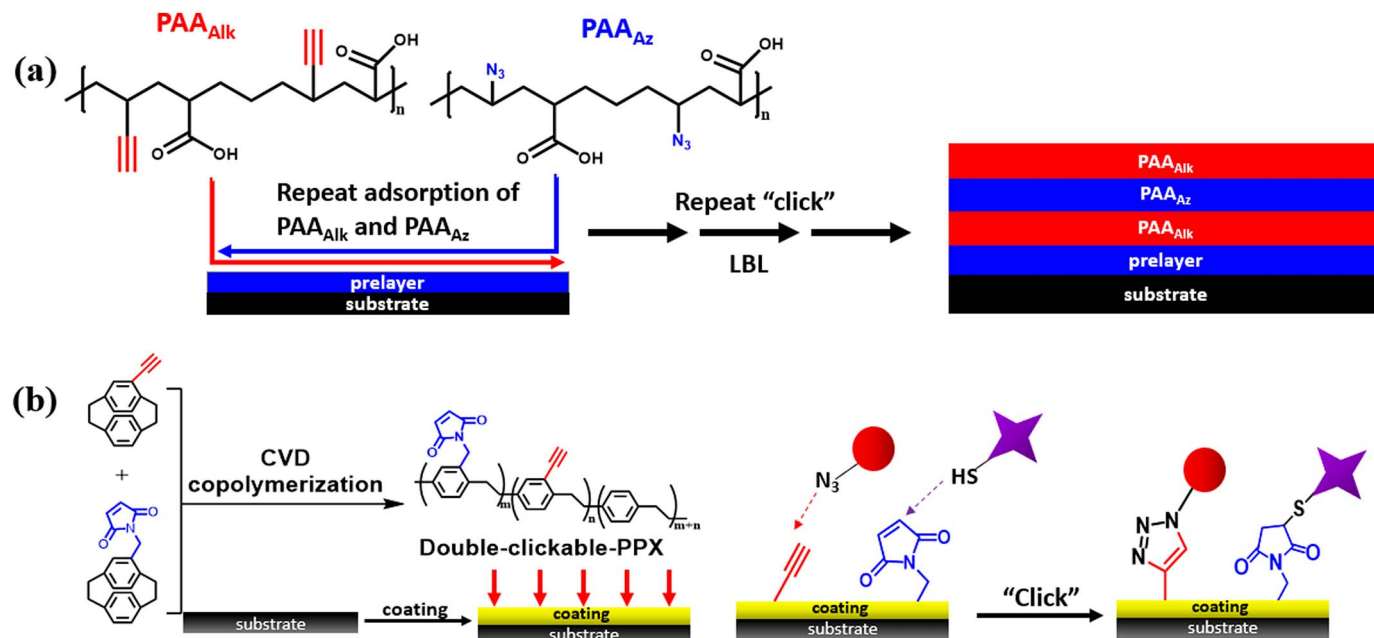


Fig. 3. (a) Synthesis of LBL assembly of Poly(acrylic acid) by CuAAC “click” reactions [6]. (b) Synthesis of double-clickable coating and the surface modified by CuAAC and thiol–maleimide “click” reactions [7].

emulsion evaporation technique which can encapsulate biomacromolecules like growth factors or proteins, giving a better cell adhesion and growth performance [74]. The combined utilization of controlled polymerization techniques and “click” reactions has been used to develop microsphere scaffolds with tunable surface functionalities [75]. Nguyen and coworkers produced microspheres by different “clickable” PEG derivatives *in situ* via CuAAC or SPAAC. Then, the scaffolds formed by crosslinking the microspheres. In the presence of endothelial cells, the scaffolds showed high viability, which were suitable for *in vivo* vascularization [76].

3. “Click” chemistry in polymeric scaffolds

3.1. “Click” toolbox

The design and preparation of multifunctional and architecturally controlled macromolecules is a prerequisite for a variety of future applications in tissue engineering. The advent of “click” chemistry indeed has led to an influx of new opportunities in this area, particularly useful in the fabrication of bioactive polymeric materials involving biomolecules like proteins and peptides [77,78]. As defined by Sharpless, “click” chemistry refers to a group of reaction that “...must be modular, wide in scope, give very high yields, generate only inoffensive by-products that can be removed by non-chromatographic methods, and be stereospecific” [21]. Their “click” features usually require simple reaction conditions (insensitive to oxygen and water), readily available starting materials and reagents, the use of minimum solvent, and non-chromatographic purification process [79]. In this section, several kinds of commonly used “click” reactions, including CuAAC, SPAAC, thiol-X reaction, DA reaction and oxime ligation (Scheme 2), will be detailly discussed on their concepts and used in polymeric scaffold fabrication.

3.1.1. CuAAC

In this cycloaddition reaction of CuAAC, a terminal alkyne reacts with an organic azido group to form a thermally and hydrolytically stable triazole ring, which is similar to the conventional Huisgen 1,3-dipolar cycloaddition [80]. In the presence of Cu(I) catalyst, the active energy for the cycloaddition step decreases from 24 kcal/mol to 11 kcal/mol. Moreover, the formation of 1,5-triazole ring is strongly disfavored based on the density functional theory calculations [22] compared with conventional Huisgen cycloaddition. Due to its versatility and efficiency, CuAAC has been widely used to construct bioactive polymeric materials *via* the conjugation of biomolecules onto polymer backbone, or “click” polymerization of biofunctional monomers [81,82]. For example, Emrick and coworkers conjugated azido-terminated PEG and arginine-glycine-aspartic acid (RGD) peptide onto polyester backbone using CuAAC reaction [77]. Guan and coworkers explored the mechanism of elastin's elasticity by CuAAC “click” polycondensation of elastin-like peptide monomers with alkyne functional group on C-terminus and azido group on N-terminus [83].

3.1.2. SPAAC

Considering that CuAAC reaction has potential drawbacks due to the toxicity of Cu(I), particularly when it is involved in biological systems, the emergence of metal-free “click” reaction expands the opportunities for use in general physiological conditions. Inspired by this demand, SPAAC reaction, developed by Bertozzi and coworkers, has gained an enormous impact due to its safe use for bioorthogonal modification of various biomolecules in living systems during the past ten years [23]. The term “strain-promoted azide-alkyne cycloaddition” derives from the concept of ring strain, which provides dramatic rate acceleration between azide and cyclooctynes compared to normal unstrained alkynes. The active energy of this ring chain reaction could decrease to 18 kcal/mol due to the bond angle deformation. Although SPAAC sometimes might produce a regioisomeric mixture of triazoles, the high efficiency of SPAAC under physiological environments makes

it an excellent alternative for biomaterials design and functionalization [23]. For example, Becker and coworkers conjugated azido-terminated peptides onto the nanofiber surface possessing cyclooctynes rapidly [84]. Anseth and coworkers developed peptide-functionalized hydrogels with controlled crosslinking densities *in situ* through SPAAC “click” reaction. The step-growth hydrogels formed in the presence of cells enabled independent and tuning bio-properties [85].

3.1.3. Thiol-X reaction

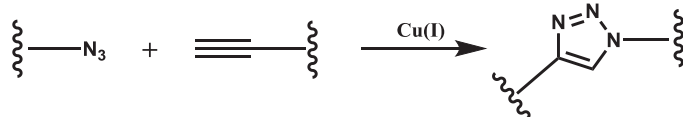
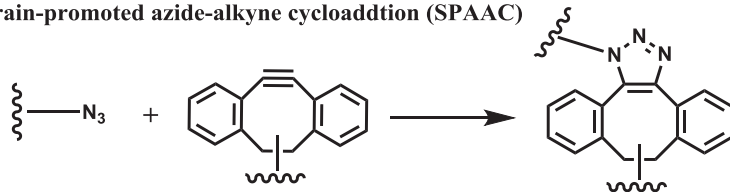
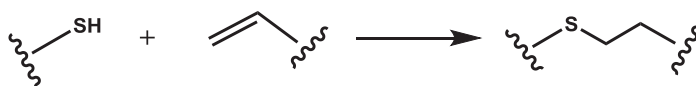
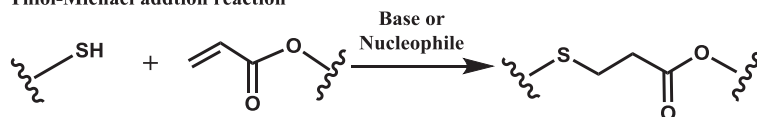
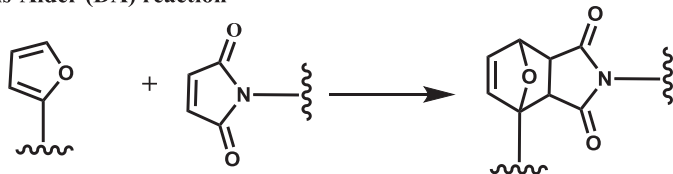
Thiol-X reactions have a long history in conventional organic synthesis and only recently have been reconsidered and exploited as a kind of “click” tool in preparing polymeric materials [24]. They are series of thiol-based reactions that meet “click” criteria, which are simple, efficient, and highly selective, yield a single product, and occur under mild condition. Particularly, radical-mediated thiol-ene/-yne are the most commonly used thiol-X reactions that can be widely employed in tissue engineering due to its metal-free feature [25]. For instance, Hawker and coworkers reported the fabrication of PEG-based hydrogel materials with enhanced mechanical properties *via* thiol-ene reaction [86]. The primary limitation of radical-mediated thiol-ene/-yne reactions is the requirement of UV source that might induce potential damages to cells and tissues. Alternatively, base/nucleophiles-mediated thiol-X reactions including thiol-Michael, thiol-isocyanate, thiol-epoxide and thiol-halide reactions have gained increasing attention [87]. In particular, thiol-Michael reaction involving the base-catalyzed coupling of thiol with active enes represents a direct way to fabricate polymeric biomaterials without additional toxic concerns. An interesting example was performed by Pritchard's group for developing an injectable hydrogels cross-linked *via* a thiol-Michael reaction with mechanical properties that resemble soft tissues [88]. However, it is also noted that several limitations, such as the lack of electron deficient alkenes in the polymer substrates as well as the presence of acid condition, might restrict its further usages in specific organs and tissues [89].

3.1.4. DA reaction

DA reaction involves a highly selective [4 + 2] cycloaddition between a diene and a dienophile to form a stable cyclohexene adduct product [26]. The scope of DA reaction is quite broad, and it could also form heteroatom-heteroatom bond, usually known as Hetero Diels-Alder reaction [79]. In addition, DA reaction proceeds reversely with temperature between 50 °C and 150 °C. This thermal reversibility is quite important that offers a simple way for the design of “self-healing” materials [90]. A variant of DA reaction, called inverse Diels-Alder tetrazine cycloadditions, gained growing interests as a next generation of “click” reaction due to its bioorthogonal feature as well as fluorogenic property which could be widely used as probes for bioimaging at the cellular level [91].

3.1.5. Oxime ligation

Carbonyl-condensation reaction between ketone/aldehyde groups and nucleophiles also represents the “click” features [21]. The resulting products contain stable chemical bonds, such as imine hydrazone and oxime with good stability under physiological condition [27]. However, the oxime ligation generally requires neutral and basic condition to minimize potential oxime exchange reaction, which limits its application in bioconjugation. Extensive work has been devoted to the development of new catalysis systems that enable oximes ligation under various pH conditions. One promising example was performed by Dawson and coworkers, by using aniline as a catalyst to exhibit a 20-fold rate enhancement at pH of 4.5 [92]. Unlike CuAAC and thiol-ene reaction, oxime ligation can be directly performed at room temperature without any metal catalyst or UV light, guarantying its safe use both *in vitro* and *in vivo*. Maynard's lab employed this reaction to develop a RGD functionalized PEG hydrogels with potential application in stem cell therapeutics [93].

Cu(I)-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC)**Strain-promoted azide-alkyne cycloaddition (SPAAC)****Thiol-X reaction****Thiol-ene reaction****Thiol-Michael addition reaction****Diels-Alder (DA) reaction****Oxime ligation****Scheme 2.** Schematic representation of various “click” reactions that have been used in preparing polymeric scaffold.

Importantly, the combination use of two or more kinds of “click” reactions usually offers a general way to fabricate bioactive polymeric scaffolds with complex chemical compositions as well as physical properties. Note that when more than one kind of reaction are involved in the material preparation, the orthogonal nature enables those multiple reactions to be processed without interfering each other, avoiding the undesirable byproducts and complicated protection-deprotection operations. Considering many promising advantages offered by multiple “click” functionalization strategy compared with individual reaction approach, like less work-up, shorter reaction time, minimum setup, and higher yield, more related work is still highly desired.

