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Recent trends in tissue engineering applications of atom transfer radical polymerization

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Outline:

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Abbreviations:

Description	Abbreviation
Atom transfer radical polymerization	ATRP
Reversible-addition fragmentation chain transfer	RAFT
Surface-initiated atom transfer radical polymerization	SI-ATRP
Activators generated electron transfer atom transfer radical polymerization	AGET ATRP
Deactivation enhanced atom transfer radical polymerization	DE-ATRP
Poly (ethylene glycol)	PEG
oligo (ethylene glycol)	OEG
oligo (ethylene glycol), methacrylate	OEGMA
Poly (poly (ethylene glycol) methacrylate)	PPEGMA
Poly (ethylene glycol) methacrylate	PEGMA
Poly (glycidyl methacrylate)	PGMA
Poly (propylene glycol) methacrylate	PPGMA
Poly (ethylene glycol) methyl ether methacrylate	PEGMEMA
Ethylene glycol dimethacrylate	EGDMA
Poly (glycidyl methacrylate)-co- (methylmethacrylate)	PGMAMMA
Poly (methyl methacrylate)	PMMA
Poly (N, N-diethyl aminoethyl methacrylate)	PDMAEMA
Poly (2-(tert-butylamino)ethyl methacrylate)	PTBAEMA
Poly (N-isopropylacrylamide)	PNIPAAm
Poly (2-hydroxyethyl methacrylate)	PHEMA
Polypropylene hollow fiber	PPHF
Polycaprolactone	PCL
Poly (ethylene glycol)methyl ether methacrylate-co-2-(2-methoxyethoxy) ethylmethacrylate-co-poly(ethylene glycol) diacrylate	$PEGMEMA_{475}$–MEO_2MA–$PEGDA_{258}$
Poly (N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane)	poly(NIPAAm-co-BMDO)
Bovine serum albumin	BSA
Magnetic nanoparticles	MNPs
Magnetic resonance	MR
Thermoset polyester	TPE
AAm polymer	PAAm
2- dimethylaminoethyl methacrylate	DMAEMA
3-sulfopropylmethacrylate	SPMA
glycidylmethacrylate	GMA
4 vinylbenzyl chloride	VBC
3-chloropropionic acid	CPA
Extra cellular matrix	ECM
Hyaluronic acid-glycidyl methacrylate	HAGM
Hyaluronic acid	HA
Free radical photopolymerization	FRP
Hydroxyapatite	HAP
HAP–poly (l-lactide)	PLLA
Poly (methyl methacrylate)	PMMA

Methyl methacrylate	MMA
Lower critical solution temperature	LCST
Lactate dehydrogenase	LDH
Radical ring-opening polymerization	RROP
Dulbecco's modified Eagle medium	DMEM
Controlled radical polymerization	CRP
Free-radical polymerizations	FRP
Polypropylene	PP
Gel permeation chromatography	GPC
2-hydroxyethyl methacrylate	HEMA
Acrylamide	AAm
2-methacryloyloxyethyl, phosphorylcholine	MPC
sulfobetaine methacrylate	SBMA
carboxybetaine methacrylate	CBMA

Tissue Engineering approach

There are numerous biomaterials, including different kinds of metals, ceramics, glasses, polymers, nanocomposites, and soft matters, introduced during the last couple of decades that has been scientifically investigated or engineered for biological and biomedical applications [1,2,3,4,5].
In order to choose a material to act as the foundation for a tissue engineering scaffold, the material should be nontoxic, mechanically similar to the native tissue, safe, capable of attachment with molecules of normal tissue, and not cause excessive immune responses [6,7,8,9,10]. Moreover, a suitable scaffold should be biocompatible and starting to degrade as cells develop and lay down the extracellular matrix [11,12]. The materials of scaffolds and their coating and surface modifications have a decisive role in the rate of degradation that affects the macroscopic shape and the appropriate development of new tissues [13,14,15,16,17]. In addition, the molecular weight of scaffold degradation products should be less than 50 kDa in order to be excreted from the body [18]. Materials with these specifications can be classified into three main categories:

- Natural polymers, which often easily fulfill these expectations such as gelatin [1922], hyaluronic acid (HA) [23], alginate [24], chitosan [25,27] and collagen [28,29],
- Synthetic polymers, mainly aliphatic polyesters[30,31], and
- Inorganic biomaterials [32,33], including hydroxyapatite [34,35].

One of the key challenges in tissue engineering techniques is creating biodegradable polymeric materials with appropriate properties that can be modified to incorporate specific proteins, growth factors or functional groups. The discovery of controlled radical polymerization (CRP) methods has recently shown the high ability of these methods for creation of well-defined materials with incorporated functional groups such as the ones required for tissue engineering constructs [36-41].

Controlled radical polymerization

Recently, there has been considerable interest in synthesizing functional polymeric surfaces with precise control over molecular weight, architecture, composition, and end groups for biomedical applications. Due to the slow initiation, fast propagation and subsequent transfer or termination in conventional free-radical polymerizations (FRP), these methods are not compatible to provide polymers with narrow molecular weight distributions and defined chain ends. Modern CRP methods display synthesis of polymers with low polydispersities and tailored molecular weights, allowing tolerance to functional groups and monomer types with direct polymerization [42,43]. Mechanistically, in CRP methods an adequately large number of activation–deactivation cycles make the total number of dead chains sufficiently smaller than that of living chains (dormant plus active chains) to attain low polydispersity [44]. This includes reversible-addition fragmentation chain transfer (RAFT) polymerization [4548], nitroxide mediated living free radical polymerization (NMP) [4952], and atom transfer radical polymerization (ATRP) [5364], which have all been used to prepare polymers with reactive side chains and applied in the surface modifications [66,67]. For example, ATRP has been employed for polymerization of a wide range of monomers such as styrenes, acrylates, methacrylates and zwitterionic [68,69], including a variety of functional monomers and varying the topology of the polymer (linear, branched, hyperbranched, stars, etc.) and block copolymers since the end groups remain active at the end of the polymerization [43,66,67,70,71]. ATRP was first reported in 1995, and showed a precise control over polymerization by using readily accessible and inexpensive catalyst components and easy experimental setups. After that it has attracted great commercial interest in many different aspects. Figure 20.1 shows a typical schematic of ATRP equilibrium. We can see the reciprocating nature of the activation and deactivation steps in order to form a high mole fraction of dormant species that still preserve the ability to grow. In fact, the control over radical polymerization can be obtained by keeping the concentration of active species or propagating radicals adequately low in the polymerization. This method can be conducted in bulk, solution or a variety of homogeneous and heterogeneous media in different ranges of emulsion, suspension, and dispersion. Furthermore, polymers can be grown from surfaces, proteins, nanoparticles etc. Overall, this method has had remarkable growth in the past 15 years, and will continue growing and entering into many other areas of science and applications [72,73,74].

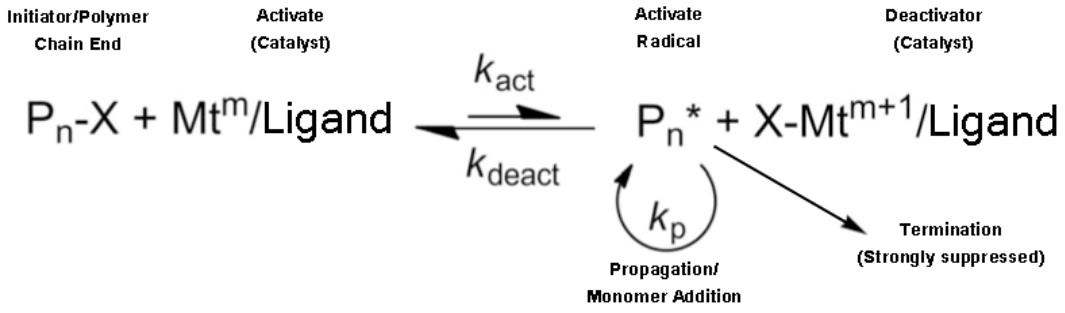

FIGURE 20.1
Normal ATRP equilibrium [55]

Tissue engineered surfaces via atom transfer radical polymerization

Functional polymeric brushes

The interactions between a biomaterial and its biological environment are overseen by its surface properties as they dominate the interactions between the material and the biological environments. In the field of tissue engineering, polymeric materials provide surfaces for the immobilization of biologically active molecules and living cells. Therefore, the ability to control the surface properties of biomaterials is of fundamental importance in the design of biomedical materials [75,76]. Polymer brushes refer to an assembly of polymer chains which are chained by one end to a surface or an interface. In comparison with other surface modification methods (e.g. self-assembled monolayers); polymer brushes can increase the spatial density of functional groups on a surface, as they extend the two-dimensional distribution of the functional compounds to a three-dimensional one. Polymer brushes are robust either in mechanical or chemical properties and exhibit a high degree of synthetic flexibility to introduce a variety of dense functional groups. Polymer brushes are considered as a central model for many polymer systems including polymer micelles, grafted polymers, adsorbed diblock copolymers and also block copolymers at fluid–fluid interfaces. All of these systems with their deformed configurations as a common feature are illustrated in Figure 20.2.

