9
Biodegradable Elastic Hydrogels for Tissue Expander Application

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9.1
Introduction

9.1.1
Hydrogels

Hydrogels are three-dimensional polymeric networks capable of absorbing a large amount of water or biological fluids while maintaining their basic structure [1, 2]. In the polymeric network, hydrophilic polymers are hydrated in an aqueous environment. The term "network" implies that crosslinked structures have to be present to avoid the dissolution of the hydrophilic polymer chains into the aqueous phase. Hydrogels can be classified into chemical and physical hydrogels based on the nature of crosslinking. In chemical hydrogels, the polymer chains are crosslinked by covalent bonding. If the polymer chains are crosslinked by non-covalent bonding, such networks are called physical hydrogels.

Since water molecules are the major component of the hydrogels, the mechanical strength of most hydrogels is rather low. That is, the storage moduli (G') of most hydrogels fall between several hundreds or several thousands pascals when the water content is high [3]. The poor mechanical strength and toughness after swelling are major disadvantages of using hydrogels. Therefore, the improvement of the elasticity of hydrogels is of great interest, since high elastic hydrogels are more suitable for application that bear mechanical loading, such as cartilage implant materials.

9.1.2
Elastic Hydrogels

Elastic hydrogels are hydrogels that are resilient and resistant to compression and elongation in their dried or water-swollen states. The elastic hydrogels possess the capability of withstanding cyclic mechanical strain without cracking or suffering significant permanent deformation [4]. The molecular weight of the polymers should be high enough, and the glass transition temperature (T_g) should be low

Handbook of Biodegradable Polymers: Synthesis, Characterization and Applications, First Edition. Edited by Andreas Lendlein, Adam Sisson.

enough, to impart elastomeric behavior of the hydrogels [5]. Shape-memory hydrogels constitute a class of elastic hydrogels that can be elastically deformed and fixed into a temporary shape, and have ability to recover the original, permanent shape on exposure to an external stimulus such as heat or light [6].

For most biomedical applications, biodegradable elastic hydrogels are favored over nondegradable hydrogels. This is because they can be removed or eliminated by natural degradation from the applied sites in the body under relatively mild conditions, thus eliminating the need for any surgical removal processes after the system fulfills its goal. Biodegradable polymeric systems also provide flexibility in the design of delivery systems for large molecular weight drugs, such as peptides and proteins, which are not suitable for diffusion-controlled release through nondegradable polymeric systems [7]. In addition, the degradation can be utilized to control the rate of drug release and the physicochemical properties of the hydrogel systems, and thus to provide flexibility in the design of biomedical devices, such as drug–biomaterials combination products. However, proper techniques for predicting hydrogel degradation rates are critical for successful application of these degradable systems as they facilitate the design of implants with optimal degradation profiles that result in proper rates of drug release or tissue regeneration and hence maximize therapeutic effects.

9.1.3
History of Elastic Hydrogels as Biomaterials

Earlier works in elastic hydrogels were mainly focused on development of shape-memory hydrogels for fabrication of devices and implant stents. The first publication mentioning shape-memory effects in hydrogels was made by Osada *et al*. in 1995, who discovered a new phenomenon of a polymer hydrogel made by radical copolymerization of acrylic acid and *n*-stearyl acrylate having elastic memory that could be stretched to at least 1.5 times of its original length when the swollen gel is heated above 50 °C [8, 9]. Since then, biodegradable shape-memory polymers have been synthesized, including network polymers formed by crosslinking oligo(ε-caprolactone) dimethylacrylate and *N*-butylacrylate [10], a multiblock copolymer of oligo(ε-caprolactone) and oligo(*p*-dioxanone)diol [11], and polyesters of poly(propylene oxide) (PPO) with polylactide or glycolide [12]. Improvement of the stiffness and recovery force of shape-memory polymers can be achieved by the synthesis of shape-memory composites. Zheng *et al*. synthesized polylactide and hydroxyapatite composites which demonstrated better shape-memory effect than pure polylactide polymer [13].