3.2. “Clickable” building blocks

In order to synthesize and fabricate polymeric scaffolds with improved functions via “click” reactions, the rational design of various building blocks with “clickable” groups is prerequisite. Those “clickable” building blocks are general simple molecular subunits possessing one or more “clickable” groups for further macromolecular construction [21]. On the basis of distinct roles of those building blocks play during the polymeric materials construction process, they can be mainly classified into four categories: “clickable” initiators, “clickable” monomers, “clickable” post-modifiers and “clickable” cross-linkers. In this section, these four “clickable” building blocks in terms of their molecular design and related macromolecular synthetic strategies will be carefully discussed.

3.2.1. “Clickable” initiators/chain transfer agents

To facilitate prepare telechelic polymers with chain-end “click” functionality, a simple and popular way is to introduce a “clickable” initiator or chain transfer agent for the macromolecular synthesis. Those “clickable” initiators should be compatible with several polymerizations methods, including atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer polymerization (RAFT), nitroxide mediated polymerization (NMP), and ring-opening polymerization (ROP), allowing the successful installation of “clickable” group(s) [94,95]. Table 2 lists several “clickable” initiators/chain transfer agents used for “clickable” polymer synthesis and fabrication. Notably, those resulting polymers from “clickable” initiators usually possess well-defined number of clickable units at the chain-end position(s), so the further “click” bioconjugation process can be easily determined and well controlled, providing a way towards well-defined polymeric biomaterials.

Recently many research groups took efforts to employ “clickable” initiators to prepare “clickable” telechelic polymers for tissue engineering usages [96–99]. For example, Fu and coworkers reported the fabrication of solvent-resistant nanofibers with a thermal-sensitive surface that could be used in scaffold-supported cell therapy for tissue engineering [96]. The “clickable” initiator, propargyl-2-bromoisobutyrate (PBiB), was employed in the synthesis of alkyne-terminated poly (*N*-isopropylacrylamide) (PNIPAM) brushes which were incorporated on the nanofibers via CuAAC “click” reaction. Then the nanofibers were endowed with thermal-sensitive property. Lian and coworkers

Table 2
“Clickable” initiators.

“Clickable” initiator	“Click” reaction	Polymerization method	Year	Ref
	CuAAC	ATRP	2009	[96]
	CuAAC	ATRP	2009	[99]
	CuAAC	RAFT	2014	[101]
	CuAAC	RAFT	2014	[102]
	CuAAC	RAFT	2010	[97]
	Thiol-ene	ATRP	2008	[103]
	Thiol-yne	ROP	2012	[98]
	SPAAC	ROP	2013	[84]
	Oxime ligation	ATRP	2007	[104]

synthesized thermoresponsive nanohydrogels with potential applications in tissue engineering by RAFT polymerization and CuAAC “click” reaction [97]. Dialkynetrithiocarbonate with two alkyne groups was employed as a RAFT agent to crosslink the gold nanoparticles with azide groups. And then thermoresponsive nanohydrogels can be *in situ* obtained by RAFT polymerization of NIPAM monomer. In addition, Luo's group designed and synthesized a new kind of chain transfer agent possessing alkyne group, [100] offering well-defined telechelic polymers with high efficiency after RAFT polymerization. These polymers can be used as drug carriers due to the properties of enzymatic biodegradation. Besides controlled radical polymerization methods, the use of “clickable” functional initiators also works well in other types of polymerization approaches, such as the application of ROP to construct biodegradable polycaprolactone [84,98] with “clickable” chain-end groups for further bioengineering.

3.2.2. “Clickable” monomers

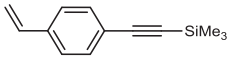
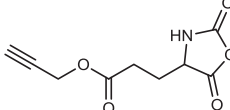
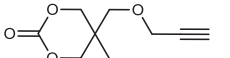
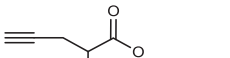
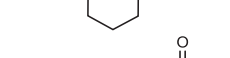
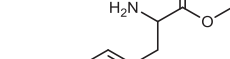
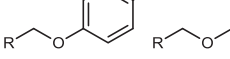
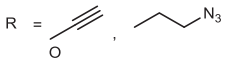
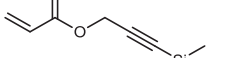
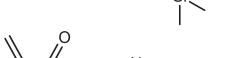
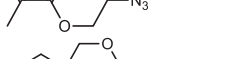
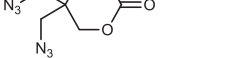
Direct polymerization of monomers with “clickable” functionality represents another useful approach for the development of multiple “clickable” groups modified pre-polymers [94]. Table 3 lists “clickable” monomers and related polymerization methods for preparing functional polymers and polymeric scaffolds. In general, the challenge of this strategy mainly lies to the incompatibility between “clickable” monomers containing ene/yne group(s) and several kinds of radical polymerization conditions, especially in high monomer concentration and high temperature. Therefore, the protection-deprotection strategy was used in those cases to avoid some undesirable side reactions. Notably, the compatibility between “clickable” monomers with ROP and condensation polymerization conditions is much better, and such protection-deprotection operations are generally not necessary [105]. The

direct polymerization of “clickable” monomers could yield functional polymers with various “clickable” units in high density, including alkyne, azide, diene, tyrosine and many others, enabling the further polymer/scaffold engineering for biomedical applications. For example, Lin et al. reported the fabrication of amino acid-based poly(ester urea)s nanofibers bearing pendent “clickable” groups for conjugation with biological functions [4]. By using different kinds of “clickable” monomers, a series of functional polymers with highly versatile “clickable” groups (alkyne, azide, alkene and others) could be successfully obtained, for further modifications with peptides and fluorescent probes. Wang and coworkers employed vinyl sulfone carbonate monomers to prepare vinyl-functionalized biodegradable polymers with different structures or compositions by ring-opening copolymerization. Kinds of biomolecules can be introduced onto the resulting vinyl-functionalized polymers by thiol-Michael “click reaction”, enabling their promising uses in tissue engineering [106]. Additionally, Engler's lab successfully synthesized poly(γ -propargyl-L-glutamate) with high alkyne density using “clickable” monomers bearing alkyne groups *via* ROP. Then PEG- N_3 chains were coupled with the alkyne groups on the backbone *via* CuAAC “click” reaction, offering functional polypeptide with high grafting PEG density that can mimic complex biomacromolecules and possess attractive features in properties and structures for biological applications [107].

3.2.3. “Clickable” post-modifiers

“Clickable” post-modifiers are defined as efficient tools for the direct functionalization of polymers with “clickable” groups, aiming to prepare desirable “clickable” pre-polymers for further bioconjugation. The related chemical transformation could occur either at pendant positions or terminal positions of polymers *via* high efficient chemical

Table 3
“Clickable” monomers.

“Clickable” monomer	“Click” reaction	Polymerization methods	Product	Year	Ref
	CuAAC	NMP	Polymer	2005	[108]
	CuAAC	ROP	Polymer	2009	[107]
	CuAAC	ROP	Hydrogel	2012	[109]
	CuAAC	ROP	Polymer	2005	[77]
	CuAAC, thiol-ene, oxime ligation	Condensation polymerization	Fiber	2013	[4]
	CuAAC, thiol-yne	RAFT	Glycopolymer	2010	[110]
	SPAAC	ATRP	Polymer film	2009	[111]
	SPAAC	ROP	Hydrogel	2011	[112]
	DA	Free radical	Pattern	2011	[113]
	Thiol-ene	RAFT	Glycopolymer	2010	[110]
	Thiol-ene	ROP	Polymer film	2011	[106]
	Thiol-ene	ROP	Copolymer	2011	[114]

reactions like esterification and amidation [115,116]. Table 4 lists several common “clickable” post-modifiers and related reactions. Although sometimes the efficiency of post-modification reaction is low due to the steric hindrance effect approaching to the polymer backbone, a unique advantage of using “clickable” post-modifiers is that it is not necessary to involve sophisticated protection and deprotection steps in this process. For example, hydroxyl terminated polymers can be successfully introduced by azide, alkyne [115], strained cyclooctyne, pyridyl-disulfide [116] and vinyl [116] “clickable” units at the polymer chain-end for further modifications. And this strategy is particularly useful for incorporating multiple “clickable” functionalities onto polymer backbone directly.

Importantly, one unique “clickable” post-modifier is called “click” adaptor, which describes a kind of molecules that are used to efficiently transfer one “clickable” group into another kind of “clickable” motif [117,118]. Table 4 shows three types of “click” adaptors for biopolymer post-functionalizations. They usually have aminooxy or other reactive groups on one side and “clickable” group(s) on the other side of the

molecule, which is quite useful for further expanding the scope of polymeric biomaterials during sequential fabrication process. For instance, Lin and coworkers utilized different aminooxy “clickable” adaptors to introduce ketone, alkyne, azide and methyl acrylate groups on the polymer film surfaces by oxime ligation [119]. Then several bioactive groups were easily immobilized and quantitated onto the polymer films via CuAAC or thiol-ene “click” chemistry and three separate bioactive groups were easily immobilized and quantitated onto the polymer scaffolds.

3.2.4. “Clickable” cross-linkers

For the *in situ* synthesis or fabrication of polymer scaffolds, cross-linking reactions are commonly used to couple with polymers or small molecules to form networks. Cross-linkers with “clickable” groups are particularly important in this aspect due to the high efficiency of “click” polymerizations. Table 5 summarized a list of “clickable” cross-linkers for nanofiber and hydrogel preparation. For example, Ellison’s group developed a green approach to synthesize chemically and thermally

Table 4
“Clickable” post-modifiers.

“Clickable” Post-modifier	“Click” reaction	Product	Year	Ref
	CuAAC	Hydrogel	2007	[72]
	CuAAC	Hydrogel	2007	[72]
	CuAAC	Nanofiber	2014	[120]
	CuAAC	Hydrogel	2006	[121]
	Thiol-Michael	Hydrogel	2014	[122]
	Thiol-yne	Hydrogel	2014	[122]
	DA	Hydrogel	2011	[16]
	SPAAC	Hydrogel	2011	[112]
	Oxime ligation	Fiber	2013	[4]
	Thiol-ene	Fiber	2014	[123]
	Thiol-ene	Fiber	2014	[123]
	Oxime ligation→CuAAC, SPAAC	Polymer film	2013	[119]
	Oxime ligation→CuAAC	Polymer film	2013	[119]
	Oxime ligation→Thiol-Michael	Polymer film	2013	[119]

* These post-modifiers are also called as “clickable” adaptors.

stable fibers via *in-situ* thiol-ene “click” polymerization of multiple acrylate-contained monomers as well as multi-thiols [124,125]. Hawker and coworkers used tetraazide-functionalized PEG as “clickable” cross-linker to synthesize PEG-based hydrogels with tunable cross-linking density by CuAAC “click” polymerization [121]. The obtained hydrogels containing unreacted azide groups can be further functionalized via the second-round CuAAC reactions. Shoichet and coworkers reported the fabrication of HA hydrogels with controlled mechanical properties and pore size by DA “click” polymerization [16]. Dimaleimide PEG and furan-modified HA derivatives were used as cross-linkers to form hydrogels, respectively. Lin and coworkers utilized oxime ligation to fabricate PEG-based hydrogels based on 4-arm aminooxy PEG cross-linkers for soft tissue engineering [126]. pH and aniline catalyst were able to influence the gel formation process due to the peculiar property of oxime ligation. Note that conventional hydrogel synthetic strategies usually involve radical polymerization or general chemical reaction of complementary groups [127], which could introduce cytotoxic chemicals which prevent further applications of the resulting gel products in tissue engineering. Alternatively, the “click” polymerization of “clickable” functional cross-linkers offered an attractive way for constructing bioactive polymeric hydrogels efficiently and safely [19,115,128–131].

4. “Click” fabrication of polymeric scaffolds for tissue engineering

The versatility and modularity of “click” reaction offers different material design and synthetic approaches to fabricate various polymeric scaffolds, which can be summarized into two kinds of general

methodologies (schematically illustrated in Fig. 4): (1) The scaffolds are directly fabricated either by polymers which are prepared and functionalized via “click” chemistry, or by “click” cross-linking polymerization of functional monomers (so-called pre-“click” fabrication strategy, see Fig. 4a); (2) The formed scaffolds are post-functionalized through *in-situ* “click” chemistry (so-called post-“click” fabrication strategy, see Fig. 4b). For the pre-“click” fabrication strategy, the bioconjugation process can be finely tuned and well controlled, resulting in the desirable scaffolds with well-distributed bioactive molecules. One possible shortcoming of this method lies in the low efficiency of incorporated biofunctionalities, especially for those inside the polymeric scaffolds. Alternatively, the post-“click” fabrication strategy offers a direct way towards surface engineering of polymer scaffolds with biomolecules *in situ*. Note that this methodology usually requires extremely high efficiency in heterogeneous reaction, and no side reaction related to denaturation of biomolecules. Thus, “click” chemistry is an ideal tool for this type of post-functionalization.