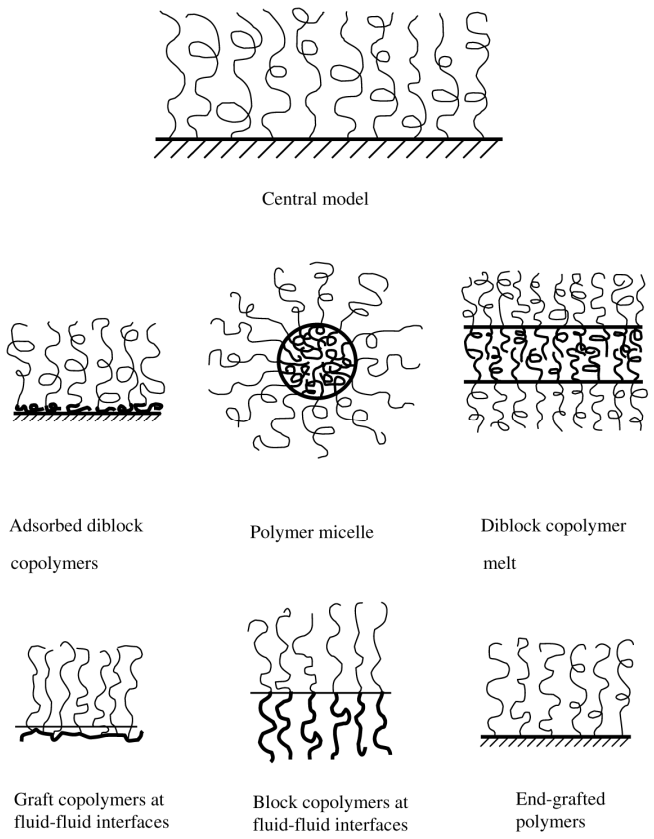

FIGURE 20.2
Examples of functional polymeric brushes [204]

The preparation methods of polymer brushes are physisorption or covalent attachment. In the first method, the sticky parts of polymer chains are adsorbed onto a suitable substrate. Non-covalent adsorption of polymers to surfaces is capable of being revoked and such polymer brushes are often unstable. Covalent chaining of polymer brushes on solid substrates is an effective method for modifying surface properties, such as antifouling ability, biocompatibility, and bimolecular recognition. It can be accomplished by either the *grafting to* or *grafting from* approaches. For the *grafting to* approach, polymer chains are attached directly on a suitable surface via reaction between end-functionalized polymers and appropriate reactive groups on the substrate surface. The method is experimentally simple, but has its limitations. For instance, it is difficult to achieve high grafting densities because of the steric gathering of the already adsorbed polymer chains on the surface reactive sites. Furthermore, the thickness of the graft layer can be determined by the molecular weight of the polymer in solution. The *grafting from* approach via polymerization from the initiators bound on substrate surfaces can be discussed as a convenient alternative to control various parameters such as functionality, density and thickness of the brushes. The substrate surface is first modified with an initiator monolayer followed by the growth of polymer chains directly from the reactive sites of the immobilized initiator layer. The screening of grafting sites is effectively reduced, because the grafted chains on the surface prevent the addition of monomers to growing chain ends or to primary radicals. This method has been attractive in recent years because of its effects in producing controllable functional polymer brushes of large thickness and high density. With the purpose of achieving maximum control over brush length, density, and composition on the surface, several *grafting from* methods have been developed, including surface-initiated cationic or anionic polymerization, ring-opening polymerization, and CRP techniques [204,77,78]. Among all above techniques, cationic, anionic and ring-opening polymerization techniques require accurate experimental conditions and sophisticated catalysts which are often moisture-sensitive. These requirements make their common application quite difficult in surface functionalization. Recently, the development of CRP techniques, including ATRP, has opened up new routes to the preparation of precise polymer brushes of controlled structures. Going through the surface-initiated CRP techniques, surface-initiated ATRP has been established to be the most versatile technique for surface functionalization [75,79]. It is easy to prepare the ATRP initiator layers on substrates using commercially available α-haloesters or benzyl halides, circumventing the multistep synthesis necessary for the introduction of functional alkoxyamine initiators of polymerization. Significantly, surface-initiated ATRP can also be carried out in the absence of sacrificial initiators to produce thick and dense polymer brushes. ATRP has been achieved from various surfaces, including surfaces of inorganic particles, planar surfaces, polymer networks, and even from dendrimers [75, 79]. In order to prepare the functional polymer brushes via surface-initiated ATRP, the presence of a uniform monolayer of initiators on the target substrate surfaces plays a vital role (Figure 20.3). Versatile immobilization methods of ATRP initiators have been developed for an extensive range of biomedical substrates, including inorganic surfaces of silicon, silica, titanium, gold and Fe_3O_4, and surfaces of films such as polypropylene (PP), aromatic ring-containing polymers, cellulose, and nylon [80-86].

As a new approach for implantable titanium substrates, bromomethyl-terminated biomimetic catechol and chloromethyl-terminated silanes can be immobilized on the oxidized titanium surfaces to serve as ATRP initiators. Bromomethyl-terminated thiol agents as ATRP initiators can be directly immobilized on gold surfaces [87,88]. The attachment of ATRP initiators on magnetic Fe_3O_4 nanoparticles can be accomplished via treatment with silanes (containing chloropropyl, chloromethyl, or chlorosulfonyl groups) and organic acids containing bromomethyl groups [Figure 20.3 (b)] [89,90]. For the common biomedical polymer films, the ATRP initiators can be introduced onto PP films via UV- or ozone-induced coupling of 2-bromoisobutyrate. Aromatic ring-containing polymer films can be functionalized via

chloromethylation, cellulose via direct coupling of 2-bromoisobutyrate, and nylon via formaldehydeinduced coupling of 2-bromoisobutyrate [91,92]. After the immobilization of ATRP initiators on the target substrates, surface-initiated ATRP can be carried out in the presence of a copper halide/nitrogen-based ligand catalyst system. The main difference between ATRP from a surface and ATRP in bulk or solution is related to the extremely low concentration of initiators immobilized on the surface. After the halogen atom transfer to the transition metal catalyst, the concentration of persistent radical (deactivator) may be too low to reversibly trap the propagating radicals, leading to uncontrolled chain growth [93]. Therefore, a sufficiently high concentration of the deactivating Cu (II) complex ($CuCl_2$ or $CuBr_2$) is required at the beginning of ATRP to rapidly establish equilibrium between the active and inactive (dormant) chains. The Cu (II) complex can be obtained by either the reaction of Cu (I) complex with the initiator or addition of the complex at the initial stage of ATRP.

Accordingly, the addition of free initiators or extra deactivating Cu (II) complex is usually chosen to guarantee the presence of an enough amount of deactivator to control the equilibrium between the inactive and the active chains during surface-initiated ATRP. In the first approach where free initiators are added, the molecular weight of free polymers formed by the free initiator in solution is used to serve as a measure of the molecular weight and polydispersity of the grafted polymers on the surface, due to the usual difficulty in obtaining the molecular weight of the grafted polymer on the solid surface. Consequently, the free initiator serves not only as a mediator for ATRP on the surface, but also as an indicator of surface graft polymerization. However, this approach enforces an intrinsic limitation to the maximum thickness of the obtained polymer brushes, because most of the monomers are utilized by homopolymerization in solution. The second method in which additional deactivating Cu(II) complex is added allows the growth of thicker polymer brushes, as the brush growth can proceed in a faster rate [94]. For the second method, the molecular weight and polydispersity of the surface-grafted polymer can be determined only with the division of grafted chains without any degradation. The amount of grafted polymer on the planar surface is minute. Only about 0.01mg of the polymer can be obtained from a 100-nm thick film grown on a $1cm^2$ flat surface. Thus, the quantity of polymers cleaved from the surface does not allow an accurate analysis of molecular weight and polydispersity by gel permeation chromatography (GPC). Compared to ATRP in bulk or in solution, in surface-initiated ATRP the problem of purifying the final products by removal of the metal catalyst has been minimized. Interestingly, the catalyst complex can be readily removed from the well-defined polymer brushes by suitable solvent extraction. Surface-initiated ATRP has been utilized widely for biomedical applications. Table I summarizes the types of bioactive surfaces prepared via surface-initiated ATRP. Details on the preparation and characteristics of these bioactive surfaces are given below [93,95].

TABLE I
Bioactive surfaces prepared via surface-initiated ATRP

Nature of Surface	Functional Monomers	Application
Antifouling Surfaces	PEG-containing methacrylate, HEMA, AAm, MPC, SBMA,CBMA	Biomedical devices, Tissue Engineering, and Filtration Membrane
Antibacterial Surfaces	DMAEMA, 4-VP, SPMA, PTBAEMA	Medical devices, Tissue Engineering, Filtration, and Fibers
Stimuli-responsive bioactive Surfaces	NIPAAm, DMAEMA, NaMA	Cell culture, drug delivery, and Tissue Engineering

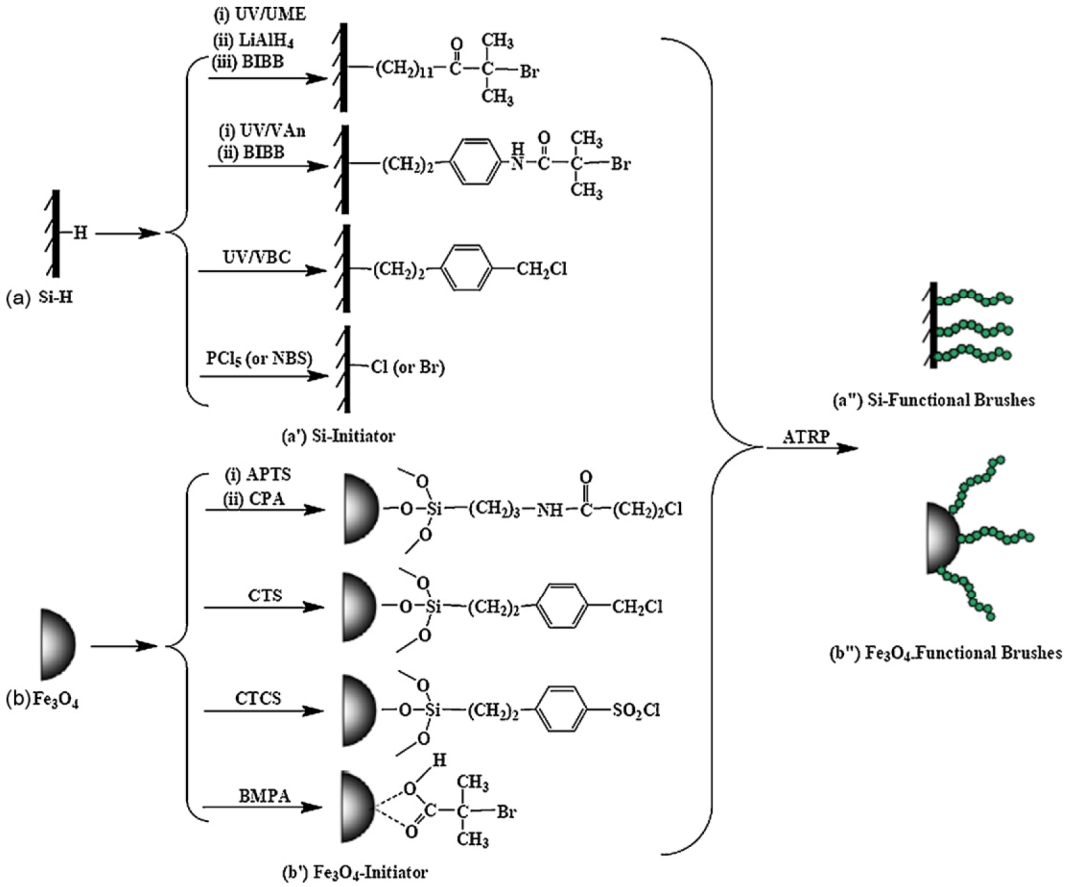

FIGURE 20.3
Methods of immobilizing ATRP initiators on various substrate surfaces for the preparation of functional polymer brushes by surface-initiated ATRP (Si–H: hydrogen-terminated Si wafer; UME: 10-undecylenic methyl ester [80]; BIBB: 2-bromoisobutyrate bromide [90]; VAn: 4-vinylaniline [89]; VBC: 4 vinylbenzyl chloride [205]; NBS: N-bromosuccinimide [81]; APTS: 3-aminopropyltriethoxysilane [76]; CPA: 3-chloropropionic acid [76]; CTS: 4-(chloromethyl)phenyl trichlorosilane [101]; CTCS: 2-(4-chlorosulfonylphenyl)ethyl trichlorosilane [84,206]; BMPA: 2-bromo-2-methylpropionic acid [83])