Recently, with the increasing interest in engineering various tissues for the treatment of many types of injuries and diseases, a wide variety of biodegradable elastic hydrogels with desirable mechanical, degradation, and cytophilic properties have been developed. Elastic superporous hydrogel hybrids exhibiting mechanical resilience and a rubbery property in the fully water-swollen state have been reported by Park *et al*. These hydrogel hybrids of acrylamide (AM) and alginate could be stretched to about 2–3 times of their original lengths and could be loaded

and unloaded cyclically at least 20 times. This property can potentially be exploited in the development of fast- and high-swelling elastic hydrogels for a variety of pharmaceutical, biomedical, and industrial applications [14, 15]. However, these systems lack biodegradable properties for various biomedical applications. Wen *et al*. developed biodegradable, biocompatible polyurethane-based elastic hydrogels by changing chain extenders. The hydrogels were highly elastic in its swollen state and comparable degradation and cytocompatible behaviors to polylactide. This may find the applications in both soft- and hard-tissue regeneration [16, 17]. In recent years, block copolymers of biodegradable polyesters such as poly(ε-caprolactone) (PCL), polylactides (PLAs), poly(glycolic acid) (PGA), and polylactide-*co*-glycolide (PLGA), and hydrophilic polyethylene glycol (PEG) have received considerable attention as potential biomaterials because of their combined advantages of the biodegradability of the polyesters and the biocompatibility of PEG [18–20]. The block copolymers also have some unique properties based on their amphiphilic nature. The block composition and structural characteristics can be utilized to modify various physicochemical properties such as biodegradation, permeability, swelling, elasticity, and mechanical properties [21–23]. Typical hydrogels are glassy and brittle in the dried state and it is difficult to change the shape and size of the dried state. Huh *et al*. have developed biodegradable PEG/PCL and PLGA–PEG–PLGA/PEG hydrogels showing flexible and elastic properties even in the dried state that they remain intact after repeated bending or stretching to twice the original length [24].

Further, elastic hydrogels with self-healing capacity were synthesized by hydrophobic association through micellar copolymerization of AM and a small amount of octyl phenol polyethoxy ether acrylate. These hydrogels showed high recovery even after extensive stretching and self-healing after being cut into two parts which can be used as shrinkable or thermal sensitive materials [25].

While covalently crosslinked hydrogels have the ability to control the elastic behaviors, one limiting factor is the difficulty in guaranteeing removal of impurities, such as unreacted monomers, sol fractions, nonaqueous solvents, and initiators. Feldstein *et al*. demonstrated the formation of water-absorbing, elastic, and adhesive hydrogels through hydrogen bonding of three pharmaceutical grade components poly(*N*-vinylpyrrolidone) (PVP), PEG, and poly[(methacrylic acid)-*co*-(ethyl acrylate)] [p(MAA-*co*-EA)] without introduction or formation of toxic by-products. The hydrogels are malleable under various processing conditions such as drawing, molding, and extrusion, suggesting a wide range of applications in the biomedical and cosmetic fields [26].

9.1.4
Elasticity of Hydrogel for Tissue Application

Most natural tissues, such as heart, blood vessels, skeletal muscle, tendon, and so forth, are very elastic and strong. If the biodegradable polymers are either too stiff/brittle with low elongation, or very soft with relatively low strength, the mechanical properties of these polymers are not compatible with natural tissues.

The hydrogels are good candidates for tissue applications when their elastic moduli are close to that of natural tissue components. For instance, articular cartilage contains ~70% water and bears loads up to 100 MPa, but most hydrogels, either synthetic or natural, can be easily broken indicating that they are much weaker than native cartilage tissue. The degradable elastic polyurethane hydrogels have elastic moduli ranging from 16.8 ± 3.3 to 26.6 ± 3.9 MPa, which are very close to the properties of native cartilage showing promise for soft- and hard-tissue regeneration [17].

For engineering of soft tissue, elastic hydrogel scaffolds are desirable since they are amenable to mechanical conditioning regimens that might be desirable during tissue development. Elasticity values of most of the single component hydrogels were lower than 10 kPa, while higher percentage of multicomponent hydrogels exhibited high elastic mechanical property up to 100 kPa [3]. The compressive modulus of hard tissue such as articular cartilage is in the range of 0.53–1.82 MPa [27]. In order to promote cartilage regeneration, a hydrogel scaffold must be able to exhibit mechanical integrity in the face of loading from the body, while at the same time guide appropriate cartilaginous tissue growth. A biodegradable hydrogel scaffold with elastic properties could be useful for application in cartilage treatment.

9.2 Synthesis of Elastic Hydrogels

9.2.1 Chemical Elastic Hydrogels

Chemical hydrogels are those that have covalently crosslinked networks. Thus, chemical hydrogels will not dissolve in water or other organic solvents unless covalent crosslinks are cleaved. There are generally two different methods to prepare chemical elastic hydrogels. Chemical elastic hydrogels can be prepared by polymerization of water-soluble monomers in the presence of bi- or multifunctional crosslinking agents. Chemical hydrogels can also be prepared by crosslinking water-soluble polymers using chemical reactions that involve functional groups of the polymer. Due to the high strength of the covalent linkages, the three-dimensional networks of hydrogels are permanent and the formation of crosslinks is usually irreversible.