4.1. Pre-“click” fabrication of polymeric scaffolds

The high efficiency of “click” reactions allows the facile synthesis and modification of biofunctional polymerization for further scaffolds preparation. As one of the typical bio-scaffolds, hydrogels are three dimensions biomaterials mimicking ECM with ideal networks for cell culture, [44] usually fabricated through *in situ* “click” reactions between functional polymers/monomers, like “clickable” HA and PEG. Note that “click” chemistry offers the high efficiency reaction and

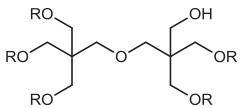
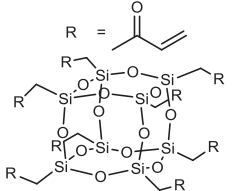
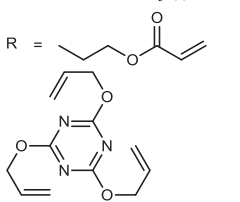
Table 5

“Clickable” cross-linker for hydrogel preparation.

“Clickable” crosslinker	“Click” reaction	Product	Year	Ref
	CuAAC	Hydrogel	2010	[115]
	CuAAC	Hydrogel	2006	[121]
	CuAAC	Hydrogel	2014	[102]
	SPAAC	Hydrogel	2009	[19]
	SPAAC	Hydrogel	2012	[128]
	SPAAC	Hydrogel	2010	[132]
	SPAAC	Hydrogel	2010	[132]
	Thiol-ene	Hydrogel	2011	[129]
	Thiol-ene	Hydrogel	2013	[130]
	DA	Hydrogel	2001	[16,133]
	DA	Hydrogel	2008	[131]
	Oxime ligation	Hydrogel	2013	[126]
	Thiol-ene	Fiber	2011	[124]
	Thiol-ene	Fiber	2011	[124]

(continued on next page)

Table 5 (continued)

"Clickable" crosslinker	"Click" reaction	Product	Year	Ref
	Thiol-ene	Fiber	2016	[125]
	Thiol-ene	Fiber	2016	[125]
	Thiol-ene	Fiber	2012	[134]

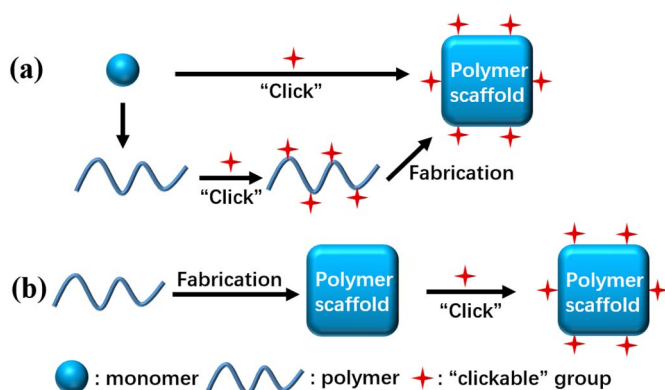


Fig. 4. Two methodologies for "click" fabrication of polymeric scaffolds: (a) pre-"click" fabrication strategy, and (b) post-"click" fabrication strategy.

excellent bond stability that enables precise control over the crosslinking reaction and density. In many cases, "click" reaction is particularly useful for the crosslinking purposes. For example, Lamanna's lab introduced azide or alkyne terminal functionality onto HA macromolecules, respectively. The resulting "clickable" HA polymers could further form the hydrogels with ideal structures and mechanical properties in the presence of Cu(I) via the efficient CuAAC crosslinking reaction [72,135]. Importantly, the hydrogels forming in drug solution or cell suspension could be used as controlled drug reservoir or scaffolds for tissue engineering, and the tiny concentration of Cu(I) showed no toxic for yeast cells and red blood cells. In addition, PEG derivatives with "clickable" groups could also be regarded as prevalent crosslinkers for hydrogel fabrication. 3-armed PEG-propiolate polymers were employed to crosslink with azide-functionalized chitosans via SPAAC at ambient temperature in aqueous solution. The mechanical strength and degradability can be finely tuned by changing the ratio between the chitosan and PEG precursors. The resulting hydrogel scaffolds were quite biocompatible and suited for cells adherence and proliferation, which could be used as injectable biomaterials for tissue engineering [136]. Furthermore, the one-pot, single-step preparation of HA-PEG hydrogels was performed by using DA reaction (Fig. 5), which does not need any additional crosslinking agents or catalysts [16]. The obtained HA-PEG hydrogels performed the shear moduli in the range of brain and nerve tissues, which are suitable for neural tissue engineering. The elastic modulus of the hydrogels can be easily controlled by adjusting

the molar ratio between furan groups and maleimide groups. It was found that the majority of cells remained on the hydrogel surface for about 14 days in the cell attachment and viability assay [16]. Also, eight-armed aminooxy PEGs were sequentially functionalized by RGD peptides, and glutaraldehyde cross-linking agents to prepare biofunctional hydrogels. They are compatible with physiological environments and useful for cell encapsulation [93]. The similar sequential "click" fabrication strategy has been widely used to synthesize multi-functionalized hydrogels. Gelatin methacrylamide (GelMA) and alkyne-modified PEG derivatives are combined to crosslink with PEG-tetra-thiol to fabricate interpenetrating networks (IPN) via thiol-ene and thiol-yne "click" reactions. The resulting bio/synthetic IPN (BioSIN) compositions are promising for cell adherence and encapsulation. The biocompatibility of BioSIN was evaluated using a Live/Dead assay of Endothelial cells (EA.hy926). The results showed a high level of cell viability (> 90%) and structural integrity for more than a week [122].

Biofibers are one kind of promising 1D scaffolds for tissue engineering due to their high surface-to-volume ratio and morphologically similarity to nature ECM [137]. Note that electrospinning nanofibers can be engineered via two different approaches: directly spinning of functional polymers with reactive and "clickable" sites, [5] and post-activating nanofiber surface after spinning [4] via wet chemical or plasma treatment. However, the later method bypassing the potential instability issues from fiber surface. Therefore, the method of directly spinning of polymers with "clickable" sites is in advance of preparing functional nanofibers and it is a facile way to fabricate nanofibers *via in situ* "click" chemistry reaction. For example, Yang and coworkers prepared thermos-sensitive fibers by thiol-ene reaction [134]. Under the UV illumination, poly((3-mercaptopropyl)methylsiloxane) (PMMS) and triallyl cyanurate are rapidly crosslinked to prepare fibers with thiol groups on the surface during electrospinning. Stimuli-responsive maleimide-terminated PNIPAM brushes were then easily introduced onto the fibers through thiol-Michael addition reaction (Fig. 6). The resulting PMMS-g-PNIPAM fibers showed thermal-sensitive property to the environment, which could be used as potential bioscaffolds. Interestingly, a green approach was developed by Shanmuganathan's group to fabricate robust fibers by thiol-ene "click" chemistry as well. A penta-functional acrylate (dipentaerythritol pentaacrylate) and a tetra-functional thiol (3-mercaptopropionate) were crosslinked *in situ* under UV light during electrospinning. The resulting functional fibers exhibited excellent chemical and thermal stability with various potential bioapplications. The resulting nanofiber structure can be further modified by "click" reactions to introduce fluorescent labels and protein tags [124].

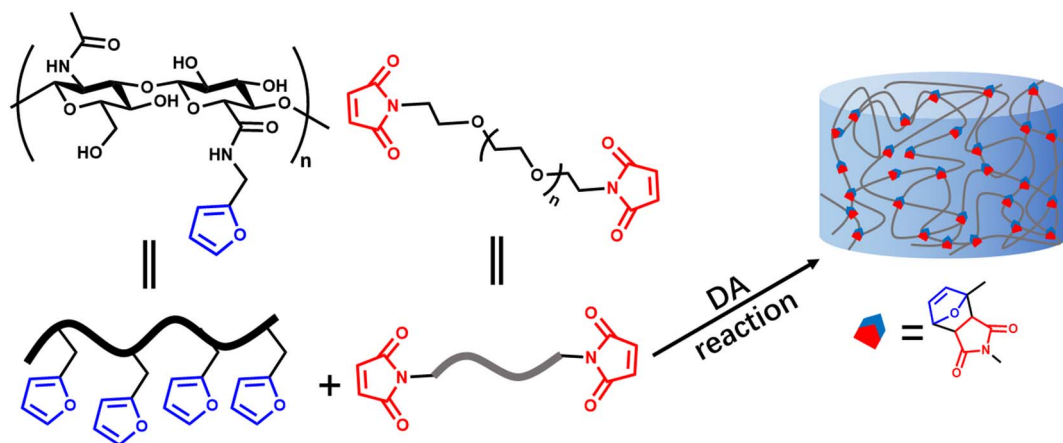


Fig. 5. DA “click” fabrication of HA-PEG hydrogels [16].

The surface functionalization of polymer film could be also performed via “click” reactions. For instance, the self-polymerization of dopamine allows for *in-situ* generating a biocompatible polydopamine layer to a wide range of materials by involving alkyne-terminated PEO [14]. The tethered alkyne groups on the film allow the further bioconjugation of various bioactive peptides using CuAAC “click” chemistry to promote a specific cell adhesion on a protein-repulsive surface. Biocompatible polymer film can be used as cell culture scaffolds with mechanical integrity and highly interconnected porosity [138]. However, when porous structures were introduced into film scaffolds, the decrease of their mechanical strength could significantly limit the practical use *in vivo*. One promising strategy to address this issue relies on the multi-functionalized “click” fabrication, which not only efficiently increases the mechanical strength of the resulting scaffolds, but also facilitates biomolecule conjugation through the “clickable” sites. Yang and coworkers also reported the use of sequentially performed thermal “click” reaction and SPAAC reactions to prepare functional porous poly (1,8-octanediol citrate) (POC) scaffold (Fig. 7) [139]. The tensile strength and Young's modulus of the resulting scaffolds are much higher than those of control samples without “click” crosslinking points. The *in vitro* cytocompatibility study against 3T3 fibroblastic cells was conducted by MTT assay. The result showed that introduction of click moieties into polymers can improve the compatibility. The authors also evaluated the *in vivo* cytocompatibility. The mild inflammatory response indicated that click moieties does not compromise the biocompatibility of POC polymers. Additionally, the residual azide groups on the surface enabled a facile and efficient conjugation of a collagen mimetic peptide (p15) to promote the adhesion and proliferation of endothelial cells.

Besides single type of “click” reaction, the combined utilization of orthogonal “click” reactions in one pot fashion offers a more rapid and efficient way to fabricate polymeric bioscaffolds. Truong and coworkers reported the facile double “click” fabrication of two interpenetrating

hydrogel networks via thiol-yne and DA reactions simultaneously. The resulting hydrogels possess high mechanical strength and biocompatibility, and the residue “clickable” groups could further convert into more kinds of bifunctionalities. The gel formation process was very fast under physiological conditions and it was regarded as a promising bio-support for human cells well-growing (Fig. 8) [140].

Double “click” synthetic protocol is a powerful methodology to directly fabricate polymeric scaffolds with a combination of different kinds of click reactions [141]. For example, Godeau et al. developed glycosyl-nucleoside-lipid (GNL) hybrid compounds via double CuAAC “click” reactions (Fig. 9) [142]. The assembly of GNL in aqueous solution could rapidly form bioactive nanofibers. The resulting nanofibers were able to further complex with nucleic acids, which greatly increased the cell uptake in serum without additional toxicity (Fig. 9). In another work, Ziane and coworkers also employed glycosyl-nucleoside-fluorinated (GNF) to self-assemble into hydrogels as scaffolds via double CuAAC reaction. The biocompatibility studies of GNF-based hydrogels in cells show good cytocompatibility since that the hydrogel doesn't release toxic compounds. Additional biocompatibility studies in tissue showed a moderate inflammation after implantation in mouse, confirming its potential in tissue engineering applications [143]. The encapsulated cells inside the formed hydrogel could differentiate into osteoblasts without osteogenic factors, offering a promising biomaterial scaffold for spongy bone regeneration. Considering the orthogonality and modularity of “click” reactions, the chemical structures and biological properties of resulting polymers and scaffolds can be respectively tuned for different therapeutic purposes.