Antifouling surfaces

Non-specific adsorption refers to the tendency of proteins or cells of being physically adsorbed on a substrate without specific receptors. This occurs at the surface after the exposure of a foreign device to a biological environment. The protein adsorption processes are complicated. The adsorption of plasma proteins plays an important role in incurring subsequent undesirable events, including platelet adhesion, thrombus formation, foreign body reaction, bacterial infection, and adhesion of macrophages through which the tissue destruction has been hidden [9698]. In addition, non-specific adsorption can typically reduce the efficiency of biosensors, single molecule detection and single cell

analysis, giving rise to undesirable features, such as high background noise and false positives. The factors governing protein surface interactions include the physical state of the material, protein properties, and solution environment. It is without question that the surface first comes into contact with the biological environment. However, the substrate surface must be modified to provide it with resistance to protein adsorption and cell adhesion. Accordingly, the functionalization of the substrate surface with antifouling coating to improve the performances of biomedical devices and biosensors is of great importance. ATRP has been widely utilized to impart various substrate surfaces with antifouling properties. The monomers used in ATRP synthesis of antifouling surfaces are: oligo(ethylene glycol), methacrylate (OEGMA), 2-hydroxyethyl methacrylate (HEMA), acrylamide (AAm), zwitterionic monomers of 2-methacryloyloxyethyl, phosphorylcholine (MPC), sulfobetaine methacrylate (SBMA), and carboxybetaine methacrylate (CBMA) [99-113].

PEG-modified surfaces

Poly (ethylene glycol) (PEG) and oligo (ethylene glycol) (OEG) have been the most commonly used antifouling materials. PEG has many remarkable features such as physical and biochemical properties, including non-toxicity, non-immunogenesis, non-antigenicity, excellent biocompatibility, and miscibility with many solvents [114,115]. PEG and its components demonstrate good antifouling effects on a wide diversity of proteins, suppress platelet adhesion, and reduce cell attachment and growth [116,121]. It is clear that the protein molecules or cells have been inhibited from approaching the substrate surface by excluded volume of PEG units and the mobility or flexibility of highly hydrated chains in water. In fact, water molecules in a range of two or three per EG unit and up to a maximum of 10 required for hydration within the PEG layers play a vital role for protein resistance. Conventional methods to immobilize the PEG coatings on substrates include direct attachment of self-assembled PEG monolayer to surfaces, graft polymerization of PEG monomers to a polymer backbone, and adsorption of PEG block copolymers at multiple sites on the surface [118]. The efficiency of each strategy for constructing a protein and cell-resistant surface not only depends on the unique antifouling properties of PEG units, but the molecular structure resulting from the surface coverage has also been important [119]. Recently, dense non-fouling polymer brushes have been synthesized via ATRP of various OEGMA macro monomers from planar substrates of gold, silica, Ti, stainless steel and hydrogel [122, 120,121,123]. The thickness of the precise OEGMA polymer, poly(poly(ethylene glycol) methacrylate) (PPEGMA) brushes was tunable, and the surfaces exhibited first-rate antifouling effects to many proteins, including fibrinogen, bovine serum albumin (BSA), globulin, lysozyme, peptide, and lactamase [124]. In general, they are resistant to platelet and cell adhesion [122,123]. The non-fouling properties of the PPEGMA brushes are stable under long-term cell culture conditions. PPEGMA brushes grafted from gold substrates have been shown to prevent nonspecific cell adhesion for up to 30 days while PEG brushes of approximately 100 nm in thickness on Ti substrates coated with catechol-anchor exhibited excellent resistance to cell fouling for up to 3 weeks independent of the EG side chain length, after which the long-term antifouling performance depended on the EG chain length [125,126].

The stability of trialkoxysilane-anchored PPEGMA brushes from silica substrates was shown to be dependent on chain density [127]. Increasing the chain densities makes the brushes separate rapidly. On the other hand, the stability of the PPEGMA brushes in cell culture medium could be improved by decreasing the grafting density from less than 1 day to more than 7 days, without cooperating the antifouling properties. Precise antifouling PPEGMA brushes from magnetic nanoparticles (MNPs) were also grafted via surface-initiated ATRP [128,130]. Although, the MNPs that were taken up by macrophage cells were less than those in the pristine MNPs, after characterization of the macrophage cells cultured with MNPs, the similar morphology and viability to those without the nanoparticles was

achieved (Figure 20.4) [128, 129]. The PEGylated MNPs demonstrated long-term colloidal stability in the physiological buffer and the nanoparticles tolerated longer circulation in the bloodstream in comparison with conventional magnetic resonance (MR) image contrasting agents [131].

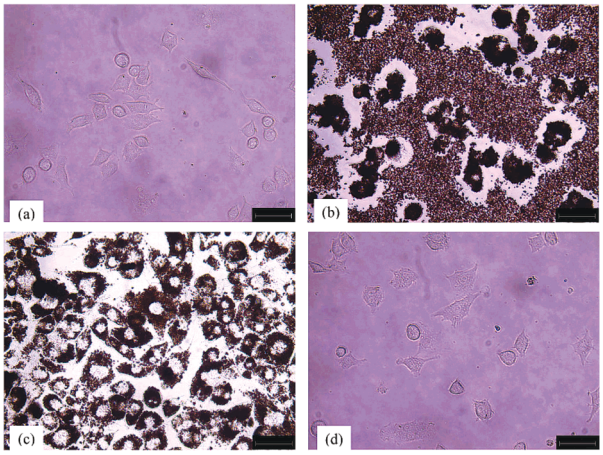

FIGURE 20.4
(a) RAW 264.7 cells in control culture (without any nanoparticles) after 1 day, (b) cells after culturing in medium containing pristine MNPs (0.2 mg/mL) for 1 day and (c) for 4 days, and (d) cells after culturing in medium containing P(PEGMA)-immobilized nanoparticles (0.2 mg/mL) for 1 day. The P(PEGMA)-immobilized nanoparticles were obtained after polymerization time of 2 h. Scale bar) 40 μm [129]

With the purpose of improving the performance of membranes in biomedical applications, ATRP of OEGMA is widely carried out to alter the membrane surfaces with antifouling properties. The membranes studied include cellulose, nylon, poly (vinylidene fluoride), and poly (phthalazinone ether sulfone ketone) membranes [132-135]. Increasing the length of the poly (ethylene glycol) methacrylate (PEGMA) brushes leads to decrease of the pore size, considering the ability to change the membrane pore size with ATRP processing time. The membranes with grafted PPEGMA brushes demonstrate good resistance to protein adsorption and fouling under continuous-flow conditions, thus prolonging the useful lifetime of the filtration membranes. Surface-initiated ATRP of OEGMA is also carried out to introduce a PPEGMA graft layer on the surfaces of poly (methyl methacrylate) (PMMA), thermoset polyester (TPE), and poly (glycidyl methacrylate)-*co*- (methylmethacrylate) (PGMAMMA) microfluidic devices. The PMMA microcapillary electrophoresis (*i*CE) devices with grafted PPEGMA brushes exhibit significantly reduced electroosmotic flow and non-specific adsorption of proteins on microchannel surfaces [136,139]. The reproducibility of column efficiency and migration time of the PPEGMA-modified PMMA microchips was improved by one order of magnitude over the untreated PMMA *i*CE chips. The PPEGMA-grafted TPE microchannel showed low and pH-stable electroosmotic flow and low non-specific protein adsorption. Capillary electrophoresis separation of amino acid and peptide mixtures in these PPEGMA-modified TPE microchips also exhibited good reproducibility. For the PEG-modified PGMAMMA microdevices, fast and efficient separations of amino acids, peptides and proteins were obtained due to denser and more uniform antifouling PEG brushes on the PGMAMMA surface [136,137,138].

PHEMA- and PAAm-modified surfaces

Dense hydrophilic poly (2-hydroxyethyl methacrylate) (PHEMA) brushes indicating remarkable biocompatibility and physical properties also exhibit excellent protein repellency [140]. It has been presented that PHEMA brushes are grafted via ATRP from silicon wafer, silica particles, and nylon membranes [141-143]. The size-exclusion effect of the dense PHEMA brushes plays an important role in suppressing protein adsorption [144]. In fact, the chains can become elongated and oriented to physically exclude the protein molecules from the entire brush layer. In addition, the PHEMA brushes can also effectively prevent cell adhesion [143]. Cell adhesion can be tuned by controlling the grafting density of PHEMA brushes. Decreasing the graft densities of PHEMA brushes leads to the adhesion followed by proliferation of cells. AAm polymer (PAAm, Figure 20.4(c)) is a biocompatible, water soluble, polar, electrically neutral and stable polymer. Highly hydrophilic PAAm brushes can also avoid the adsorption of proteins and prevent cell growth [145]. Well-defined PAAm brushes have been prepared via surface-initiated ATRP from silica microfluidic chips [146], poly (dimethylsiloxane) [147], and silicon wafer [148]. Antifouling PAAm brushes are attached on electrophoretic microfluidic chips via surface initiated ATRP for improving protein separation [146]. In fact, the higher the density the much less forces between the surface and microorganisms [148].