9.2.1.1 Polymerization of Water-Soluble Monomers in the Presence of Crosslinking Agents

Polymerization of water-soluble monomers in the presence of crosslinking agents results in the formation of chemical hydrogels. Typical water-soluble monomers for the preparation of chemical elastic hydrogels include acrylic acid, AM, hydroxyethyl methacrylate, and so on. The crosslinking agents for the synthesis of elastic hydrogels are not only low-molecular-weight agents such as *N*,*N*′-methylenebisacrylamide but also inorganic agents such as hectorite clay.

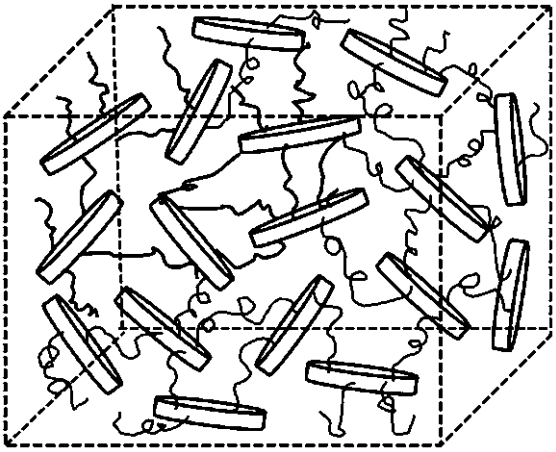


Figure 9.1 Structure of nanocomposite hydrogel using Clay-S by *in situ* polymerization.

For example, a novel highly resilient nanocomposite hydrogel with ultra-high elongation was prepared by polymerization of monomer (AM or *N*-isopropylacrylamide (NIPAAm)) in the presence of the inorganic hectorite clay as a crosslinker (Clay-S), initiator (potassium persulfate), and accelerator (tetramethyldiamine) [28]. As shown in Figure 9.1, Clay-S forms a stable uniform dispersion in a solution that contains monomer and other reagents. Polymerization is initiated on the surfaces of the clay, and polymer chains are attached to the clay surface to form clay-brush particles, and finally, the aqueous dispersion is converted into a nanocomposite hydrogel of the uniform polymer network of Clay-S and AM, which can distribute stress evenly on each chain. The hydrogel could be elongated to 10 times of its original length and recovered to initial state.

In another approach, a hybrid of chemical and physical hydrogels was prepared from polyacrylamide and sodium alginate [14]. The copolymerization of AM monomer and *N*,*N*′-methylenebisacrylamide as a crosslinker and other necessary ingredients formed superporous polyacrylamide hydrogels. The crosslinking density of the hydrogel was increased by the physical crosslinking of sodium alginate with Ca^{2+}. The mechanical properties of the superporous hydrogels can be significantly increased through this interpenetrating network formation.

9.2.1.2 **Crosslinking of Water-Soluble Polymers**

Crosslinking of water-soluble polymers by the addition of bifunctional or multifunctional reagents results in chemical elastic hydrogels. Macromers are macromolecular monomers or polymers that contain two or more vinyl groups, acrylates and methacrylates being the most common. The crosslinking reactions can be catalyzed chemically, thermally, or photolytically. Photopolymerization is an increasingly common way to drive the crosslinking reaction.

Degradable polyurethane-based light-curable elastic hydrogels were synthesized from polycaprolactone diol, PEG as soft segment, lysine diisocyanate as hard

segment, and 2-hydroxylethyl methacrylate as chain terminator through UV-light-initiated polymerization. The hydrogels were formed through the crosslinking of methacrylate groups in 2-hydroxylethyl methacrylate via UV light. The PCL:PEG ratios in soft segments were responsible in determining elasticity as well as the strength of the hydrogels [17].

The formation of degradable hydrogels by crosslinking macrodimethacrylates was also reported by Choi *et al.* [12]. Triblock copolymers of PLA–PPO–PLA containing polylactic acid (PLA) blocks and acrylate end groups of PPO were used to create photopolymerizable hydrogels showing shape-memory property.