4.2. Post-“click” fabrication of polymeric scaffolds

Post-“click” fabrication strategy has been widely employed for the surface engineering of various polymeric scaffolds, including hydrogels, nanofibers and films via *in-situ* “click” reactions [4,11,84,126,144].

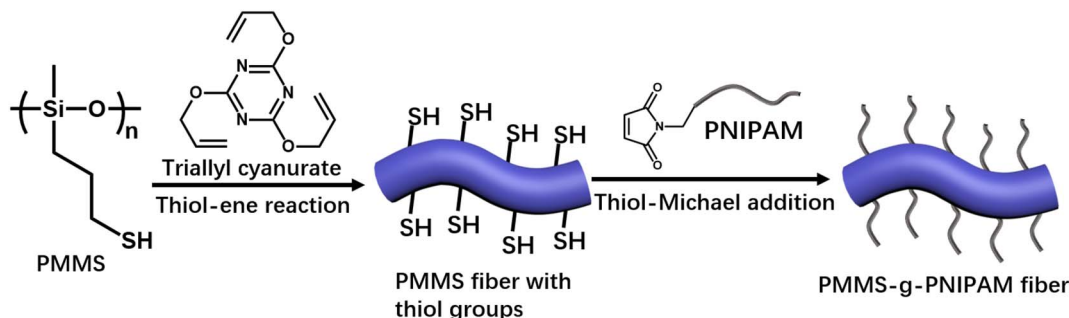


Fig. 6. “Click” preparation and fabrication of electrospinning functional PMMS fibers [134].

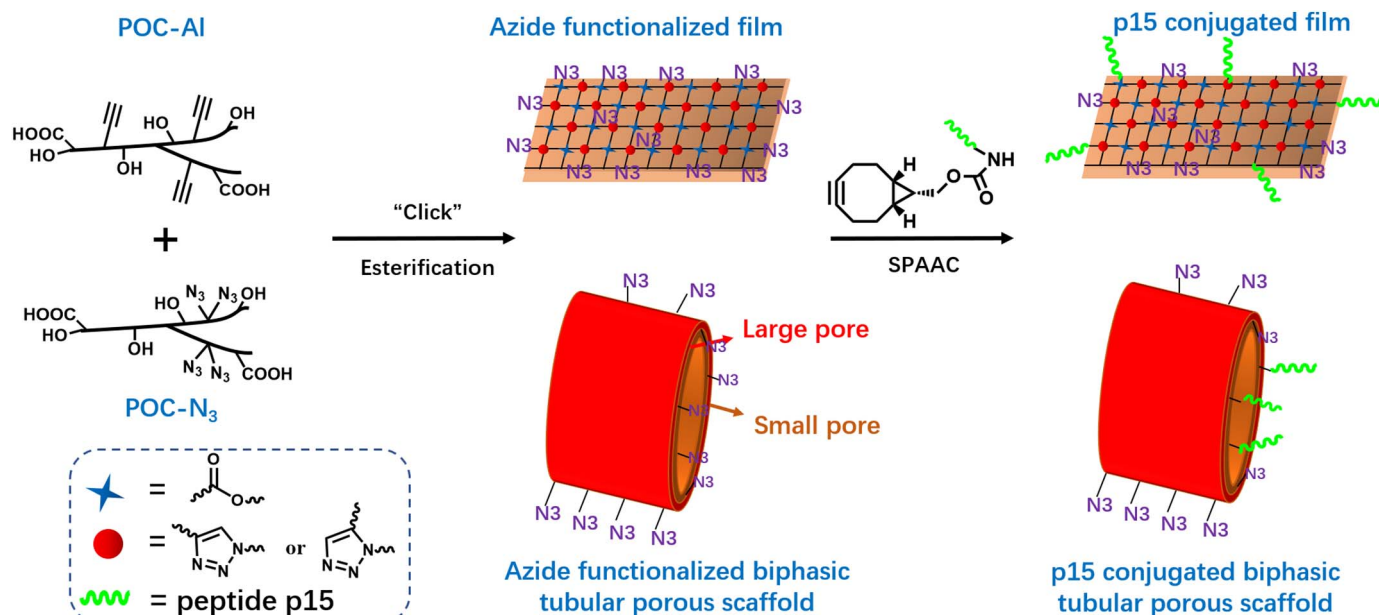


Fig. 7. Sequential "click" fabrication of p15 conjugated film and biphasic tubular porous scaffolds [[139].].

Conventional chemical methods for the surface modification fails to precisely control the incorporated functionality since the reaction usually involved the uncontrollable pre-treatments such as plasmas or sodium naphthalene etching, while "clickable" sites on the surface can be easily introduced during the scaffold formation process. More importantly, the high efficiency of "click" reactions could enable the successful heterogeneous bioconjugations onto the scaffolds to achieve the desirable biological functions. Their mimic of heterogeneity and biofunctionality in human tissues can also be easily controlled.

"Click" chemistry is widely used for hydrogel functionalization at specific sites and specific time, creating biomolecule patterned hydrogels [145,146]. This is generally regarded as the first step study to mimic the complexity of natural biological environment. Anseth and her co-workers developed a series of work on creating 3D patterned hydrogel networks using "click" reactions [19,128,145,147]. For example, they have successfully fabricated photo-degradable hydrogel materials with bioactive peptides *via* thiol-ene reaction. When the gel

products were exposed to UV-light, specific peptides were removed from the networks through photocleavage reaction, leading to a spatiotemporal control of peptide concentration. The cells attachment, proliferation and motility can be finely controlled after the encapsulation of cells inside hydrogel platforms. In addition, they have also used two kinds of sequential performed metal-free "click" reactions to generate reversible biopattern gels which were potentially served for drug release (Fig. 10) [128]. The SPAAC reaction was employed for the hydrogel formation, and thiol-ene reaction served as the efficient tool for biomolecule conjugation with thiol-derived biomolecules. In the cell assay, it was found that cell behavior can be selectively confined to patterned regions within each single gel. In another work, thiol-ene "click" reaction was again employed to functionalized PEG gel scaffolds with glucocorticoid dexamethasone (Dex) and matrix metalloproteinase (MMP)-cleavage peptides. The resulting cell-mediated delivery performance can be simply tuned by varying the peptide sequence [147].

Conventional chemical modifications of nanofiber surface, such as

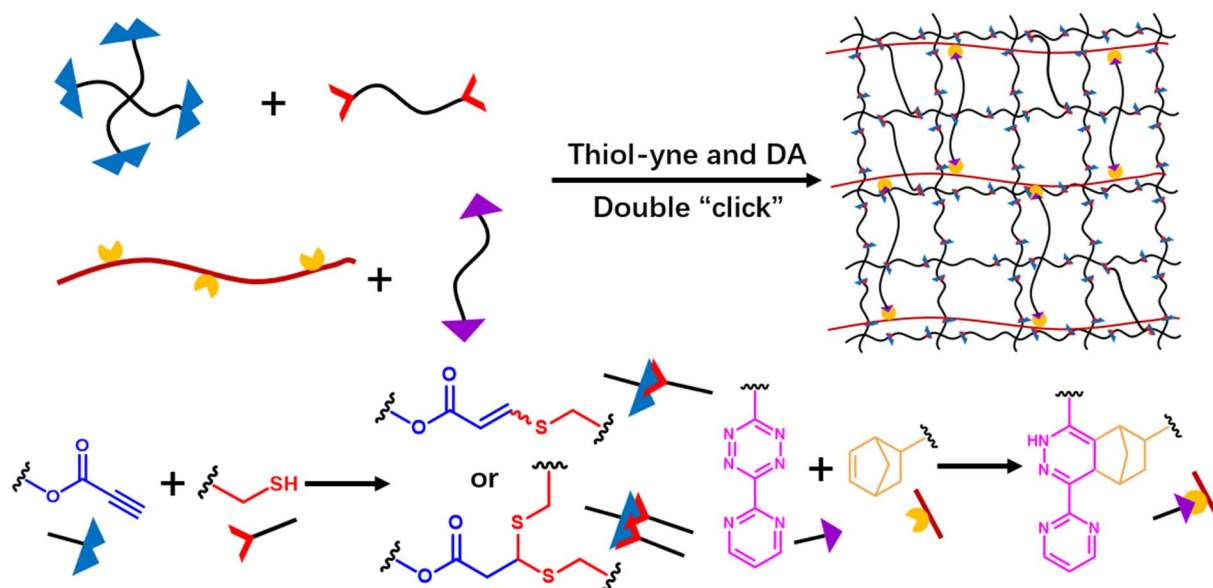


Fig. 8. One-pot double "click" fabrication of hydrogels through thiol-yne and DA reactions [[140].].

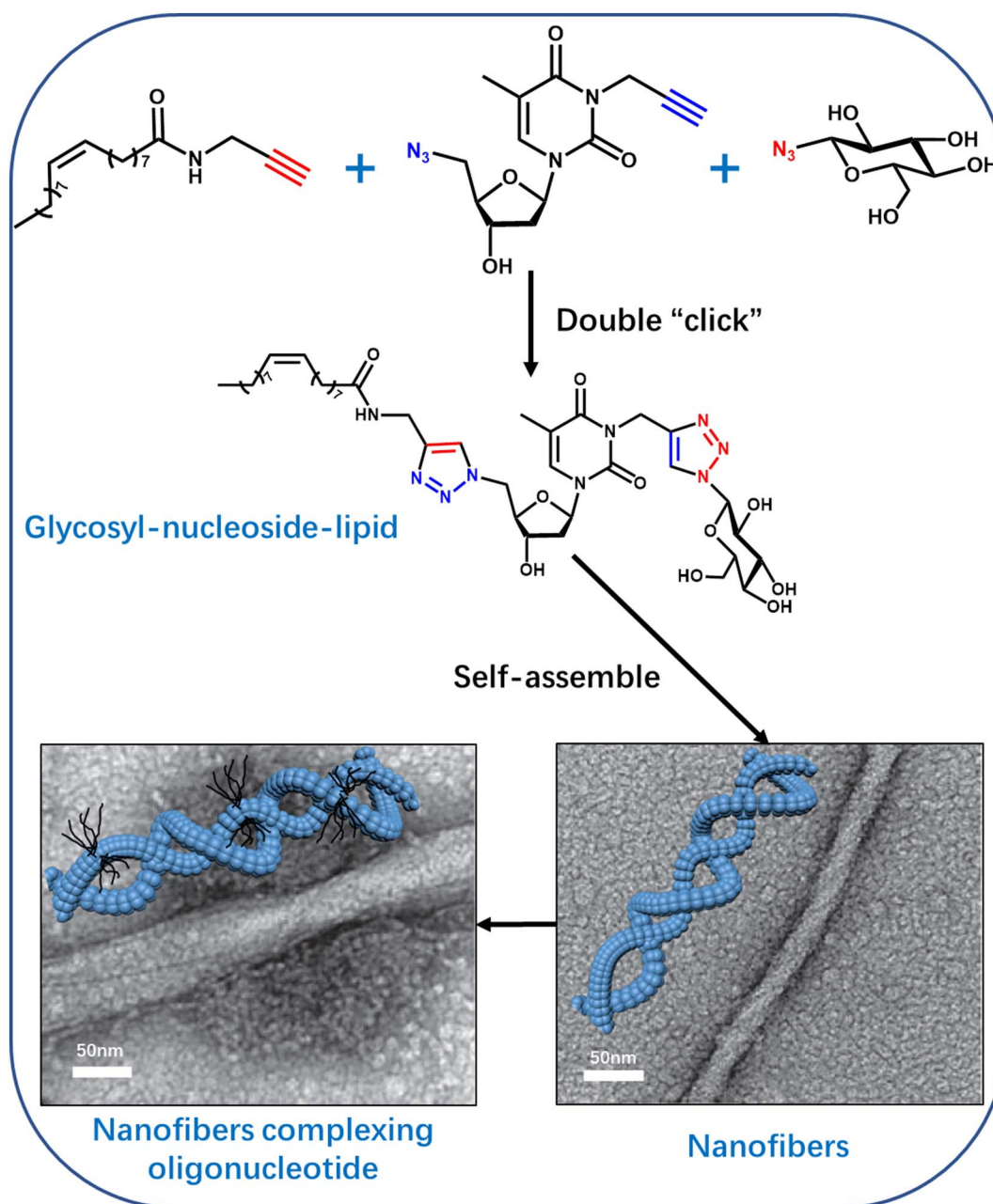


Fig. 9. Bioactive nanofibers formed by self-assembling GNL prepared via double CuAAC reactions. Reprinted with permission from Ref [142] with permission from (Copyright 2009, Royal Society of Chemistry).