Antibacterial surfaces

Infections caused by microorganisms still remain as a major concern, especially in the healthcare sector where bacterial infections arising from implants and medical devices result in increased suffering, lengthy hospital visits, regular operations, and sometimes even death. In spite of the high success rate in dental and orthopedic implant surgery, many studies have reported bacterial infections associated with implants. To reduce the risk of infection, much research has been applied in the production of antibacterial surfaces [149,150]. Antimicrobial surfaces are broadly used to avoid microbial infection in a wide range of industrial, medical and private settings. Different strategies have been developed to realize the necessity for antibacterial surfaces. One of the most useful approaches for active antimicrobial agents is being permanently attached on the surface through covalent interactions. The antibacterial action results from the contact of the microorganisms with the biocidal surface without releasing the biocide into the environment. This reduces the possibility of generating drug resistance to the active agent through the microbial segment. In general, antimicrobial surfaces have been prepared via covalent immobilization of antimicrobial polymers onto different substrates [151,153]. The antimicrobial polymers generally contain cationic groups, such as alkyl pyridinium or quaternary ammonium moieties. The interaction of the cationic sites of quaternized groups with the negatively charged membrane of bacteria has an adverse effect on the integrity of the bacterial cell, as the quaternary ammonium groups can disrupt the plasma membrane to cause the release of intracellular substances. Hence, cationic antimicrobials play a vital role in the development of permanent and non-leaching antibacterial surfaces. The conventional antibacterial surfaces have been synthesized by either classical free-radical polymerization or by simple coupling reactions [86]. These methods possessed less control over the polydispersity, molecular weight, and density of functional groups.

In order to fabricate a successful antibacterial surface on a tissue scaffold, the facilitation of tissue repair and regeneration by enabling the localized production of therapeutic drugs is of crucial importance. Although polycaprolactone (PCL) has been extensively employed as a scaffold biomaterial, its undesirable cell-adhesion property still needs to be improved. ATRP has been used to impart antibacterial surfaces to filter paper, titanium, gold, glass, silicon, polyolefin, fibers, polymer microspheres and poly (vinylidene fluoride) [154,161].

Recent research has shown that some vinyl monomers containing tertiary amino groups, such as 2-dimethylaminoethyl methacrylate (DMAEMA) and 4-vinyl pyridine, can be polymerized or copolymerized via ATRP, followed by quaternization, to induce the antimicrobial activity. Control over both the polymer length and the effective number of quaternary ammonium groups could result in a highly effective biocidal polymer [155, 156,162]. According to a recent study [162] the PCL film surface was conjugated with poly ((2-dimethyl amino) ethyl methacrylate) (P (DMAEMA))/gelatin complexes via surface-initiated atom transfer radical polymerization (SI-ATRP) for improving cell immobilization and subsequent gene transfection. Matyjaszewski *et al*. recently proposed the original idea of preparing permanent, non-leaching antibacterial surfaces via ATRP [155]. Polymerization of tertiary amine-including DMAEMA via ATRP is illustrated in Figure 20.5. The process is performed directly from the filter paper to produce polymer chains of controlled molecular weight and low polydispersity. The biocidal functionality on the surfaces has been produced by subsequent quaternization of the amino groups of poly (N,N-diethyl aminoethyl methacrylate) (PDMAEMA) brushes. The modified surfaces exhibited considerable antimicrobial capacity over *E-coli* and Bacillus subtilis. It has been demonstrated that the available surface of PDMAEMA brushes synthesized by ATRP was the critical element in designing a surface for maximum efficiency [163,156]. Most biocidal surfaces had charge densities greater than 10^{15} accessible quaternary groups/cm^2 [156]. The antimicrobial surfaces of PDMAEMA/poly (3-(trimethoxysilyl) propyl methacrylate) can also be prepared by ATRP by the *grafting onto* technique. [157]. The higher concentrations of quaternary groups achieve a higher biocidal functionality. Considering the same density of quaternary groups, the biocidal activity of surfaces prepared by the grafting onto technique has been higher than those of surfaces prepared by the grafting from technique [108]. Hence, biocidal activity was not strongly affected by polymer architecture. This phenomenon could be described by the non-uniform coverage of the polymer on the former surfaces, and the biocidal activity was affected by localized patches of high concentration of quaternary ammonium groups.

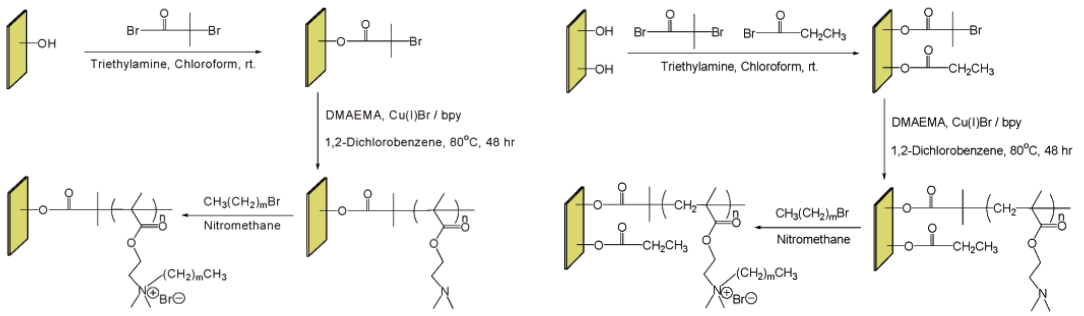

FIGURE 20.5
Surface-initiated ATRP of DMAEMA, and subsequent quaternization of PDMAEMA, from cellulose surfaces [155]

Viologen has been used to quaternize the tertiary amino groups of the monodispersed PDMAEMA brushes from ATRP. Antimicrobial capability and inhibition of biofilm formation were enhanced by the substantially increased polycation concentration on the surface. For example, compared to quaternized PDMAEMA brushes exhibited by an alkyl halide, the viologen-quaternized PDMAEMA brushes exhibited enhanced antimicrobial functionality. Recently, novel surfaces with grafted block copolymer brushes of

PEGMA and DMAMEA (PPEGMA-b-PDMAEMA) were prepared via consecutive surface-initiated ATRPs from PP hollow fiber (PPHF) membranes [164].
The ATRP time can affect the length of the PPEGMA-b-PDMAEMA brushes resulting in the regulation of the pore size of PPHF. Quaternization of the PDMAEMA block by alkyl bromides demonstrated both antibacterial and antifouling effects due to the hydrophilic antifouling nature of the PPEGMA blocks. Moreover, 3-sulfopropylmethacrylate (SPMA) incorporation with silver ions inside the polyelectrolyte film was synthesized via ATRP followed by fabrication of polyelectrolyte brushes of poly (3-sulfopropylmethacrylate) [165]. The silver-incorporated brushes successfully hinder the growth of bacteria. Furthermore, during leaching, the brushes were able to keep the silver ions on the surface. Consequently, the silver-incorporated brushes exhibited highly desirable properties of an antibacterial surface. In addition to PDMAEMA, antibacterial surfaces of the neutral polymeric biocide, poly (2-(tert-butylamino)ethyl methacrylate) (PTBAEMA), were also prepared via ATRP [166,167]. Thus, ATRP is a versatile tool for creating antibacterial surfaces, with the antibacterial properties derived from cationic or neutral polymeric biocides.

Stimuli-responsive surfaces

The design and synthesis of materials so that their physico-chemical properties respond to any external stimuli highlights the potential of these materials in biomedical areas. Grafting the stimuli-responsive materials containing polymers may yield surfaces that control biological interactions such as bioadhesions. The applications include surface adhesion modifiers, cell culture, and tissue engineering [168]. Different stimuli-responsive polymer brushes such as pH-responsive [169,170], and temperature-responsive [171,173] have been prepared via ATRP method. In the second group, temperature has been recognized as one of the most widely used physical stimuli in environmentally responsive polymer systems. Not only can the changes of temperature be easily controlled, but it is also readily applicable both *in vivo* and *in vitro*. Poly (*N*-isopropylacrylamide) (PNIPAAm) is the most broadly employed thermo-responsive polymer, which exhibits a lower critical solution temperature (LCST) of about 32° C in aqueous media. The state of PNIPAAm can be changed; it undertakes hydrophilic state below the LCST while a hydrophobic state is happening above the LCST due to rapid, reversible chain dehydration and aggregation [174,175]. Because of this remarkable property, precise PNIPAAm brushes have been extensively used in the preparation of stimuli-responsive surfaces in order to control the cell adhesion [171, 172,173].
In tissue engineering without the participation of biochemical or releasing of chemical reagents, it is desirable to do cell culture and then detach the cells at an appropriate stage to be harvested. PNIPAAm-based thermo-responsive polymers have been extensively used as surface mediators for cell attachment [176,177]. The adhesion and detachment of cultured cells on these surfaces can be controlled using only temperature variation. On the PNIPAAm surfaces, cells can adhere followed by growth and proliferation at 37° C. However, at temperatures below the LCST of PNIPAAm, the cultured cells can be detached at the same time from the hydrophilic surfaces. For the purpose of controlling cell response, many PNIPAAm surfaces have been prepared via conventional graft polymerizations, made by electron beam [177] or plasmas [178]. These methods offered less control over chain density, length, and flexibility of the grafted PNIPAAm on the substrate surfaces. To get control of the cell adhesion/detachment, ATRP has been used to prepare well-defined PNIPAAm brushes [176,179,180]. Xu *et al.* [179] prepared well-defined functional PNIPAAm brushes via surface-initiated ATRP from silicon surfaces resulting in steady development of chains of PNIPAAm in controlled process. By adjusting the cell culture temperature, the cell adhesion/detachment on the PNIPAAm-grafted silicon surfaces can be changed.

Rapid detachment of cultured cells from substrates plays a vital role in fabrication of functional tissue-mimicking structures. Hence, the hydration of the underlying PNIPAAm grafted on the surface can affect the rate-limiting step to cell substrate recovery. It is also reported that the incorporation of PEGMA units into the NIPAAm chains could enhance the hydration of the cell-cultured surfaces resulting in quick cell detachment during the temperature transition. However, the introduction of PEG derivatives has also resulted in a dramatic decrease in growth or adhesion of cells at temperatures above the LCST [181]. Accordingly, to accelerate cell detachment at lower temperatures, without affecting cell adhesion and growth at temperatures above the LCST, attempts have been made to prepare novel thermo-responsive copolymer brushes from silicon surfaces via ATRP[182]. Figure 20.6 illustrates the mechanism of preparation of precise poly (glycidyl methacrylate) (PGMA) brushes on silicon surfaces via surface-initiated ATRP of glycidylmethacrylate (GMA) from the 4 vinylbenzyl chloride (VBC)-coupled silicon surface. In fact, the epoxy groups of the PGMA brushes were employed for the direct coupling of 3-chloropropionic acid (CPA) followed by ATRP of NIPAAm.