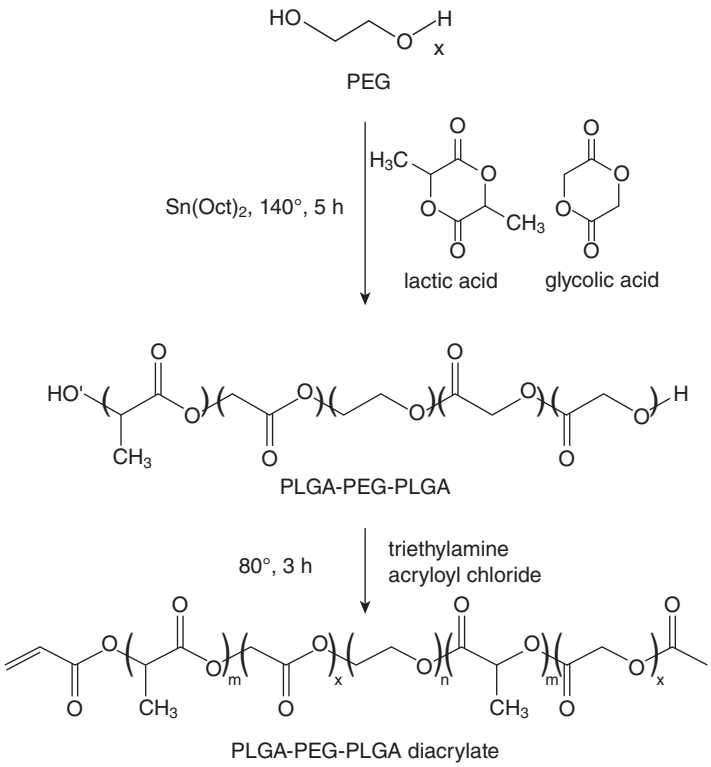
Recently, the formation of elastic hydrogels from block copolymer of PEG and biodegradable polyesters has been extensively investigated. PEG is a hydrophilic polymer and its glass transition temperature is very low due to the flexible chain structure. When PEG was used as a building block for preparing hydrogels with other biodegradable polyesters such as PGA, PLA, and PCL, the hydrogels can show flexible and/or elastic properties [4, 24, 27]. PEG has two hydroxyl groups at both ends of the polymer that can be modified with a vinyl group to form a divinyl macromer. PEG acrylates are the major type of macromers for the preparation of PEG-based elastic hydrogels. For example, chemically crosslinked biodegradable elastic PEG/PCL or PLGA–PEG–PLGA/PEG hydrogels were prepared via radical crosslinking reaction of PEG-diacrylate with PCL-diacrylate or PLGA-PEG-PLGA-diacrylate in the presence of a radical initiator 2,2-azobisisobutyronitrile in a drying oven at 65 °C for 12 h [24]. Scheme 9.1 illustrates the synthetic method of PLGA-PEG-PLGA diacrylate using for crosslinking reaction with PEG-diacrylate under thermal catalyst.

9.2.2 Physical Elastic Hydrogels

Physical gels are the continuous, disordered, three-dimensional networks formed by associative forces capable of forming noncovalent crosslinks [29]. Noncovalently crosslinked hydrogels are formed when primary polymer chains contain chemical moieties capable of electrostatic, hydrogen bonding, ion dipole, or hydrophobic interaction [26]. Physical crosslinking of polymer chains can also be achieved using a variety of environment triggers (pH, temperature, and ionic strength). In physical elastic hydrogels, association of certain linear segments of long polymer molecules forms extended "junction zones." The junction zones are expected to maintain ordered structure. Although noncovalent association are reversible and weaker than chemical crosslinking, they allow solvent casting and thermal processing, and the resulting polymers often show elastic or viscoelastic properties [30].

9.2.2.1 Formation of Physical Elastic Hydrogels via Hydrogen Bonding

Examples of elastic and adhesive hydrogels via the formation of hydrogen bonding are triple blends of PVP, PEG, and p(MAA-*co*-EA). Ternary polymer blends were dissolved in ethanol under vigorous stirring, and then casted into film. The PVP/PEG/p(MAA-*co*-EA) hydrogel was formed via the stable three-dimensional



Scheme 9.1 Synthetic methods of PLGA–PEG–PLGA diacrylate.

hydrogen-bonded network in which p(MAA-*co*-EA) contains H-bond donor groups, PVP contains H-bond acceptors, and PEG contains both. The hydrogel films are malleable and retain their integrity upon hydration – a feature characteristic of covalently crosslinked hydrogels. The polymer blend films remained intact at pH 5.6 but underwent dissolution at pH 7.4 due to loss of hydrogen bonding and development of charge repulsion [26].