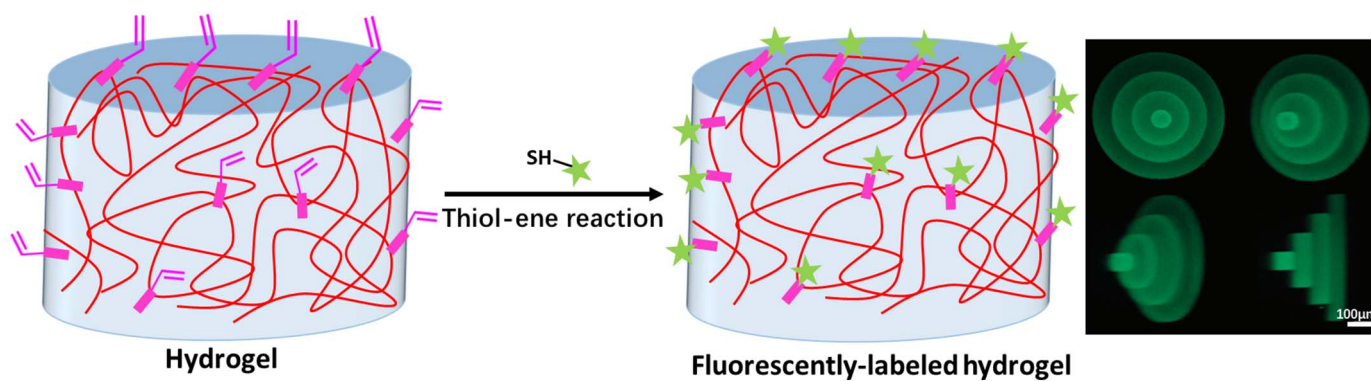


Fig. 10. Thiol-ene "click" post-fabrication of fluorescently-labeled peptide hydrogels. Reprinted with permission from Ref [19] with permission from (Copyright 2009, Nature Publishing Group).

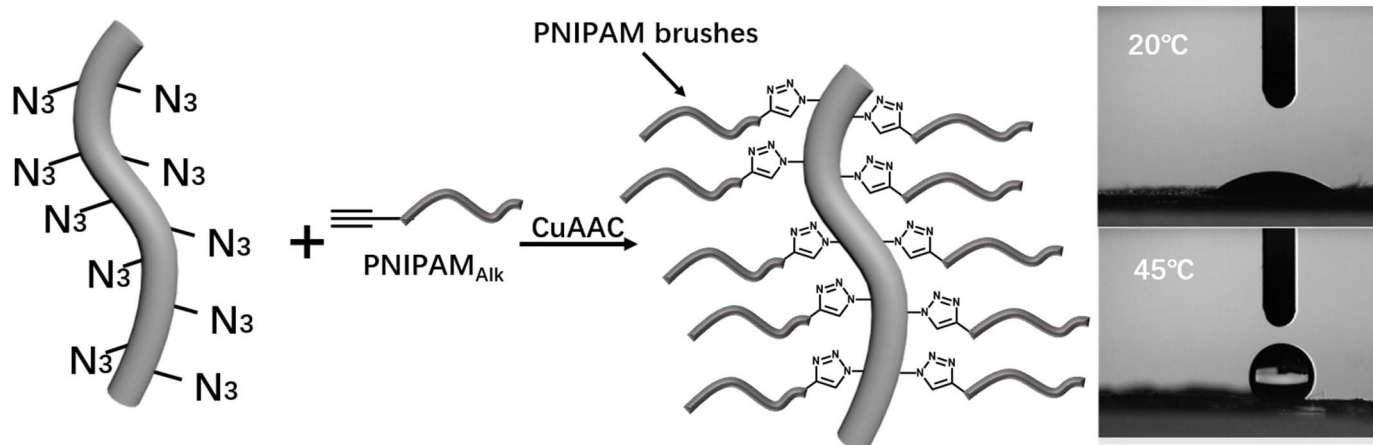


Fig. 11. CuAAC “click” fabrication of thermal-responsive nanofiber surface with PNIPAM brushes. Reprinted with permission from Ref [96] with permission from (Copyright 2009, American Chemical Society).

wet chemical method and plasma treatment, have been proved as an efficient way to introduce chemical functionalities onto the surface [148]. However, those methods could also induce surface damages on the fibers. In order to retain the integrity of nanofibers while introducing broad functional moieties in a controlled manner, direct post-modifications using “click” reactions were purposed to achieve the diverse surface biofunctionalities on the 1D polymeric scaffolds [4]. For instance, an electrospinning poly(4-vinylbenzyl chloride) and poly(glycidyl methacrylate) copolymer (PVBC-b-PGMA) nanofiber were efficiently conjugated with alkyne-terminated PNIPAM on the surface through CuAAC reaction, finally resulting in solvent-resistant nanofibers with a thermal-sensitive surface. The thermal-responsive behavior was explored by measuring water contact angles at different temperatures. As shown in Fig. 11, at low temperature, nanofiber exhibited a hydrophilic surface property, possessing a water contact angle $< 30^\circ$; while the temperature increased to 45°C , the surface nanofibers changed to be hydrophobicity, and its water contact angle increased to 140° . Those surface smart nanofibers can be potentially used as scaffold-supported cell therapy for tissue engineering [96]. In another work developed by Becker and his coworkers, azide derived peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) was *in-situ* functionalized onto PLLA nanofiber surface containing alkyne groups via SPAAC [84]. Those surface biofunctional nanofiber scaffolds were found to increase the level of neurite extension and gene expression of mouse embryonic stem cells (mESC). Additionally, a series of amino acid-based poly(ester urea) (PEU) bearing different kinds of “clickable” groups such as alkyne, azide, alkene, tyrosine–phenol and ketone group were prepared and the grafting densities of these functional groups are rigorously tuned via copolymerization strategy [4]. The corresponded PEU nanofibers were generated via electrospinning technology. Fluorescein-labeled peptides possessing “clickable” groups reacted with the PEU nanofibers onto the surface in aqueous solution through thiol–ene, oxime ligation and CuAAC “click” reactions orthogonally. The resulting biofunctional nanofiber scaffolds possessing ECM-like on the surface and biodegradable property without toxic byproducts which show great potential in regenerative medicine applications.

Recently, significant efforts have also been devoted to the rapid surface functionalization of polymer films with bioactive molecules via “click” reactions [106,149,150]. For instance, Zhong and coworkers fabricated a series of polymer films by copolymerizing of ϵ -caprolactone/L-lactide/trimethylene carbonate and vinyl sulfone carbonate (VSC), which were further modified with peptides using thiol–Michael “click” reaction (Fig. 12) [106,151]. They observed improved cell adhesion and growth of MG63 cells on PCL films after treated with non-fouling polymers or GRGDC peptide. On the other hand, the combination of *in situ* “click” fabrication concept with physical processing

methods, such as spin coating, show great ability for both quantitative composition and functionality control in surface bioengineering of polymer films. For example, wafers spin-coated polymer films were patterned by E-beam resulting in micropatterned hydrogels with aminoxy groups which can react with ketone-bearing RGD peptide via oxime ligation [149]. Cell can adhere and proliferate in the hydrogel film in the presence of the integrin ligands and growth factors. Furthermore, the bioactive peptides enlarged the cell area and created an appropriate microenvironment which was suitable for tissue engineering application potentially. In addition, a solid cellulose substrate tether with cyclopentadienes on the surface has been used to react with peptides bearing thioamides through hetero-DA reaction. Size exclusion chromatography (SEC) and SEC-electrospray ionization mass spectrometry (SEC-ESI-MS) were employed to confirm the high efficiency and compatibility of “grafting-to” reaction in aqueous solution. These bioactive substrate surfaces offer new opportunities in tissue engineering fields [150].

Double “click” strategy has been regarded as an important tool to immobilize complex biofunctionalities onto polymer scaffolds, which could minimize synthetic steps and reaction times. For example, Zheng et al. introduced GRGDS and YIGSR peptides onto nanofiber surfaces simultaneously via SPAAC and oxime ligation in a one-pot reaction [152]. The DIBO groups were derived from the initiators and the reactive ketone groups appeared upon deprotection. The bound peptides of the resulting scaffolds were bioactive for cell attachment and proliferation, which suggested the applications for peripheral nerve regeneration. Moreover, a 2D orthogonal concentration gradient film with azide and alkene “clickable” groups was generated via a sequential deposition process by Ma and coworkers. Multiple kinds of peptides were conjugated onto the solid surface through metal-free SPAAC and thiol–ene reactions sequentially. Peptides introduced onto the scaffolds enabled excellent concentration identification and the synergistic interactions assessment. These products potentially provided profitable information as to scaffolds for tissue engineering applications (Fig. 13) [153].

5. Summary and perspectives

The advent of “click” chemistry has led to an influx of new ideas and new tools in the fabrication of polymeric scaffolds for tissue engineering applications. Those chemical ligations possess quite high efficiency, orthogonality and biocompatibility, allowing the synthesis and functionalization of well-defined polymers with tailored properties, and offering many attractive possibilities for bioconjugations. The combination of “click” chemistry toolbox with “clickable” building block library simplifies the manufacturing process as well as brings new

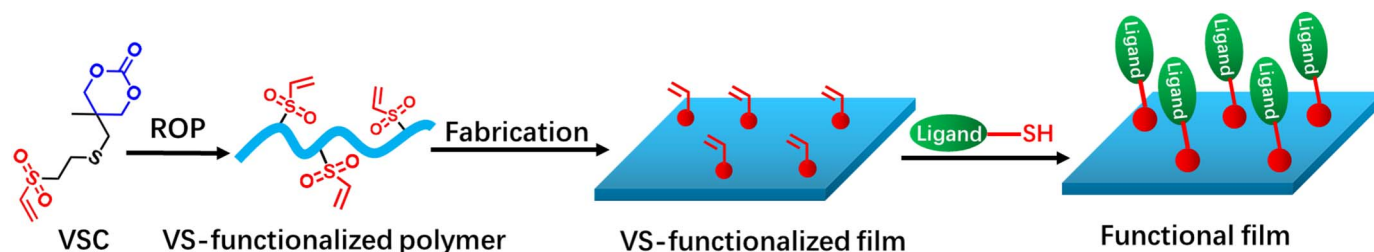


Fig. 12. Thiol-ene surface “click” fabrication of polymer film scaffolds [106].

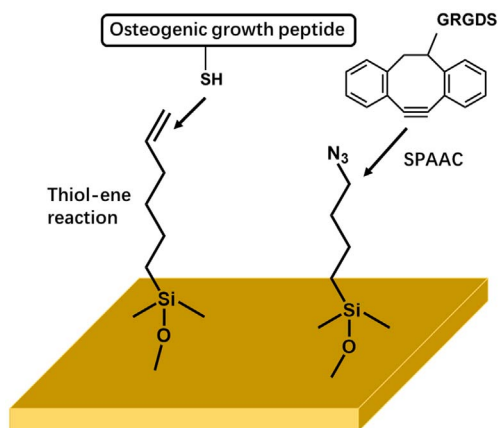


Fig. 13. Orthogonal peptide gradient “click” fabrication of polymer film [153].

features to the polymeric scaffold, including better biocompatibility, controlled cell behaviors, enhanced mechanical properties and many others.

As the family of “click” chemistry continues to expand, more types of polymeric materials with desirable chemical functionalities and hierarchical structures can be widely exploited as biomaterial scaffolds. Notably, the current design rules for polymeric scaffolds usually emphasize the specific and desired functions to surrounding cells and tissues. However, the dynamic feature of biological system highly requires fast response of scaffold material to adapt to physiological environment change. Although there have been a few examples of single stimuli-responsive bio-scaffold systems reported in recent years, the need for alternative solutions meeting the demand for an active response to the multiple and complex environmental changes could continue to drive advances in tissue engineering. And the combined utilization of multiple biorthogonal and “click” reactions, and other efficient chemical transformation will become one of the central topics in this innovation. In another context, many of the current works focus on the proof of concept *in vitro* while examples of translation these scaffolds into clinical trial are still rare due to the existence of significant technical and business barriers. More importantly, the safety issue of “click” based materials has been still under investigation since “click” concept was introduced in 2001, particularly in many “click” functionalization FDA approved biodegradable polymers. Less attention has been put on to evaluate the stability and degradation mechanism of the “click” based polymers in living systems [154]. Moreover, the contribution of “click” groups and additives (catalysts and initiators) towards biological systems needs careful evaluation when going into clinical trials [79]. For example, the use of toxic Cu(I) is always a concern when applying to *in vivo* studies. While the metal-free SPAAC can avoid this circumstance, the time and cost of synthesizing the a strained cyclooctyne, especially in industrial scale, still needs more considerations when applied to the clinical applications. The radical initiator left in the thiol-ene reaction has been found to induce toxic response in the cell level. Therefore, the development of cost-efficient purification methods to get rid of toxic metals as well as initiators might be an alternative way to solve this

issue. With the development of “click” chemistry to adequate the active response to the complex environmental, we can anticipate that all the complexity requirement from the technical demands can addressed but also control the cost and implementation time in an acceptable range.