The PGMA main chains with hydroxyl groups provided a local hydrophilic microenvironment for accelerated hydration of PNIPAAm side chains at temperatures below the LCST, while the grafted PNIPAAm chains acted as the thermo-responsive side chains of the comb copolymer brushes. The comb copolymer brushes assisted cell recovery below LCST without restraining cell attachments and growth at 37° C due to its unique microstructure. The well-defined PNIPAAm brushes were examined to investigate the complicated relations between the biophysical response of cells and the physiochemical properties of PNIPAAm brushes [180,176]. The longer polymerization time can cause a higher initial rate of cell detachment below the LCST [180]. Increasing the density of PNIPAAm brushes during initial cell recovery caused a reduction in the degree of cell deformation and average adhesion energy. In addition, to control cell adhesion and separation of biomolecules, PNIPAAm-functionalized surfaces were also used to get control of drug release [183]. In fact, PNIPAAm brushes were prepared inside the pores for the control of drug release in response to temperature. It was speculated that the PNIPAAm brushes inside the pores could form internal holes for loading the drug molecules, while responding to external temperature stimuli.

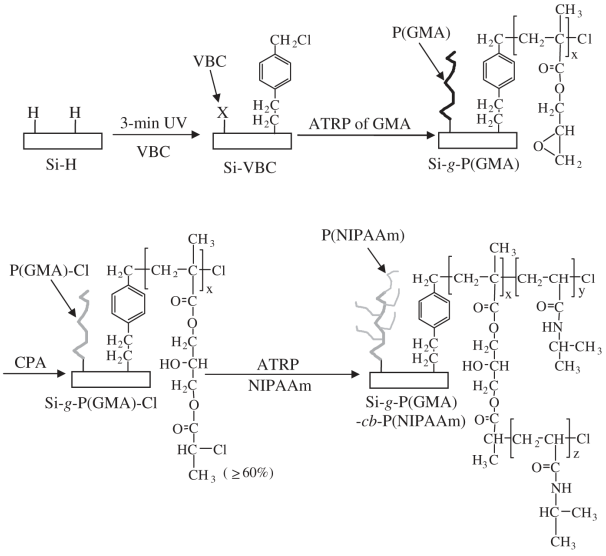

FIGURE 20.6
Process of preparing comb-shaped copolymer brushes via successive surface-initiated ATRPs of GMA and NIPAAm for accelerated cell detachment below the LCST of PNIPAAm [182]

Hydrogel scaffolds via ATRP

Hydrogels with cross-linked polymer networks are appropriate materials for tissue engineering scaffolds because of their similar mechanical and mass transfer properties with native tissues. Due to their cross-linked polymer network, this class of materials allows small molecules such as proteins to diffuse in and out of the matrix of hydrogel. On the other hand, larger molecules as plasmid DNA are often entrapped within their network and can only be set free subsequent to the degradation of hydrogel. Ratner *et al.* [184] recently reported the synthesis of biodegradable PHEMA hydrogels for tissue engineering using ATRP technique, a degradable cross-linker and a macro-initiator. Since PCL is a hydrolytically and enzymatically degradable polymer, they used oligomeric blocks of PCL as a cross-linking agent and a degradable macro-initiator that also contained oligomeric PCL to initiate the ATRP, in order to prepare biodegradable scaffold. As a result, they have observed that the degradation rate is a variable of the cross-linking density, the PCL chain length, and the PHEMA backbone chain length. The combination of a macroscopic hydrogel and nanogel to form nanostructured hybrid hydrogels could lead to enormous progress in the field of tissue engineering to synthesize scaffolds that are able to deliver single growth factor or a combination of different growth factors simultaneously. These biodegradable nanostructured hydrogels containing growth factors, drug and other useful low molecular weight biomolecules could be used as an artificial extra cellular matrix (ECM) for tissue regeneration.

In another study, Matyjaszewski *et al.* [185] prepared nanostructured hybrid hydrogels by synthesizing $POEO_{300}MA$ nanogels using activators generated electron transfer atom transfer radical polymerization (AGET-ATRP) in cyclohexane inverse miniemulsion at ambient temperature and subsequent FRP of hyaluronic acid-glycidyl methacrylate (HAGM). In the synthesis procedure of $POEO_{300}MA$ nanogels with ATRP approach, they have used hydroxyl-containing water-soluble ATRP as an initiator (oligo(ethylene oxide)-functionalized bromoisobutyrate, HO–EO–Br) in order to produce functional nanogels with the capacity for further chemical modifications. As shown in Figure 20.7, they functionalized hydroxyl-containing nanogels with methacrylated groups to generate photo reactive nanospheres. They also introduced disulfide moieties into the polymerizable groups to gain a controllable release of nanogels from cross-linked HAGM hydrogels under a reducing environment.

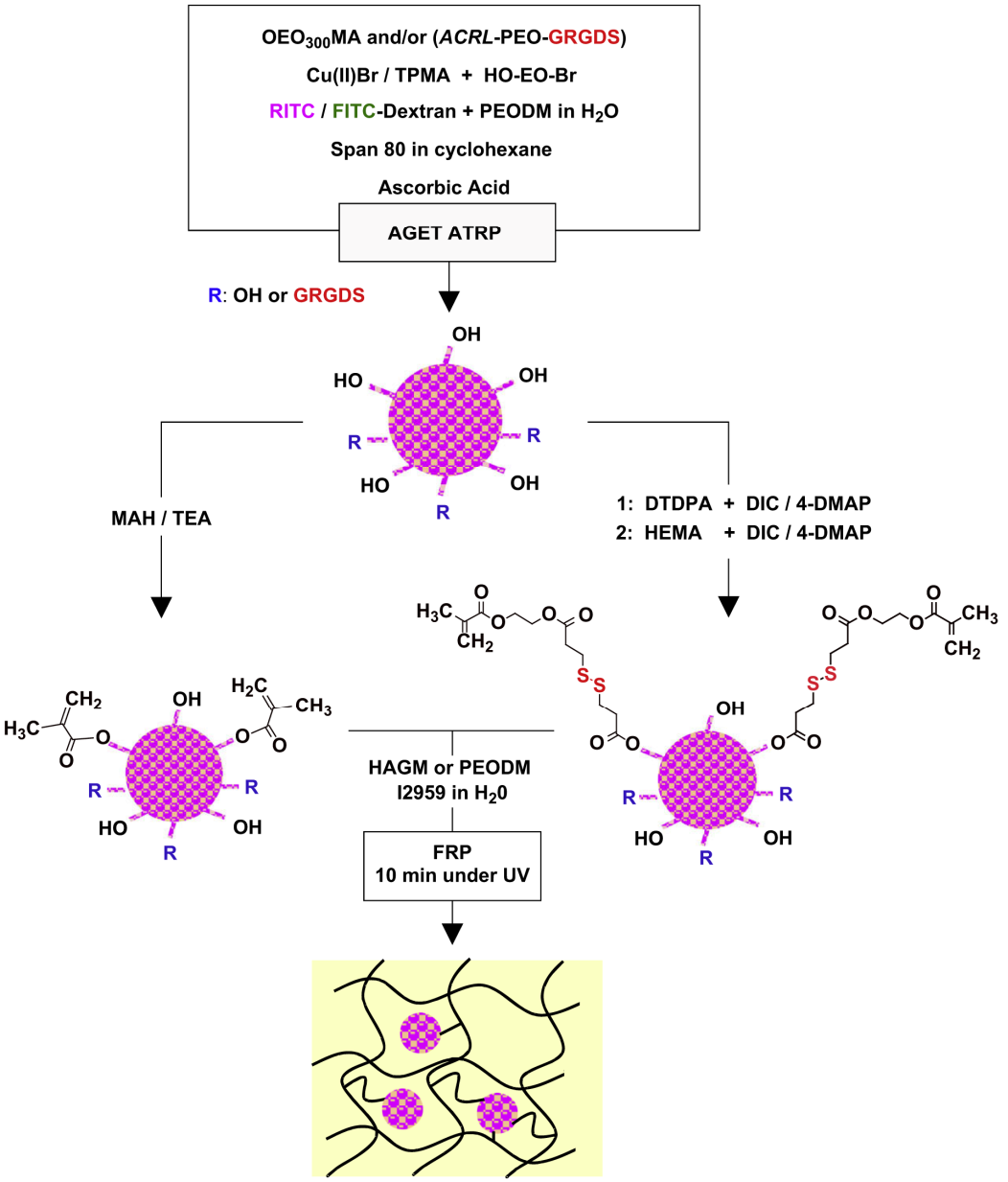

FIGURE 20.7
Synthesis of well-defined fluorescent dye-loaded GRGDS-POEO$_{300}$MA nanogels by AGET-ATRP in inverse miniemulsion of water/cyclohexane at ambient temperature. The nanogels were subsequently substituted with MAH or coupled with DTPA/HEMA to incorporate cleavable photopolymerizable groups. Nanostructured hybrid hydrogels were prepared by covalent incorporation of methacrylated POEO$_{300}$MA nanogels into macroscopic HAGM or PEODM hydrogels via FRP under ultra-violet (UV) irradiation [185]

In order to monitor the cytotoxicity and cell adhesion, they covalently incorporated GRGDS (Gly–Arg–Gly–Asp–Ser) contained MA-nanogelsinto PEODM scaffolds. The results showed that GRGDS in the nanogel structure promoted the cell–substrate interactions within 4 days of incubation and cells could recognize the integrin-binding motif whether GRGDS grafted to nanogels or to a macroscopic scaffold. They concluded that the cells did not show any reaction to the variations of molecular architecture (Figure 20.8).