Hydrogen-bonding interaction can also be used to produce hydrogels by freeze-thawing. A novel double-network elastic hydrogel fabricated with PVP and PEG was prepared through a simple freezing and thawing method. PVA/PEG hydrogel structure was formed by a PVA-rich first network and a PEG-rich second component, in which hydrogen bonding existed. The two polymers were dissolved in ultrapure water and exposed to repeated cycles of freezing at –20 °C for 8 h and thawing at room temperature for 4 h. Figure 9.2 illustrates the structural formation of elastic PVA/PEG double-network hydrogels. The condensed PVA-rich phase forms microcrystals first, which bridge with one another to form a rigid and inhomogenous net backbone to support the shape of the hydrogels, and the dilute PEG-rich phase partially crystallizes among the cavities of voids of the backbone. PEG clusters in the cavities of PVA networks absorb the crack energy and relax

Figure 9.2 Schematic representation of the structural model of PVA/PEG double-network hydrogel.

the local stress either by various dissipations or by large deformation of the PEG chains. The crystalline regions of PVA essentially serve as physical crosslinks to redistribute external stresses [31].

9.2.2.2 Formation of Physical Elastic Hydrogels via Hydrophobic Interaction

Polymers with hydrophobic domains can crosslink in aqueous environment via reverse thermal gelation. Temperature increase promotes hydrophobic interactions resulting in the association of hydrophobic polymer chains. The physical association of hydrophobic domains holds swollen soft domains together and makes the polymers stable in water [32]. The common hydrophobic blocks which can undergo reverse thermal gelation at or near physiological temperature are PPO, PLGA, poly(*N*-isopropylacrylamide), PCL, and poly(urethane) [33].

For example, multiblock copolymers of polyethylene oxide and PCL or PLA were synthesized for the preparation of polymer films by solvent casting method. The multiblock copolymers formed thermoplastic hydrogels via hydrophobic interaction. The block copolymer films were rubbery in both dried and swollen states. The interesting property of these multiblock copolymers was that the swelling increased by increasing temperature and increased further, rather than decreasing, when the temperature was lowered to the initial temperature [30]. Other types of amphiphilic block copolymers of PCL with PLA and PGA were also synthesized to prepare elastic PCL/PLA and PCL/PGA physical hydrogels [4, 27].

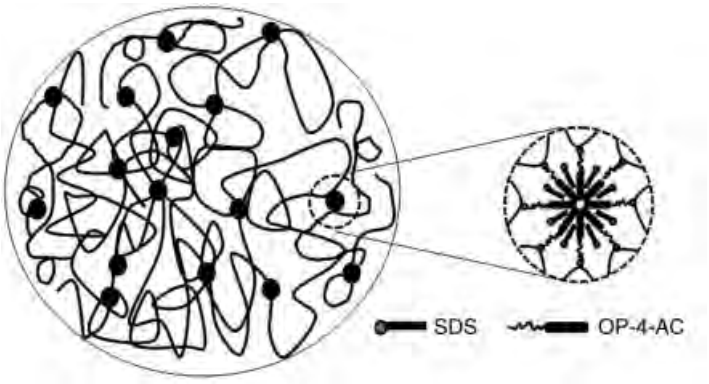


Figure 9.3 Schematic illustration of the hydrophobic association of hydrogels, which consists of associated micelles and flexible polymer chains connected by neighboring associated micelles.

In another example, a new type of physically crosslinked hydrogel via hydrophobic interaction was prepared. An elastic hydrogel with self-healing property was synthesized through micellar copolymerization of AM and a small amount of octylphenol polyethoxyether acrylate in an aqueous solution containing sodium dodecyl sulfate at 50 °C. The hydrophobically modified polyacrylamide was synthesized by the copolymerization of AM and octylphenol polyethoxyether acrylate. After polymerization, hydrophobic association of SDS and hydrophobic microblocks of hydrophobically modified polyacrylamide leads to the formation of associated micelles. These micelles act as crosslinking points, so three-dimensional polymer networks were constructed as shown in Figure 9.3 [25]. Because of the large distance between the associated micelles, all polymer chains between the crosslinking points in the hydrogels were sufficiently long and flexible.

9.3 Physical Properties of Elastic Hydrogels

Some of the most important properties of elastic hydrogels are: the gel mechanical properties, to withstand the physiological strains *in vivo* or mechanical conditioning *in vitro*; gel swelling properties to maintain cell viability; and the degradation profiles to match tissue regeneration.