Acknowledgment

The authors are indebted to many scientists whose research works are discussed here. This work was supported by National Natural Science Foundation of China (21774079 to Y. L., and 21474064 to M. D.). Y. L. thanks the financial support from State Key Laboratory of Silicate Materials for Architectures, Wuhan University of Technology (SYSJJ2017-01) and State Key Laboratory of Polymer Materials Engineering, Sichuan University (No. sklpm2016-3-03).

References

- [1] D.F. Williams, *The Williams Dictionary of Biomaterials*, Liverpool University Press, 1999.
- [2] L.S. Nair, C.T. Laurencin, Biodegradable polymers as biomaterials, *Prog. Polym. Sci.* 32 (2007) 762–798.
- [3] C.A. DeForest, K.S. Anseth, Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions, *Nat. Chem.* 3 (2011) 925–931.
- [4] F. Lin, J. Yu, W. Tang, J. Zheng, S. Xie, M.L. Becker, Postelectrospinning “click” modification of degradable amino acid-based poly (ester urea) nanofibers, *Macromolecules* 46 (2013) 9515–9525.
- [5] J.M. Poolman, C. Maity, J. Boekhoven, L. van der Mee, V.A. le Sage, G.M. Groenewold, S.I. van Kasteren, F. Versluis, J.H. van Esch, R. Eelkema, A toolbox for controlling the properties and functionalisation of hydrazone-based supramolecular hydrogels, *J. Mater. Chem. B* 4 (2016) 852–858.
- [6] G.K. Such, J.F. Quinn, A. Quinn, E. Tjpto, F. Caruso, Assembly of ultrathin polymer multilayer films by click chemistry, *J. Am. Chem. Soc.* 128 (2006) 9318–9319.
- [7] T.-P. Sun, C.-H. Tai, J.-T. Wu, C.-Y. Wu, W.-C. Liang, H.-Y. Chen, Multifaceted and route-controlled “click” reactions based on vapor-deposited coatings, *Biomater. Sci.* 4 (2016) 265–271.
- [8] Y. Hu, W. Dan, S. Xiong, Y. Kang, A. Dhinakar, J. Wu, Z. Gu, Development of collagen/polydopamine complexed matrix as mechanically enhanced and highly biocompatible semi-natural tissue engineering scaffold, *Acta Biomater.* 47 (2017) 135–148.
- [9] M.E. Furth, A. Atala, M.E. Van Dyke, Smart biomaterials design for tissue engineering and regenerative medicine, *Biomaterials* 28 (2007) 5068–5073.
- [10] M. Ding, J. Li, X. He, N. Song, H. Tan, Y. Zhang, L. Zhou, Q. Gu, H. Deng, Q. Fu, Molecular engineered super-nanodevices: smart and safe delivery of potent drugs into tumors, *Adv. Mater.* 24 (2012) 3639–3645.
- [11] M. Ding, N. Song, X. He, J. Li, L. Zhou, H. Tan, Q. Fu, Q. Gu, Toward the next-generation nanomedicines: design of multifunctional multiblock polyurethanes for effective cancer treatment, *ACS Nano* 7 (2013) 1918–1928.
- [12] X. You, Y. Kang, G. Hollett, X. Chen, W. Zhao, Z. Gu, J. Wu, Polymeric nanoparticles for colon cancer therapy: overview and perspectives, *J. Mater. Chem. B* 4 (2016) 7779–7792.
- [13] S.-o. Han, R.I. Mahato, Y.K. Sung, S.W. Kim, Development of biomaterials for gene therapy, *Mol. Ther.* 2 (2000) 302–317.
- [14] V. Proks, J. Jaroš, O. Pop-Georgievski, J. Kučka, Š. Popelka, P. Dvořák, A. Hampl, F. Rypáček, “Click & Seed” approach to the biomimetic modification of material surfaces, *Macromol. Biosci.* 12 (2012) 1232–1242.
- [15] D. Shendi, A. Dede, Y. Yin, C. Wang, C. Valmikinathan, A. Jain, Tunable, bioactive protein conjugated hyaluronic acid hydrogel for neural engineering applications, *J. Mater. Chem. B* 4 (2016) 2803–2818.
- [16] C.M. Nimmo, S.C. Owen, M.S. Shoichet, Diels-Alder click cross-linked hyaluronic acid hydrogels for tissue engineering, *Biomacromolecules* 12 (2011) 824–830.
- [17] J. Yang, T. Long, N.-F. He, Y.-P. Guo, Z.-A. Zhu, Q.-F. Ke, Fabrication of a chitosan/bioglass three-dimensional porous scaffold for bone tissue engineering applications, *J. Mater. Chem. B* 2 (2014) 6611–6618.