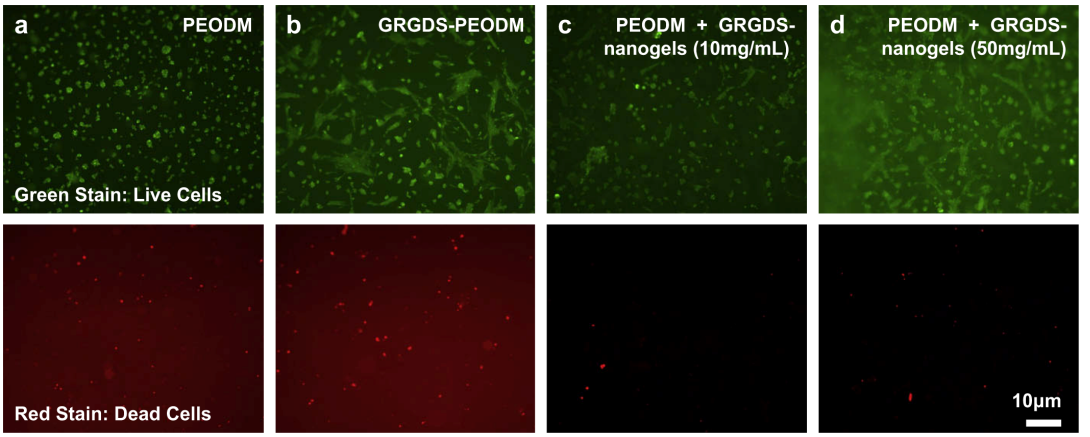

FIGURE 20.8
Mouse myoblast cells (C2C12) seeded on a series of 10% (wt/v) hydrogels; PEODM (a), GRGDS-modified PEODM (b), PEODM-co-GRGDS-MA-nanogels (nanogel content: 10 mg/mL) (c), and PEODM-co-GRGDS-MA-nanogels (nanogel content: 50 mg/mL) (d). The cells were cultured for 4 days and stained with the live stain on top (green) and dead cell stain on the bottom (red) [185]

Following the aforementioned study, Matyjaszewski *et al.* [186] synthesized a nanostructured HA hydrogel by a combination of ATRP and Michael-type addition reaction. The intent of this was to take advantage of having a second delivery carrier in addition to the macroscopic scaffold matrix for the controlled delivery of growth factors, drug and other useful low molecular weight biomolecules. With this perspective, they have applied AGET-ATRP in cyclohexane inverse mini emulsion in the presence of a hydrolytically labile cross-linker to create biodegradable $POEO_{300}$MA-co-PHEMA nanogels with pendent hydroxy groups. These hydroxy groups were subsequently altered with acrylated segments to produce reactive nanogels that could make covalent bonds with nucleophilic thiols via a Michael-type addition reaction. Carbodiimide-mediated coupling reaction of HA with cysteamine hydrochloride occurred leading to thiol-derivatization of HA (HA-SH) to prepare nanostructured hybrid hydrogel by mixing HA-SH with acrylated-nanogels under physiological conditions. As shown in Figure 20.9, the HA-based nanostructured hybrid hydrogel was obtained by the nucleophilic thiolene addition between the thiol functionalized HA chains and the vinyl moieties contained in grafted acrylic nanogels.

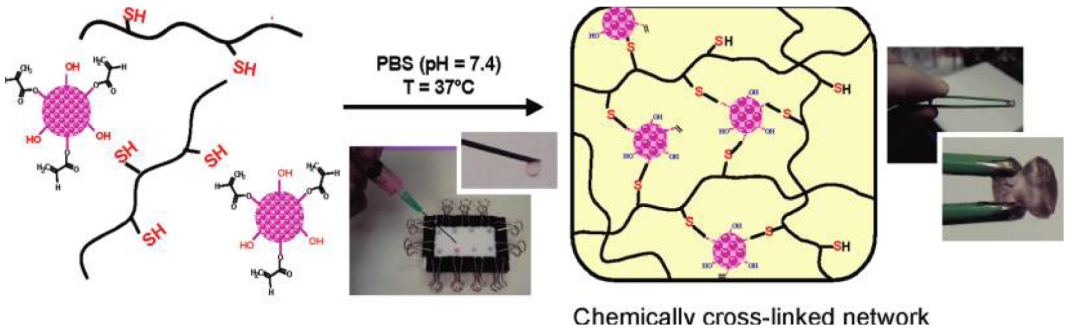

FIGURE 20.9
Gel formation via Michael-type addition reaction under physiological conditions. Formation of the nanostructured hybrid hydrogel was visually observed with digital images before and after gelation [186]

In addition, as shown in Figure 20.10, the SEM photomicrographs of the prepared nanostructured HA hydrogel demonstrated uniform distribution of nanogels on the surface and in the interior structure of the nanostructured hydrogel and porous three-dimensional structure of scaffolds.

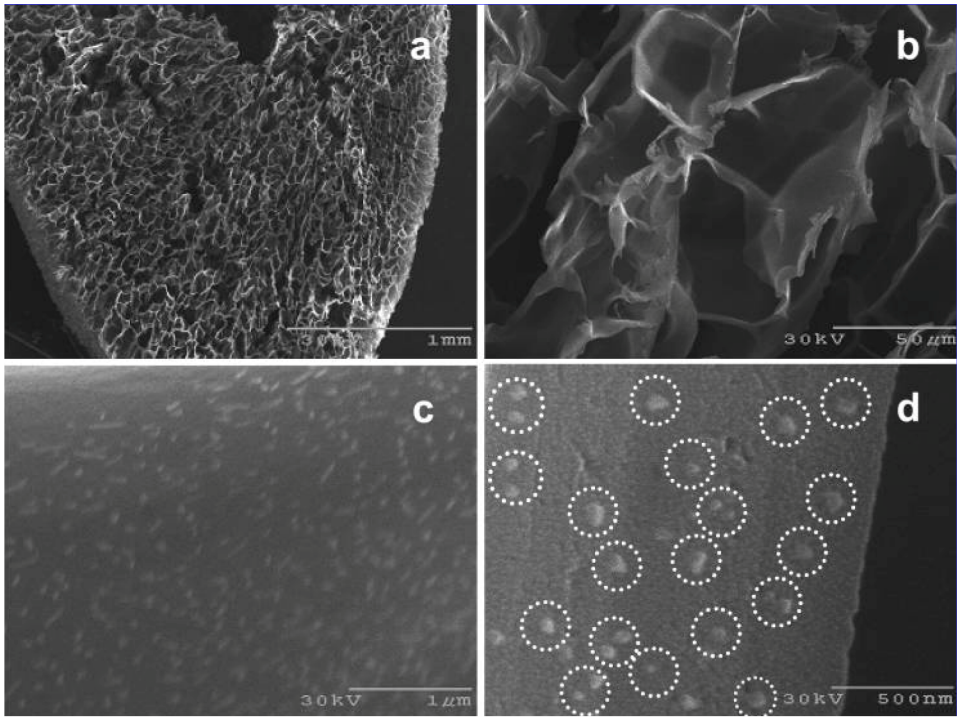

FIGURE 20.10
SEM photomicrographs of nanostructured HA hydrogel. Morphology and structure of SEM images: (a) overall image, (b) crosssection of interior, and surface hybridized with nanogels (c,d) at different magnifications. Dashed circles (d) denote nanogels homogeneously dispersed in the macroscopic HA hydrogel. The images are shown at the following scales: 1 mm (a), 50 μm (b), 1 μm (c), and 500 nm (d) [186]

Besides using the nanogels to make the properties of scaffolds more and more similar to extracellular matrix, adding inorganic nanomaterials such as hydroxyapatite to the matrix of scaffolds can improve applicability of engineered bone tissue scaffolds. However, hydroxyapatite (HAP) is widely used as inorganic nanomaterial fillers to prepare scaffolds for bone-like tissues because of its great properties such as high bioactivity, biocompatibility and osteoconductivity; the intrinsic brittleness and low mechanical strength of pure HAP and the low compatibility of HAP with the surrounding matrix compel its application without any further surface modification. In order to overcome this issue, Qing Cai et al. [187] have reported a new strategy to prepare HAP–poly (l-lactide) (PLLA) nano hybrids, where the oligomers were grafted from the nanoparticle surfaces via SI-ATRP of methylacrylate group terminated PLLA macro monomers (PLLA-MA).

As shown in Figure 20.11, by increasing the compatibility of the matrix, after the surface modification of HAP with PLLA segments through SI-ATRP, mineralized depositions occurred much faster in comparison to the control samples. This is due to better dispersibility of the modified HAP nanoparticles in the matrix of polymer, which causes more nucleation sites for apatite formation on the film surface.

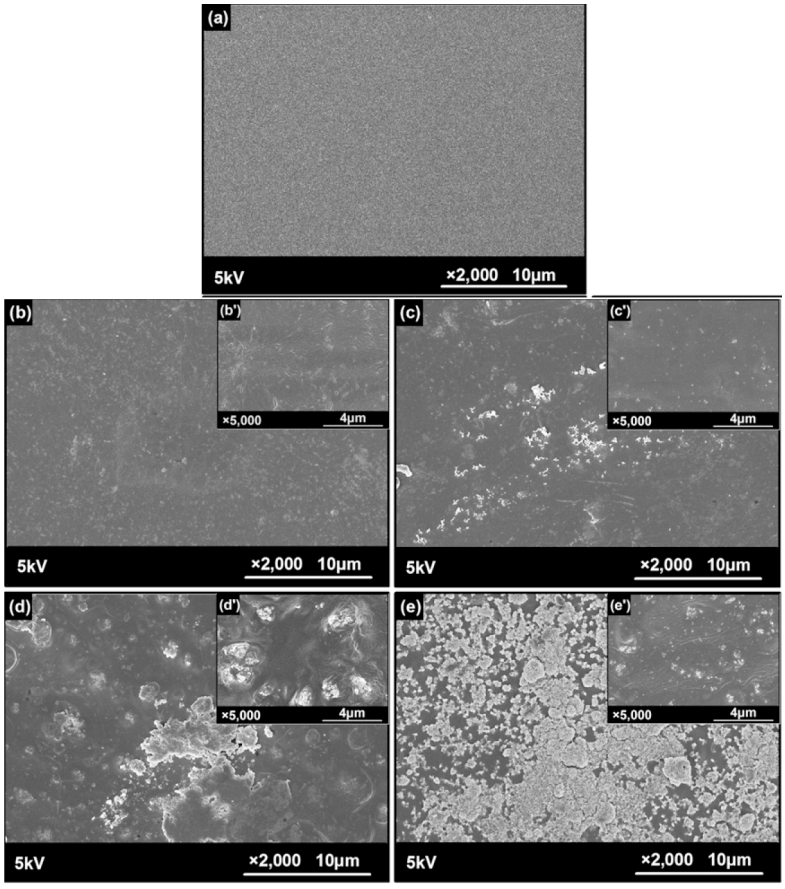

FIGURE 20.11

Surface morphologies of different composites cultured in 1.5 SBF at 37 ◦C for 2 days: (a) pure PLLA, (b) PLLA/HAP (containing 10 wt% of HA), (c) PLLA/HAP–PLLA (containing 10 wt% of HAP), (d) PLLA/HAP (containing 30 wt% of HAP), and (e) PLLA/HAP–PLLA (containing 30 wt% of HAP). The insets show the morphologies of different composites before biomineralization [187]

PMMA is repeatedly used for tissue engineering purposes as bone cement or orthopedic devices. Incorporation of HAP with PMMA can generate synergistic effects of both materials but, as HAP has low compatibility and very poor interfacial adhesion with PMMA, the preparation of PMMA composites and pristine HAP may result in agglomeration of HAP. Due to the agglomeration of HAP inside the matrix, the mechanical properties of the prepared composites may dramatically reduce (because of stress concentration). In order to eliminate this phenomenon, it is necessary to modify the surface of HAP with PMMA chains to prevent HAP nanoparticles from aggregating. For the surface modification of HAP by grafting PMMA to avoid the agglomeration of HAP particles, Lang *et al.* [188] reported grafting of PMMA chains using "grafting from" approach on the HAP surfaces through ATRP method. They first modified the HAP particles with aminopropyltriethoxysilane and then with 2-bromoisobutyryl bromide to introduce the appropriate amount of Br groups on the surface of HAP particles. Then, the polymerization of methyl methacrylate (MMA) on the surface of HAP particles was carried out using SI-ATRP. Results showed an increase in the compatibility with PMMA and improvement in the dispersibility and hydrophobicity of HAP particles.