9.3.1 Mechanical Property

Mechanical properties of elastic hydrogels are evaluated by the measurement of elasticity and stress relaxation. Elasticity is estimated from the tensile strength,

elongation at break, and recovery after stretching. The mechanical tests are performed with hydrogel samples in a fixed cross-sectional area by pulling with a controlled, gradually increasing force until the sample changes shape or breaks. When a constant strain is applied to a rubber material, the force necessary to maintain that strain is not constant but decreases with time, this behavior is called "stress relaxation." Stress relaxation of hydrogels was determined by the following equation:

$$(\text{maximum stress at a constant strain}/\text{stress at the constant strain after holding for a determined time}) \times 100.$$

The tensile strength of elastic hydrogels is dependent on the crosslinking density and the flexibility of the water-soluble monomer or macromers in water. The elastic hydrogels exhibit rubberlike profiles in the stress–strain curves with very high elongation at break as shown in Figure 9.4. The recovery after stretching is usually more than 90% when applied up to the tensile strain at break.

Stress relaxation describes how polymers relieve stress under constant strain. In viscoelastic materials, stress relaxation occurs due to polymer chain rearrangement allowing permanent deformation of the materials. By this method, low values for stress relaxation indicate that polymer chain rearrangement is occurring. Example of stress relaxation of elastic hydrogel is given in Figure 9.5. The stress relaxation of all samples was more than 90%, indicating that polymer chain rearrangement is occurring only minimally. Since these hydrogels are highly crosslinked, there is little freedom for rearrangement and, as such, these materials do not deform under stress.

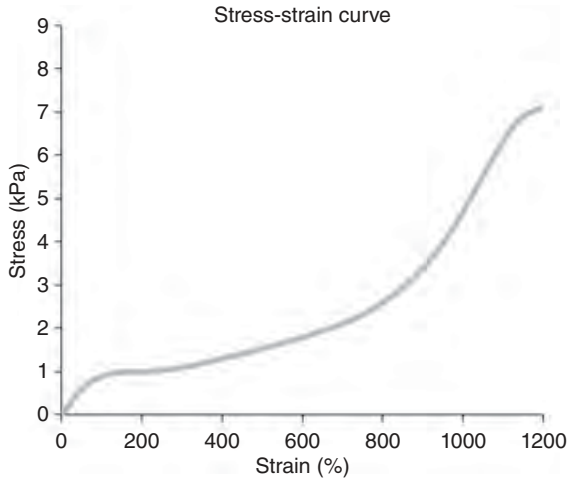


Figure 9.4 Stress–strain curve of elastic film prepared from poly(L-lactide-*co*-ε-caprolactone).

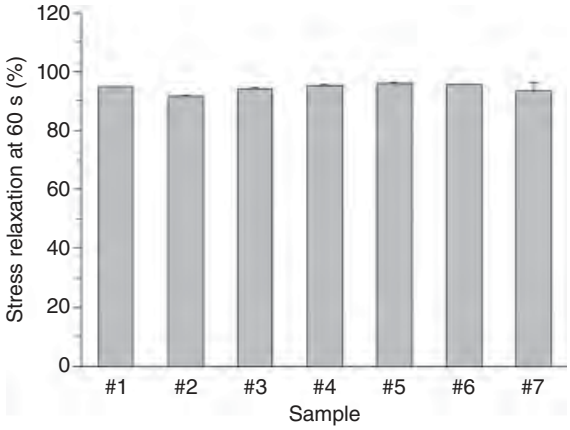


Figure 9.5 Stress relaxation of PLGA–PEG–PLGA/PEG elastic hydrogels.

9.3.2 Swelling Property

The swelling property of hydrogels is usually characterized by measuring their capacity to absorb water or aqueous solutions. The swelling ratio (R_s), which is the most commonly used parameter to express the swelling capacity of hydrogels, is defined as follows:

$$R_s = (W_s - W_d)/W_d \tag{9.1}$$

where W_s and W_d are the weights of swollen and dried hydrogels, respectively.

It is well known that the swelling ratio of hydrogels not only depends on the hydrophilic ability of the functional groups but also on the network space of the hydrogels. Generally, the hydrogel with higher network space presents higher water content. Ionization often provokes swelling due to electrostatic/osmotic repulsion of polyelectrolyte chains.