- [18] H.D. W, Scaffolds in tissue engineering bone and cartilage, *Biomaterials* 21 (2000) 2529–2543.
- [19] C.A. Deforest, B.D. Polizzotti, K.S. Anseth, Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments, *Nat. Mater.* 8 (2009) 659–664.
- [20] L.L. Hench, J.M. Polak, Third-generation biomedical materials, *Science* 295 (2002) 1014–1017.
- [21] H.C. Kolb, M. Finn, K.B. Sharpless, Click chemistry: diverse chemical function from a few good reactions, *Angew. Chem. Int. Ed.* 40 (2001) 2004–2021.
- [22] F. Himo, T. Lovell, R. Hilgraf, V.V. Rostovtsev, L. Noodleman, K.B. Sharpless, V.V. Fokin, Copper(I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates, *J. Am. Chem. Soc.* 127 (2004) 210–216.
- [23] N.J. Agard, J.A. Prescher, C.R. Bertozzi, A strain-promoted [3 + 2] azide–alkyne cycloaddition for covalent modification of biomolecules in living systems, *J. Am. Chem. Soc.* 126 (2004) 15046–15047.
- [24] Weixian Xi, Timothy F. Scott, Christopher J. Kloxin, Christopher N. Bowman, Click chemistry in materials science, *Adv. Funct. Mater.* 24 (2014) 2572–2590.
- [25] A.B. Lowe, Thiol–ene “click” reactions and recent applications in polymer and materials synthesis, *Polym. Chem.* 1 (2010) 17–36.
- [26] M.A. Tasdelen, Diels–Alder “click” reactions: recent applications in polymer and material science, *Polym. Chem.* 2 (2011) 2133–2145.
- [27] J. Kalia, R.T. Raines, Hydrolytic stability of hydrazones and oximes, *Angew. Chem. Int. Ed.* 120 (2008) 7633–7636.
- [28] D.M. Yoon, J.P. Fisher, Natural and synthetic polymeric scaffolds, *Biomed. Mater.*, Springer US (2009) 415–442.
- [29] B.D. Ulery, L.S. Nair, C.T. Laurencin, Biomedical applications of biodegradable polymers, *J. Polym. Sci. B Polym. Phys.* 49 (2011) 832–864.
- [30] P.B. Malafaya, G.A. Silva, R.L. Reis, Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications, *Adv. Drug Deliv. Rev.* 59 (2007) 207–233.
- [31] D.W. Hutmacher, Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives, *J. Biomater. Sci. Polym. Ed.* 12 (2001) 107–124.
- [32] F. Alfonso, New drug-eluting stents: polymer-free, biodegradable polymers or bioabsorbable scaffolds? *Rev. Esp. Cardiol.* (1970) 423–426.
- [33] J.F. Nelson, H.G. Stanford, D.E. Cutright, Evaluation and comparisons of biodegradable substances as osteogenic agents, *Oral Surg., Oral Med., Oral Pathol.* 43 (1977) 836–843.
- [34] C.T. Hung, E.G. Lima, R.L. Mauck, E. Taki, M.A. LeRoux, H.H. Lu, R.G. Stark, X.E. Guo, G.A. Ateshian, Anatomically shaped osteochondral constructs for articular cartilage repair, *J. Biomech.* 36 (2003) 1853–1864.
- [35] M. Kawase, N. Michibayashi, Y. Nakashima, N. Kurikawa, K. Yagi, T. Mizoguchi, Application of glutaraldehyde-crosslinked chitosan as a scaffold for hepatocyte attachment, *Biol. Pharm. Bull.* 20 (1997) 708–710.
- [36] E.M. Noah, J. Chen, X. Jiao, I. Heschel, N. Pallua, Impact of sterilization on the porous design and cell behavior in collagen sponges prepared for tissue engineering, *Biomaterials* 23 (2002) 2855–2861.
- [37] R.G. Payne, M.J. Yaszemski, A.W. Yasko, A.G. Mikos, Development of an injectable, in situ crosslinkable, degradable polymeric carrier for osteogenic cell populations. Part 1. Encapsulation of marrow stromal osteoblasts in surface crosslinked gelatin microparticles, *Biomaterials* 23 (2002) 4359–4371.
- [38] S. Sofia, M.B. McCarthy, G. Gronowicz, D.L. Kaplan, Functionalized silk-based biomaterials for bone formation, *J. Biomed. Mater. Res.* 54 (2001) 139–148.
- [39] T. Shinoka, P.X. Ma, D. Shum-Tim, C.K. Breuer, R.A. Cusick, G. Zund, R. Langer, J.P. Vacanti, J.E. Mayer Jr., Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model, *Circulation* 94 (1996) 1164–1168.
- [40] B. Seal, T. Otero, A. Panitch, Polymeric biomaterials for tissue and organ regeneration, *Mater. Sci. Eng. R* 34 (2001) 147–230.
- [41] G. Ciapetti, L. Ambrosio, L. Savarino, D. Granchi, E. Cenni, N. Baldini, S. Pagani, S. Guizzardi, F. Causa, A. Giunti, Osteoblast growth and function in porous poly-ε-caprolactone matrices for bone repair: a preliminary study, *Biomaterials* 24 (2003) 3815–3824.
- [42] N. Kumar, R.S. Langer, A.J. Domb, Polyanhydrides: an overview, *Adv. Drug Deliv. Rev.* 54 (2002) 889–910.
- [43] J. Choueka, J.L. Charvet, K.J. Koval, H. Alexander, K.S. James, K.A. Hooper, J. Kohn, Canine bone response to tyrosine-derived polycarbonates and poly (L-lactic acid), *J. Biomed. Mater. Res.* 31 (1996) 35–41.
- [44] S.J. Bryant, K.S. Anseth, Controlling the spatial distribution of ECM components in degradable PEG hydrogels for tissue engineering cartilage, *J. Biomed. Mater. Res., Part A* 64 (2003) 70–79.
- [45] S. Grad, L. Kupcsik, K. Gorna, S. Gogolewski, M. Alini, The use of biodegradable polyurethane scaffolds for cartilage tissue engineering: potential and limitations, *Biomaterials* 24 (2003) 5163–5171.
- [46] T. Garg, O. Singh, S. Arora, R. Murthy, Scaffold: a novel carrier for cell and drug delivery, *Crit. Rev. Ther. Drug Carrier Syst.* 29 (2012).
- [47] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, D.S. Kumar, Polymeric scaffolds in tissue engineering application: a review, *Int. J. Polym. Sci.* 2011 (2011).
- [48] C.E. Hoyle, C.N. Bowman, Thiol–ene click chemistry, *Angew. Chem. Int. Ed.* 49 (2010) 1540–1573.
- [49] P. Roach, D. Eglin, K. Rohde, C.C. Perry, Modern biomaterials: a review—bulk properties and implications of surface modifications, *J. Mater. Sci-Mater. Med.* 18 (2007) 1263–1277.
- [50] K.Y. Lee, D.J. Mooney, Hydrogels for tissue engineering, *Chem. Rev.* 101 (2001) 1869–1880.
- [51] K.S. Anseth, A.T. Metters, S.J. Bryant, P.J. Martens, J.H. Elisseeff, C.N. Bowman, In situ forming degradable networks and their application in tissue engineering and drug delivery, *J. Control. Release* 78 (2002) 199–209.
- [52] R. Landers, U. Hübner, R. Schmelzeisen, R. Mülhaupt, Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering, *Biomaterials* 23 (2002) 4437–4447.
- [53] E.M. Ahmed, F.S. Aggor, A.M. Awad, A.T. El-Aref, An innovative method for preparation of nanometal hydroxide superabsorbent hydrogel, *Carbohydr. Polym.* 91 (2013) 693–698.
- [54] R. Dong, Y. Pang, Y. Su, X. Zhu, Supramolecular hydrogels: synthesis, properties and their biomedical applications, *Biomater. Sci.* 3 (2015) 937–954.
- [55] A.S. Hoffman, Hydrogels for biomedical applications, *Adv. Drug Deliv. Rev.* 64 (2012) 18–23.
- [56] Q. Wang, J.L. Mynar, M. Yoshida, E. Lee, M. Lee, K. Okuro, K. Kinbara, T. Aida, High-water-content mouldable hydrogels by mixing clay and a dendritic molecular binder, *Nature* 463 (2010) 339–343.
- [57] T. Sakai, T. Matsunaga, Y. Yamamoto, C. Ito, R. Yoshida, S. Suzuki, N. Sasaki, M. Shibayama, U.-i. Chung, Design and fabrication of a high-strength hydrogel with ideally homogeneous network structure from tetrahedron-like macro-monomers, *Macromolecules* 41 (2008) 5379–5384.
- [58] N.C. Hunt, L.M. Grover, Cell encapsulation using biopolymer gels for regenerative medicine, *Biotechnol. Lett.* 32 (2010) 733–742.
- [59] H. Yoshimoto, Y. Shin, H. Terai, J. Vacanti, A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering, *Biomaterials* 24 (2003) 2077–2082.
- [60] O.I. Kalaoglu-Altan, R. Sanyal, A. Sanyal, Reactive and ‘clickable’ electrospun polymeric nanofibers, *Polym. Chem.* 6 (2015) 3372–3381.
- [61] X. Mo, C. Xu, M. Kotaki, S. Ramakrishna, Electrospun P (LLA-CL) nanofiber: a biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation, *Biomaterials* 25 (2004) 1883–1890.
- [62] M. Li, Y. Guo, Y. Wei, A.G. MacDiarmid, P.I. Lelkes, Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications, *Biomaterials* 27 (2006) 2705–2715.
- [63] R. Vassita, D.S. Katti, Nanofibers and their applications in tissue engineering, *Int. J. Nanomedicine* 1 (2006) 15.
- [64] D. Liang, B.S. Hsiao, B. Chu, Functional electrospun nanofibrous scaffolds for biomedical applications, *Adv. Drug Deliv. Rev.* 59 (2007) 1392–1412.
- [65] N. Bhardwaj, S.C. Kundu, Electrospinning: a fascinating fiber fabrication technique, *Biotechnol. Adv.* 28 (2010) 325–347.
- [66] J.Z. Luk, J. Cooper-White, L. Rintoul, E. Taran, L. Grøndahl, Functionalised polycaprolactone films and 3D scaffolds via gamma irradiation-induced grafting, *J. Mater. Chem. B* 1 (2013) 4171–4181.
- [67] J.H. Lee, H.W. Jung, I.-K. Kang, H.B. Lee, Cell behaviour on polymer surfaces with different functional groups, *Biomaterials* 15 (1994) 705–711.
- [68] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings, *Science* 318 (2007) 426–430.
- [69] M. Tanaka, M. Takebayashi, M. Miyama, J. Nishida, M. Shimomura, Design of novel biointerfaces (II). Fabrication of self-organized porous polymer film with highly uniform pores, bio-med, *Mater. Eng.* 14 (2004) 439–446.
- [70] G. Chen, T. Ushida, T. Tateishi, Development of biodegradable porous scaffolds for tissue engineering, *Mater. Sci. Eng. C* 17 (2001) 63–69.
- [71] A.G. Mikos, J.S. Temenoff, Formation of highly porous biodegradable scaffolds for tissue engineering, *Electron. J. Biotechnol.* 3 (2000) 23–24.
- [72] V. Crescenzi, L. Cornelio, C. Di Meo, S. Nardecchia, R. Lamanna, Novel hydrogels via click chemistry: synthesis and potential biomedical applications, *Biomacromolecules* 8 (2007) 1844–1850.
- [73] S. Caldwell, D.W. Johnson, M.P. Didsbury, B.A. Murray, J.J. Wu, S.A. Przyborski, N.R. Cameron, Degradable emulsion-templated scaffolds for tissue engineering from thiol–ene photopolymerisation, *Soft Matter* 8 (2012) 10344–10351.
- [74] W. Huang, X. Li, X. Shi, C. Lai, Microsphere based scaffolds for bone regenerative applications, *Biomater. Sci.* 2 (2014) 1145–1153.
- [75] L. Barner, Synthesis of microspheres as versatile functional scaffolds for materials science applications, *Adv. Mater.* 21 (2009) 2547–2553.
- [76] P.K. Nguyen, C.G. Snyder, J.D. Shields, A.W. Smith, D.L. Elbert, Clickable poly (ethylene glycol)-microsphere-based cell scaffolds, *Macromol. Chem. Phys.* 214 (2013) 948–956.
- [77] B. Parrish, R.B. Breitenkamp, T. Emrick, PEG- and peptide-grafted aliphatic polyesters by click chemistry, *J. Am. Chem. Soc.* 127 (2005) 7404–7410.
- [78] H. Shao, M.M. Crnogorac, T. Kong, S.-Y. Chen, J.M. Williams, J.M. Tack, V. Gueriouan, E.N. Cagle, M. Carnevali, D. Tumelty, X. Paliard, L.P. Miranda, J.A. Bradburne, G.G. Kochendoerfer, Site-specific polymer attachment to a CCL-5 (RANTES) analogue by oxime exchange, *J. Am. Chem. Soc.* 127 (2005) 1350–1351.
- [79] H.C. Kolb, K.B. Sharpless, The growing impact of click chemistry on drug discovery, *Drug Discov. Today* 8 (2003) 1128–1137.
- [80] R. Huisgen, Kinetics and reaction mechanisms: selected examples from the experience of forty years, *Pure Appl. Chem.* 61 (1989) 613–628.
- [81] N. Song, M. Ding, Z. Pan, J. Li, L. Zhou, H. Tan, Q. Fu, Construction of targeting-clickable and tumor-cleavable polyurethane nanomaterials for multifunctional intracellular drug delivery, *Biomacromolecules* 14 (2013) 4407–4419.
- [82] J. Wei, X. Shuai, R. Wang, X. He, Y. Li, M. Ding, J. Li, H. Tan, Q. Fu, Clickable and imageable multiblock polymer micelles with magnetically guided and PEG-switched targeting and release property for precise tumor theranosis, *Biomaterials* 145 (2017) 138–153.
- [83] Y. Chen, Z. Guan, Bioinspired modular synthesis of elastin-mimic polymers to probe the mechanism of elastin elasticity, *J. Am. Chem. Soc.* 132 (2010) 4577–4579.
- [84] L.A. Smith Callahan, S. Xie, I.A. Barker, J. Zheng, D.H. Reneker, A.P. Dove,