In comparison to normal ATRP, components of the initial system (transition metal complex) such as Cu(II) of reverse ATRP, are more tolerant of exposure to air. In a recent study, the feasibility of reverse ATRP on the HAP surface and the control of polymerization has been investigated [189]. Surface-initiated reverse ATRP has been employed by covalent attachment of peroxide initiator moiety to the surface of HAP through the surface hydroxyl groups (g-HAP). In the next step, end bromide groups of grafted PMMA initiated normal ATRP of MMA to form further modification of HAP nanoparticles (b-HAP). The TEM images of HAP, g-HAP and b-HAP show that pristine HAP tends to be aggregated as a result of inter-particle van der Waals interactions, although the surface modification of HAP showed a strong improvement in dispersion of the nanoparticles (Figure 20.12).

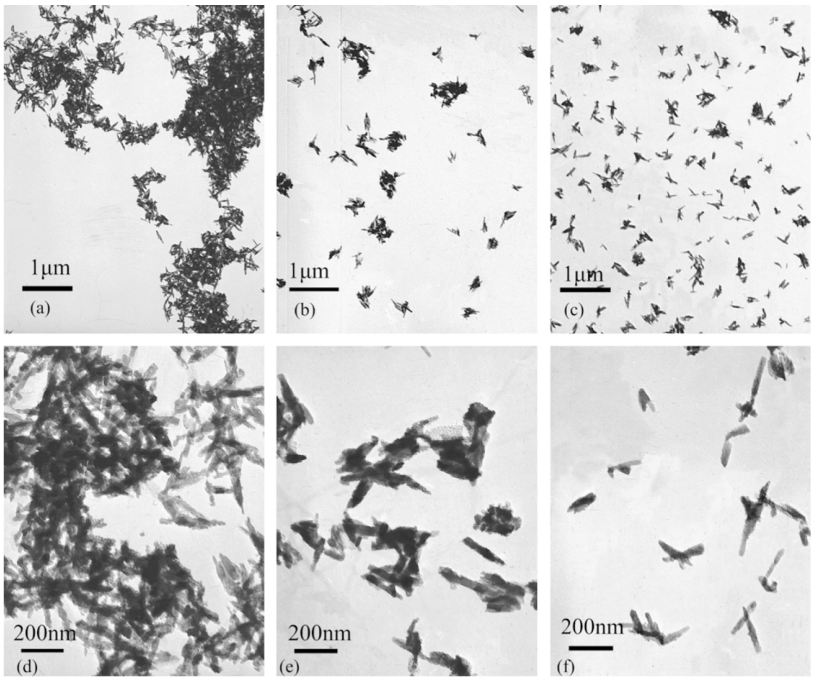

FIGURE 20.12
TEM micrographs of (a and d) HAP, (b and e) g-HAP-8h and (c and f) b-HAP-12h dispersed in chloroform [189]

Injectable thermo-responsive scaffolds via ATRP

Over the last two decades, stimuli-responsive macromolecules (i.e., pH-, thermo-, photo-, chemo-, and bio-responsive polymers) have attracted the scrutiny and considerable interest of materials science, nanotechnology and biotechnology scientists. Stimuli-responsive polymers provide a wide range of design and application of smart scaffolds for tissue engineering and regenerative medicine. They can adapt to surrounding environments and rapidly change their microstructure, wettability, dimension or physical properties, etc. Thermo-responsive polymers for biomedical applications, including drug delivery, tissue engineering and gene delivery, have drawn much attention among all the other smart polymers. In particular, thermo-responsive polymers that have cloud point or LCST around 32°C in water, which is close to physiological temperature, are useful for biomedical applications. Thermo-responsive polymers switch from hydrophilic to hydrophobic states above LCST in response to temperature changes, which makes them dehydrate and aggregate. Besides the advantages of thermo-responsive polymers, disadvantages of other stimuli-responsive polymers offer a wealth of reasons to use thermo-responsive polymers in the tissue engineering field. Whereas light is not a suitable stimulus for turbid biological fluids like blood, and changes in ionic strength and/or pH require transport of matter and can provoke sensitive reactions of biological systems, moderate temperature changes can be applied to most specimens without an adverse effect [190-199].

In recent years, development of in situ curing gels, also called injectable scaffolds, has been introduced, heralding in a new era. This is because they facilitate the injection of even large implant hydrogels directly into cavities with irregular shapes and sizes via minimally invasive surgery. Another advantage of in situ hydrogels is their ability to encapsulate cells, nutrients and other important biomolecules throughout the scaffolds. In situ cross-linking of stimuli-responsive hydrogels in the body generally occurs in two steps. First, with response to external stimuli, such as temperature, macromolecules form hydrogels via physical cross-linking. In this stage the physical interactions help the scaffold hold its shape, but it would still have weak mechanical properties. In the next step, gelation via chemical cross-linking, initiated by either photo-cross linking or chemical compounds, should be conducted to exhibit much better mechanical performance.

Using thermo-responsive copolymers to produce injectable scaffolds, Tai *et al.* [200] synthesized water-soluble thermo-responsive copolymers containing multiple methacrylate groups via one-step deactivation enhanced ATRP (DE-ATRP) of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, Mn=475), poly(propylene glycol) methacrylate (PPGMA, Mn=375), and ethylene glycol dimethacrylate (EGDMA) to form covalent cross-linked hydrogels by photo-polymerization. The copolymers can also be cross-linked by photo-polymerization through their multivinyl functional groups. In their study, they have used photo-polymerization, which is a mild cross-linking method, since chemical cross-linking can cause a harsh reaction. In fact, by using thermo-responsive polymers besides the photo-polymerization method advanced injectable biomaterials could be obtained. To prove the biocompatibility of the synthesized samples, mouse C2C12 myoblast cells were cultured in the presence of the copolymers. Figure 20.13 indicates that the majority of the cells remained viable, as assessed by Alamar Blue, lactate dehydrogenase (LDH), and Live/Dead cell viability/cytotoxicity assays [201,186].

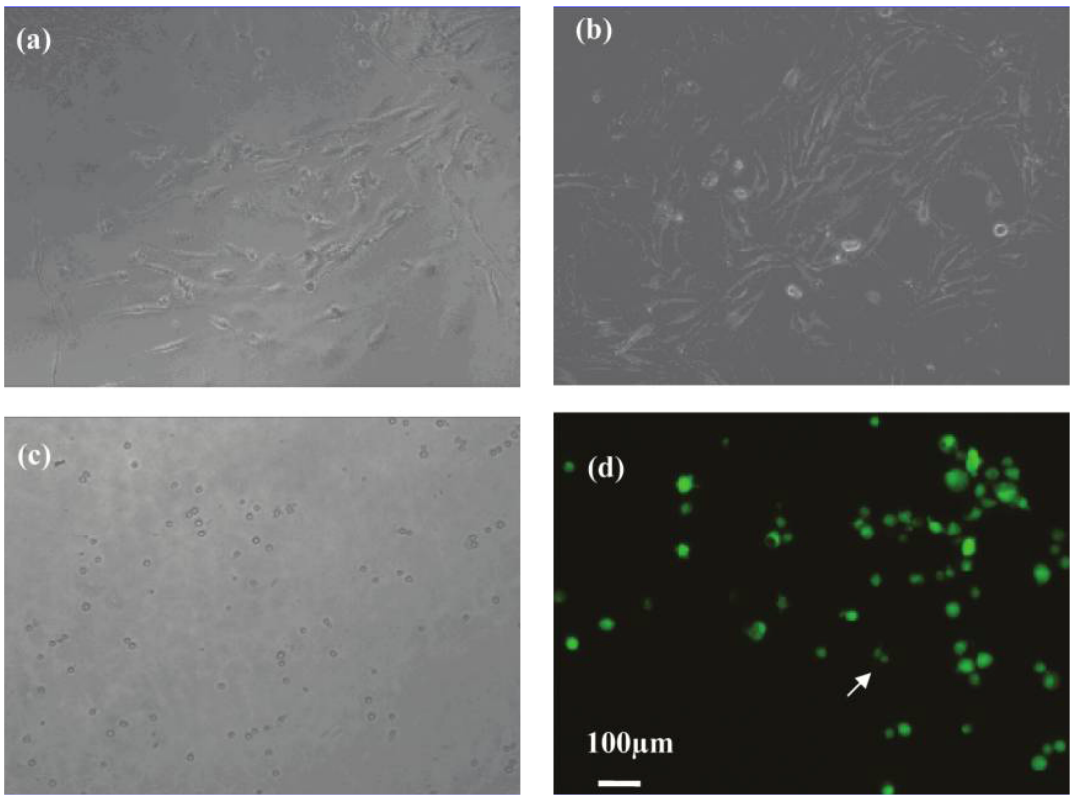

FIGURE 20.13
Light phase control microscope images for the cells cultured (a) in PEGMEMA-PPGMA-EGDMA copolymer (**1** in Table 1) culture media solutions (750 μg/mL); (b) in the culture media without polymers, (c) on the photo-cross-linked polymer films. (d) Live/Dead viability assay for the cells cultured in the copolymer/culture media solutions after 5 days. The viable cells fluoresce green, whereas the nonviable cells fluoresce red (pointed by the arrow) [200]

In another similar work by Tai *et al.* [202] a thermo-responsive hyper-branched copolymer system of poly(ethylene glycol) methyl ether methacrylate-co-2-(2-methoxyethoxy) ethylmethacrylate-co-poly(ethylene glycol) diacrylate ($PEGMEMA_{475}$–MEO_2MA–$PEGDA_{258}$) has been developed using DE-ATRP approach. Figure 20.14 demonstrates the procedure of forming this new injectable and in situ cross-linking hybrid hydrogel from the combination of thiolated hyaluronan and PEG-based thermo-responsive hyper-branched functional groups. These functional groups can react with HA–SH via efficient Michael-type thiolene reaction at physiological condition. In order to investigate the biological response of the hydrogels, 3D cell culture study was done and the results demonstrated good cell viability after the cells were embedded inside the samples (see Figure 20.15).