Swelling pressure is the pressure exerted on the swelling hydrogels due to the osmotic effect or the degradation of the crosslinking structure. The swelling pressure (π_{sw}) of a neutral polymer gel is determined by two opposing effects: the osmotic pressure (π_{osm}) that expands the network and the elastic pressure (π_{el}) that acts against expansion [34]:

$$\pi_{sw} = \pi_{osm} + \pi_{el} \tag{9.2}$$

where $\pi_{el} = -G'$, G' being the elastic (shear) modulus of the hydrogel.

Swelling pressure is usually measured during the degradation of hydrogels at the accelerated condition close to physiological condition, for example, in the

isotonic solution of 0.154 M HCl. Up to present, no relationship between swelling pressure and swelling ratio of neutral hydrogels has been reported. However, it is known that swelling pressure gradually increases in the course of the degradation process and depends on the mechanism of the degradation. For example, the dextran gels are degraded at their backbone, the swelling pressure increases rather continuously; in the case where they are degraded at the crosslinks, it increases more discontinuously and a sudden increase occurs when the gels are completely degraded [35]. Similarly, an increase in swelling ratio of hydrogels occurs in the first period of the hydrogel degradation due to the decrease of the crosslinking density of hydrogels. As the degradation proceeded further, the swelling ratio decreases to zero since the network structure of hydrogels broke down. The swelling ratio and the swelling pressure of a hydrogel depend on internal and external factors. The internal factors are the polymer network of the hydrogel. The swelling ratio and the swelling pressure of a gel are determined by: (i) osmotic pressure, (ii) the rubber elasticity, and (iii) polymer–polymer interaction of the polymer network [36]. The external factors are parameters of the solution like the concentration and electric charge of the solute molecules or ions.

Swelling pressure of hydrogels is also measured under the osmotic condition to determine the ability of the hydrogels as a tissue expander. Osmosis is the main driving force of the volume expansion of an anhydrous gel body in the solutions of living tissue [36]. Mechanical work can be done when hydrogels transform osmosis into real pressure. In this case, the role of a gel to act as a pressure-generating device is based on the balancing of the osmotic pressure and the rubber elasticity. If the swelling pressure can overcome the resistance of the adjacent tissue, it is sufficient to dilate the surrounding tissue at the expected rate [37]. Previous research has established that a pressure close to 100 mmHg is ideal for tissue expansion [38]. As an example, the swelling pressure of PLGA–PEG–PLGA/PEG elastic hydrogels is given in Figure 9.6. All these hydrogels can create swelling pressure more than 400 mmHg which is sufficient for tissue expansion.

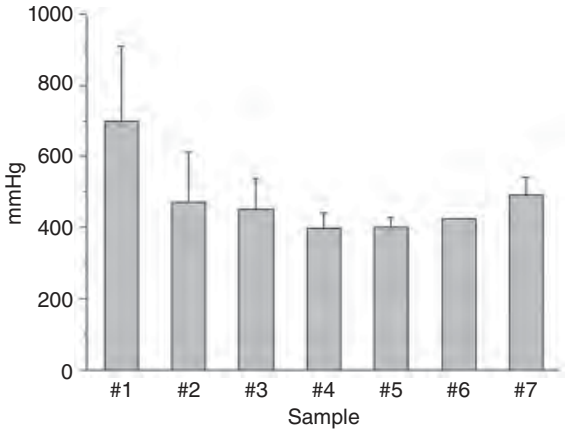


Figure 9.6 Swelling pressure of PLGA–PEG–PLGA/PEG elastic hydrogels.

9.3.3
Degradation of Biodegradable Elastic Hydrogels

Degradation of polymer hydrogel networks can occur by different mechanisms: (i) by hydrolysis of side chains or pendant groups, (ii) by cleavage of the polymeric backbone, and (iii) by cleavage of labile groups in the crosslinks [35]. Biodegradation of hydrogels occurs by either simple hydrolysis or by enzyme-catalyzed hydrolysis. *In vitro* degradation rate of biodegradable elastic hydrogels was generally evaluated by measuring the weight loss and swelling ratio of the samples in phosphate buffered saline solution at multiple time points at 37 °C with gentle shaking to mimic the *in vivo* environment. The degradation of the gel is a function of the crosslink density, as well as the hydrolytic susceptibility of chemical bonds. Hydrogels made from lower molecular weight precursors are more tightly crosslinked and thus degrade more slowly than hydrogels made from higher molecular weight precursors. Degradation of the biodegradable hydrogel network led to decreased crosslinking density, which increased the hydrogel swelling ratio. Figure 9.7 gives an example of *in vitro* degradation properties of PLGA-PEG-PLGA/PEG elastic hydrogels at different ratios of lactic acid/glycolic acid. The hydrogels showed various lag times before swelling depending on the chemical composition of the triblocks and the PLGA-PEG-PLGA/PEG block composition ratio. The hydrogels with higher content of PEG block showed lower degradation rates due to higher crosslinking density of low-molecular-weight PEG. As the degradation proceeded further, the network structure finally broke down so that the hydrogel mass was disintegrated into soluble degradation products.