- M.L. Becker, Directed differentiation and neurite extension of mouse embryonic stem cell on aligned poly(lactide) nanofibers functionalized with YIGSR peptide, *Biomaterials* 34 (2013) 9089–9095.
- [85] C.A. DeForest, E.A. Sims, K.S. Anseth, Peptide-functionalized click hydrogels with independently tunable mechanics and chemical functionality for 3D cell culture, *Chem. Mater.* 22 (2010) 4783–4790.
- [86] C.-C. Lin, A. Raza, H. Shih, PEG hydrogels formed by thiol-ene photo-click chemistry and their effect on the formation and recovery of insulin-secreting cell spheroids, *Biomaterials* 32 (2011) 9685–9695.
- [87] Charles E. Hoyle, Andrew B. Lowe, Christopher N. Bowman, Thiol-click chemistry: a multifaceted toolbox for small molecule and polymer synthesis, *Chem. Soc. Rev.* (4) (2010) 1355–1387.
- [88] C.D. Pritchard, T.M. O'Shea, D.J. Siegwart, E. Calo, D.G. Anderson, F.M. Reynolds, J.A. Thomas, J.R. Slotkin, E.J. Woodard, R. Langer, An injectable thiol-acrylate poly(ethylene glycol) hydrogel for sustained release of methylprednisolone sodium succinate, *Biomaterials* 32 (2011) 587–597.
- [89] C.E. Hoyle, A.B. Lowe, C.N. Bowman, Thiol-click chemistry: a multifaceted toolbox for small molecule and polymer synthesis, *Chem. Soc. Rev.* 39 (2010) 1355–1387.
- [90] Y. Zhang, A.A. Broekhuis, F. Picchioni, Thermally self-healing polymeric materials: the next step to recycling thermoset polymers? *Macromolecules* 42 (2009) 1906–1912.
- [91] J. Yang, J. Šečutė, C.M. Cole, N.K. Devaraj, Live-cell imaging of cyclopropene tags with fluorogenic tetrazine cycloadditions, *Angew. Chem. Int. Ed.* 124 (2012) 7594–7597.
- [92] A. Dirksen, S. Dirksen, T.M. Hackeng, P.E. Dawson, Nucleophilic catalysis of hydrazine formation and transimination: implications for dynamic covalent chemistry, *J. Am. Chem. Soc.* 128 (2006) 15602–15603.
- [93] G.N. Grover, J. Lam, T.H. Nguyen, T. Segura, H.D. Maynard, Biocompatible hydrogels by oxime click chemistry, *Biomacromolecules* 13 (2012) 3013–3017.
- [94] R.K. Iha, K.L. Wooley, A.M. Nyström, D.J. Burke, M.J. Kade, C.J. Hawker, Applications of orthogonal “click” chemistries in the synthesis of functional soft materials, *Chem. Rev.* 109 (2009) 5620–5686.
- [95] U. Mansfeld, C. Pietsch, R. Hoogenboom, C.R. Becer, U.S. Schubert, Clickable initiators, monomers and polymers in controlled radical polymerizations—a prospective combination in polymer science, *Polym. Chem.* 1 (2010) 1560–1598.
- [96] G.D. Fu, L.Q. Xu, F. Yao, K. Zhang, X.F. Wang, M.F. Zhu, S.Z. Nie, Smart nanofibers from combined living radical polymerization, “click chemistry”, and electrospinning, *ACS Appl. Mater. Interfaces* 1 (2009) 239–243.
- [97] X. Lian, J. Jin, J. Tian, H. Zhao, Thermoresponsive nanohydrogels cross-linked by gold nanoparticles, *ACS Appl. Mater. Interfaces* 2 (2010) 2261–2268.
- [98] T. Cai, M. Li, K.-G. Neoh, E.-T. Kang, Preparation of stimuli responsive polycaprolactone membranes of controllable porous morphology via combined atom transfer radical polymerization, ring-opening polymerization and thiol-yne click chemistry, *J. Mater. Chem.* 22 (2012) 16248–16258.
- [99] C.-J. Huang, F.-C. Chang, Using click chemistry to fabricate ultrathin thermoresponsive microcapsules through direct covalent layer-by-layer assembly, *Macromolecules* 42 (2009) 5155–5166.
- [100] K. Luo, J. Yang, P. Kopečková, J. Kopeček, Biodegradable multiblock poly [N-(2-hydroxypropyl) methacrylamide] via reversible addition-fragmentation chain transfer polymerization and click chemistry, *Macromolecules* 44 (2011) 2481.
- [101] Z. Jia, V.A. Bobrin, N.P. Truong, M. Gillard, M.J. Monteiro, Multifunctional nanoworms and nanorods through a one-step aqueous dispersion polymerization, *J. Am. Chem. Soc.* 136 (2014) 5824–5827.
- [102] S.K. Pasale, B. Cerroni, S.V. Ghugare, G. Paradossi, Multiresponsive hyaluronan-p (nipaam) “click”-linked hydrogels, *Macromol. Biosci.* 14 (2014) 1025–1038.
- [103] L.M. Campos, K.L. Killops, R. Sakai, J.M. Paulusse, D. Damiron, E. Drockenmuller, B.W. Messmore, C.J. Hawker, Development of thermal and photochemical strategies for thiol-ene click polymer functionalization, *Macromolecules* 41 (2008) 7063–7070.
- [104] K.L. Heredia, Z.P. Tolstyka, H.D. Maynard, Aminooxy end-functionalized polymers synthesized by ATRP for chemoselective conjugation to proteins, *Macromolecules* 40 (2007) 4772–4779.
- [105] J.K. Kammeyer, A.P. Blum, L. Adamiak, M.E. Hahn, N.C. Gianneschi, Polymerization of protecting-group-free peptides via ROMP, *Polym. Chem.* 4 (2013) 3929–3933.
- [106] R. Wang, W. Chen, F. Meng, R. Cheng, C. Deng, J. Feijen, Z. Zhong, Unprecedented access to functional biodegradable polymers and coatings, *Macromolecules* 44 (2011) 6009–6016.
- [107] A.C. Engler, H.-i. Lee, P.T. Hammond, Highly efficient “grafting onto” a polypeptide backbone using click chemistry, *Angew. Chem. Int. Ed.* 48 (2009) 9334–9338.
- [108] M. Malkoch, R.J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T.P. Russell, C.J. Hawker, Orthogonal approaches to the simultaneous and cascade functionalization of macromolecules using click chemistry, *J. Am. Chem. Soc.* 127 (2005) 14942–14949.
- [109] J.C. Dyke, K.J. Knight, H. Zhou, C.-K. Chiu, C.-C. Ko, W. You, An investigation of siloxane cross-linked hydroxyapatite-gelatin/copolymer composites for potential orthopedic applications, *J. Mater. Chem.* 22 (2012) 22888–22898.
- [110] M. Semsarilar, V. Ladmiral, S. Perrier, Highly branched and hyperbranched glycopolymers via reversible addition – fragmentation chain transfer polymerization and click chemistry, *Macromolecules* 43 (2010) 1438–1443.
- [111] L.A. Canalle, S.S. van Berkel, L.T. de Haan, J. van Hest, Copper-free clickable coatings, *Adv. Funct. Mater.* 19 (2009) 3464–3470.
- [112] T.M. F. J. Xu, F. Piffti, et al., Cytocompatible poly (ethylene glycol)-co-poly-carbonate hydrogels cross-linked by copper-free, strain-promoted click chemistry, *Chem.-Asian. J.* 6 (2011) 2730–2737.
- [113] C. Subramani, N. Cengiz, K. Saha, T.N. Gevrek, X. Yu, Y. Jeong, A. Bajaj, A. Sanyal, V.M. Rotello, Direct fabrication of functional and biofunctional nanostructures through reactive imprinting, *Adv. Mater.* 23 (2011) 3165–3169.
- [114] K. Kempe, R. Hoogenboom, M. Jaeger, U.S. Schubert, Three-fold metal-free efficient (“click”) reactions onto a multifunctional poly (2-oxazoline) designer Scaffold, *Macromolecules* 44 (2011) 6424–6432.
- [115] H. Altin, I. Kosif, R. Sanyal, Fabrication of “clickable” hydrogels via dendron-polymer conjugates, *Macromolecules* 43 (2010) 3801–3808.
- [116] R. Lösel, D. Grafahrend, M. Möller, D. Klee, Bioresorbable electrospun fibers for immobilization of thiol-containing compounds, *Macromol. Biosci.* 10 (2010) 1177–1183.
- [117] Y. Li, X.-H. Dong, Y. Zou, Z. Wang, K. Yue, M. Huang, H. Liu, X. Feng, Z. Lin, W. Zhang, Polyhedral oligomeric silsesquioxane meets “click” chemistry: rational design and facile preparation of functional hybrid materials, *Polymer* 125 (2017) 303–329.
- [118] H. Su, Y. Li, K. Yue, Z. Wang, P. Lu, X. Feng, X.-H. Dong, S. Zhang, S.Z. Cheng, W.-B. Zhang, Macromolecular structure evolution toward giant molecules of complex structure: tandem synthesis of asymmetric giant gemini surfactants, *Polym. Chem.* 5 (2014) 3697–3706.
- [119] F. Lin, J. Zheng, J. Yu, J. Zhou, M.L. Becker, Cascading “tricklick” functionalization of poly(caprolactone) thin films quantified via a quartz crystal microbalance, *Biomacromolecules* 14 (2013) 2857–2865.
- [120] G.T. 7714, W.J. Kim, Jordan A.M. S. E, et al., Surface modification of melt extruded poly (ε-caprolactone) nanofibers: toward a new scalable biomaterial scaffold, *ACS Macro Lett.* 3 (2014) 585–589.
- [121] V.R. Malkoch, M. Gupta, N. et al., Synthesis of well-defined hydrogel networks using click chemistry, *Chem. Commun.* (2006) 2774–2776.
- [122] A.A. A. M.A. Daniele, J. Naciri, et al., Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds, *Biomaterials* 35 (2014) 1845–1856.
- [123] E.P. B. M.W. Thielke, D.L. Wong, et al., Thiol-ene modification of electrospun polybutadiene fibers crosslinked by UV irradiation, *Polymer* 55 (2014) 5596–5599.
- [124] K. Shanmuganathan, R.K. Sankhagowit, P. Iyer, C.J. Ellison, Thio-ene chemistry: a greener approach to making chemically and thermally stable fibers, *Chem. Mater.* 23 (2011) 4726–4732.
- [125] Y. Fang, H. Ha, K. Shanmuganathan, C.J. Ellison, Polyhedral oligomeric silsesquioxane-containing thiol-ene fibers with tunable thermal and mechanical properties, *ACS Appl. Mater. Interfaces* 8 (2016) 11050–11059.
- [126] F. Lin, J. Yu, W. Tang, J. Zheng, A. Defante, K. Guo, C. Wesdemiotis, M.L. Becker, Peptide-functionalized oxime hydrogels with tunable mechanical properties and gelation behavior, *Biomacromolecules* 14 (2013) 3749–3758.
- [127] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, *Adv. Drug Deliv. Rev.* 54 (2002) 13–36.
- [128] C.A. DeForest, K.S. Anseth, Photoreversible patterning of biomolecules within click-based hydrogels, *Angew. Chem. Int. Ed.* 124 (2012) 1852–1855.
- [129] S.B. Anderson, C.-C. Lin, D.V. Kuntzler, K.S. Anseth, The performance of human mesenchymal stem cells encapsulated in cell-degradable polymer-peptide hydrogels, *Biomaterials* 32 (2011) 3564–3574.
- [130] H. Shih, A.K. Fraser, C.-C. Lin, Interfacial thiol-ene photoclick reactions for forming multilayer hydrogels, *ACS Appl. Mater. Interfaces* 5 (2013) 1673–1680.
- [131] B.J. Adzima, H.A. Aguirre, C.J. Kloxin, T.F. Scott, C.N. Bowman, Rheological and chemical analysis of reverse gelation in a covalently cross-linked Diels-Alder polymer network, *Macromolecules* 41 (2008) 9112–9117.
- [132] J. Zheng, L.A. Smith Callahan, J. Hao, K. Guo, C. Wesdemiotis, R.A. Weiss, M.L. Becker, Strain-promoted cross-linking of peg-based hydrogels via copper-free cycloaddition, *ACS Macro Lett.* 1 (2012) 1071–1073.
- [133] C.M. Nimmo, M.S. Shoichet, Regenerative biomaterials that “click”: simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning, *Bioconjug. Chem.* 22 (2011) 2199–2209.
- [134] Z.Q. Yang, H. Lin B, et al., Thermo-sensitive electrospun fibers prepared by a sequential thiol-ene click chemistry approach, *J. Polym. Sci. Part A Polym. Chem.* 50 (2012) 4182–4190.
- [135] B.D. Polizzotti, B.D. Fairbanks, K.S. Anseth, Three-dimensional biochemical patterning of click-based composite hydrogels via thiolene photopolymerization, *Biomacromolecules* 9 (2008) 1084–1087.
- [136] V.X. Truong, M.P. Ablett, H.T. Gilbert, J. Bowen, S.M. Richardson, J.A. Hoyland, A.P. Dove, In situ-forming robust chitosan-poly (ethylene glycol) hydrogels prepared by copper-free azide-alkyne click reaction for tissue engineering, *Biomater. Sci.* 2 (2014) 167–175.
- [137] C.T. L. W.J. Li, E.J. Caterson, et al., Electrospun nanofibrous structure: a novel scaffold for tissue engineering, *J. Biomed. Mater. Res., Part A* 60 (2002) 613–621.
- [138] S. Shi, X.H. Wang, G. Guo, M. Fan, M.J. Huang, Z.Y. Qian, Preparation and characterization of microporous poly (D, L-lactic acid) film for tissue engineering scaffold, *Int. J. Nanomedicine* 5 (2010) 1049.
- [139] J. Guo, Z. Xie, R.T. Tran, D. Xie, D. Jin, X. Bai, J. Yang, Click chemistry plays a dual role in biodegradable polymer design, *Adv. Mater.* 26 (2014) 1906–1911.
- [140] V.X. Truong, M.P. Ablett, S.M. Richardson, J.A. Hoyland, A.P. Dove, Simultaneous orthogonal dual-click approach to tough, in-situ-forming hydrogels for cell encapsulation, *J. Am. Chem. Soc.* 137 (2015) 1618–1622.
- [141] H. Durmaz, A. Sanyal, G. Hizal, U. Tunca, Double click reaction strategies for polymer conjugation and post-functionalization of polymers, *Polym. Chem.* 3 (2012) 825–835.
- [142] G. Godeau, J. Bernard, C. Staedel, P. Barthélémy, Glycosyl-nucleoside-lipid based supramolecular assembly as a nanostructured material with nucleic acid delivery capabilities, *Chem. Commun.* (2009) 5127–5129.

- [143] S. Ziane, S. Schlaubitz, S. Miraux, A. Patwa, C. Lalande, I. Bilem, S. Lepreux, B. Rousseau, J.-F. Le Meins, L. Latxague, A thermosensitive low molecular weight hydrogel as scaffold for tissue engineering, *Eur. Cell. Mater.* 23 (2012) 147–160.
- [144] J. Zheng, K. Liu, D.H. Reneker, M.L. Becker, Post-assembly derivatization of electrospun nanofibers via strain-promoted azide alkyne cycloaddition, *J. Am. Chem. Soc.* 134 (2012) 17274–17277.
- [145] M.A. Azagarsamy, K.S. Anseth, Bioorthogonal click chemistry: an indispensable tool to create multifaceted cell culture scaffolds, *ACS Macro Lett.* 2 (2012) 5–9.
- [146] L.A. Sawicki, A.M. Kloxin, Design of thiol–ene photoclick hydrogels using facile techniques for cell culture applications, *Biomater. Sci.* 2 (2014) 1612–1626.
- [147] C. Yang, P.D. Mariner, J.N. Nahreini, K.S. Anseth, Cell-mediated delivery of glucocorticoids from thiol-ene hydrogels, *J. Control. Release* 162 (2012) 612–618.
- [148] H.S. Yoo, T.G. Kim, T.G. Park, Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery, *Adv. Drug Deliv. Rev.* 61 (2009) 1033–1042.
- [149] C.M. Kolodziej, S.H. Kim, R.M. Broyer, S.S. Saxer, C.G. Decker, H.D. Maynard, Combination of integrin-binding peptide and growth factor promotes cell adhesion on electron-beam-fabricated patterns, *J. Am. Chem. Soc.* 134 (2011) 247–255.
- [150] T. Tischer, A.S. Goldmann, K. Linkert, V. Trouillet, H.G. Börner, C. Barner-Kowollik, Modular ligation of thioamide functional peptides onto solid cellulose substrates, *Adv. Funct. Mater.* 22 (2012) 3853–3864.
- [151] J. Zhou, P. Chen, C. Deng, F. Meng, R. Cheng, Z. Zhong, A simple and versatile synthetic strategy to functional polypeptides via vinyl sulfone-substituted L-cysteine N-carboxyanhydride, *Macromolecules* 46 (2013) 6723–6730.
- [152] J. Zheng, D. Kontoveros, F. Lin, G. Hua, D.H. Reneker, M.L. Becker, R.K. Willits, Enhanced Schwann cell attachment and alignment using one-pot “dual click” GRGDS and YIGSR derivatized nanofibers, *Biomacromolecules* 16 (2014) 357–363.
- [153] Y. Ma, J. Zheng, E.F. Amond, C.M. Stafford, M.L. Becker, Facile fabrication of “dual click” one- and two-dimensional orthogonal peptide concentration gradients, *Biomacromolecules* 14 (2013) 665–671.
- [154] J.A. Prescher, C.R. Bertozzi, Chemistry in living systems, *Nat. Chem. Biol.* 1 (2005) 13–21.