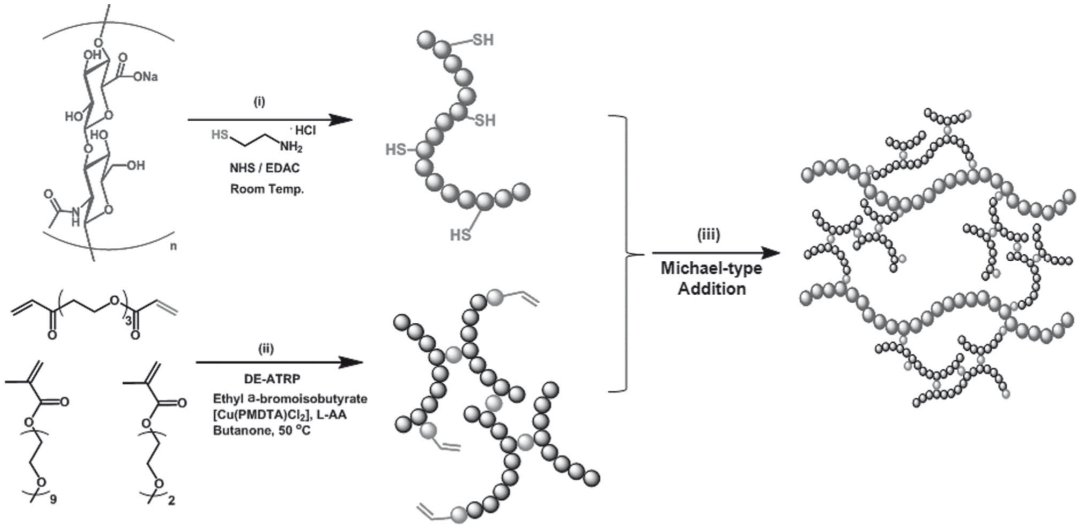

FIGURE 20.14
Synthetic route and chemical structures of $PEGMEMA_{475}$–MEO_2MA–$PEGDA_{258}$and HA–SH hydrogel. (i) modification of HA with cysteamine (EDAC–NHS) carbodiimide coupling; (ii) 'One-pot and One-step' DE-ATRP in butanone at 50°C; (iii) hydrogel from in situ crosslinking of components *via* Michael-type thiolene addition at pH 7.4 [202]

Vinyl polymers made by ionic or radical polymerization cannot efficiently degrade. In order to demonstrate the concept of preparing degradable polymers based on a combination of controlled radical polymerization of vinyl monomers and radical ring-opening polymerization (RROP), Matyjaszewski *et al.* [203], reported the development of an injectable thermo-sensitive hydrogel consisting of polyacrylamides with degradable units as an injectable scaffold to improve fracture repair. They have synthesized poly (N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane) (poly(NIPAAm-co-BMDO)) by ATRP and RAFT polymerization.

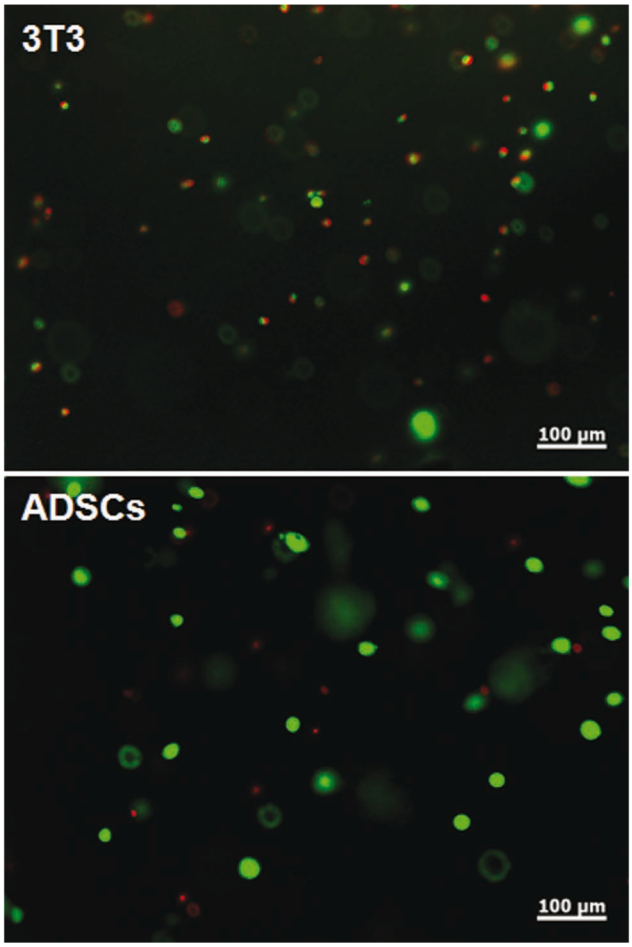

FIGURE 20.15
LIVE/DEAD viability assay for 3D cell embedded study. The hydrogels were formed from 5 wt% polymer solution and the HA–SH (2%, w/v) was added with molar ratio of thiol to vinyl group 4:1. 5 × 10^5 cells mL^{-1} of 3T3 fibroblast cells and rabbit ADSCs were embedded into hydrogels separately. After 7 d, the hydrogels fluorescently labeled with a dye that fluoresces green upon the presence of intracellular esterase activity in living cells (calcein-AM) and a dye that fluoresces red when bound to the DNA of dead membrane compromised cells. The samples were directly visualized on an Olympus IX81 inverted microscope [202]

After conducting the in vitro cell viability assay by culturing C2C12 cells on the top of a surface coated with the synthesized polymer and by adding the polymer directly to growing cells, they showed that both methods had no apparent cytotoxicity by live/dead and CyQUANT assays, as is clearly shown in Figure 20.16 and Figure 20.17.

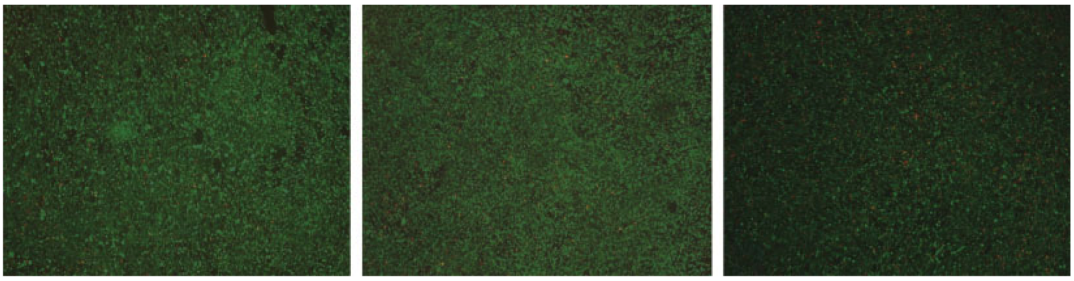

FIGURE 20.16
Combined fluorescence microscope images of C2C12 cells at 37°C. 95% ±1% viability was measured for the control (no polymer on surface) after 2 days (left image). 94% ± 2% viability was measured for cells cultured on top of poly(NIPAAm-co-BMDO) (Table I, expt. 3) after 2 days (center image) and 90% ± 2% viability was observed after 5 days (right image) [203]

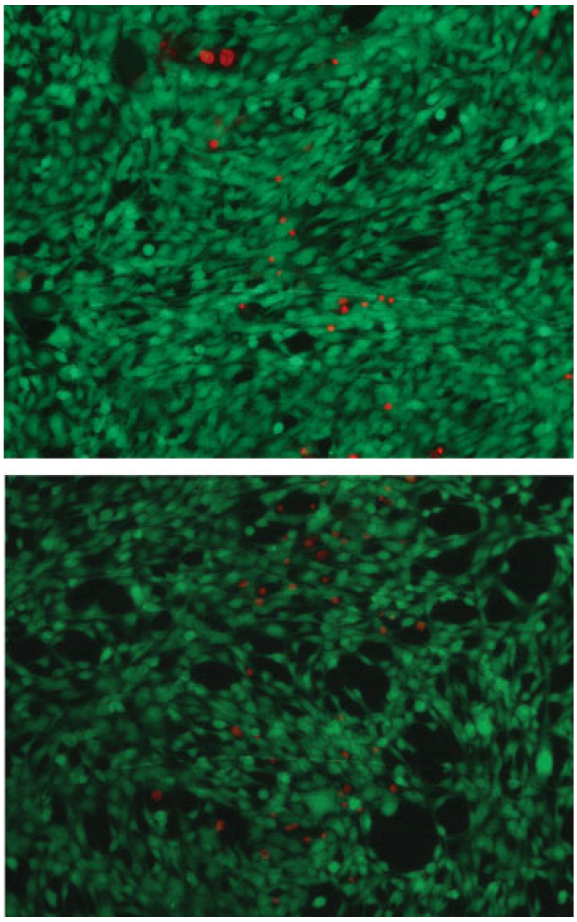

FIGURE 20.17
Combined live/dead fluorescence microscope images of C2C12 cells after 24 h without added polymer (control), showing 96% ± 1% cell viability (left), and with added poly(NIPAAm-co-BMDO) (Table 2, expt. 6), showing 94% ± 2% cell viability (right). [203]

Furthermore, the synthesized polymer was degraded completely in Dulbecco's modified Eagle medium (DMEM) and was added directly to the culturing cells confirming that the degradation products are not toxic in biological environments. The degradation products in the medium were also nontoxic indicating the high potential of this material for tissue engineering applications.

Conclusion

Advanced polymer chemistry allows careful tailoring of new biomaterials for different applications. New approaches in this field have led to the formation of complex architectures of defined molecular weight and polydispersity. As a new technique, ATRP has recently gained much attention for the design and synthesis of functional surfaces due to its ability to produce antifouling, antibacterial, or stimuli responsive surfaces. This technique has been recognized as a robust and versatile tool for the development of different kinds of functional bioactive scaffolds used in tissue engineered constructs which we discussed in this article. It is expected that these sophisticated polymeric structures with specific characteristics for clinical applications will lead to the development of new techniques for synthesis of tissue engineering scaffolds in the future.

Acknowledgements

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