9.4
Applications of Elastic Hydrogels

9.4.1
Tissue Engineering Application

Due to the high mechanical property, most biodegradable elastic hydrogels are attractive for development or regeneration of both soft- and hard tissues. For example, PEG/PCL and poly(lactide-*co*-caprolactone) (PLCL) elastic hydrogels have been investigated for use as scaffolds for cartilage regeneration [27, 39]. Chondrocyte cells were found to be dispersed evenly through the scaffold material without any further prewetting treatment, and remained viable after 3 weeks of culture. The PEG/PCL scaffold-seeded chondrocytes enhanced the gene expression of chondrogenic differentiation in a time-dependent manner. The formation of neocartilage was increased over 4 weeks after implantation in nude mice [39]. The elastic PLCL scaffolds maintained their mechanical integrity after implantation and guided cartilaginous tissue growth *in vivo* [27].

The application of cyclic mechanical strain during the smooth muscle tissue-engineering process has been found to show increased elastin and collagen

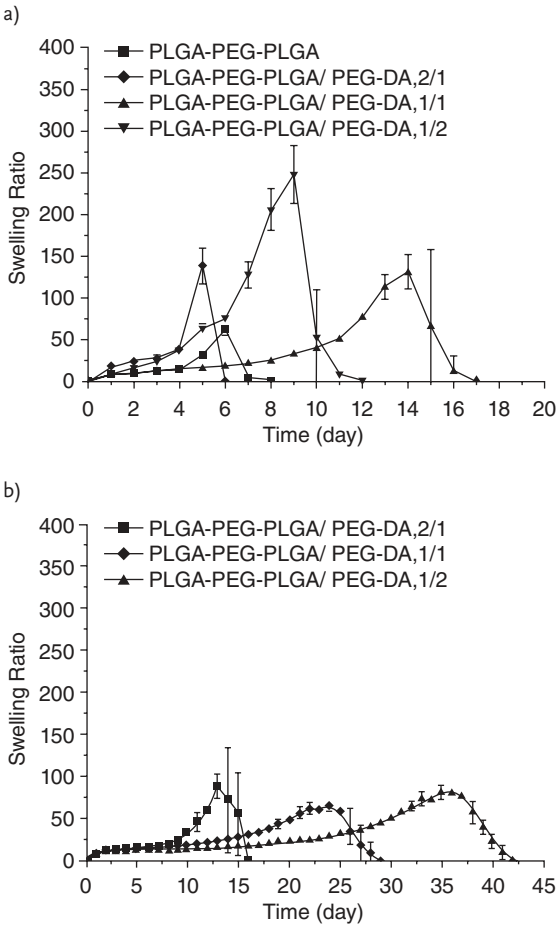


Figure 9.7 *In vitro* degradation test of PLGA–PEG–PLGA/PEG elastic hydrogels (a) LA/GA = 1 and (b) LA/GA = 4 at 37 °C.

production and tissue organization [40]. To achieve this, scaffolds must be elastic and capable of withstanding cyclic mechanical strain without cracking or suffering significant permanent deformation. Elastic biodegradable poly(glycolide-*co*-caprolactone), PLCL, and polyurethane scaffold could be used to engineer smooth-muscle-containing tissue (e.g., blood vessels and bladders) in mechanical dynamic environments [4, 5, 41]. The elastic scaffolds allowed for appropriate smooth muscle cell adhesion and subsequent tissue formation.

9.4.2
Application of Elastic Shape-Memory Hydrogels as Biodegradable Sutures

The medical application of shape-memory polymers is of great interest due to a combination of biocompatibility, tailorable transition temperature, large shape

deformation and complete recovery, and elastic properties of the materials [42]. For example, the mechanical characteristics and degradability of shape-memory, multiblock copolymers can be used for the preparation of smart surgical suture. Lendlein and Langer fabricated a self-tightenable biodegradable suture from a biodegradable, elastic shape-memory polymer. The suture can be loosely connected and then heated above critical temperature to trigger the shape recovery and tighten the suture [11].

9.5
Elastic Hydrogels for Tissue Expander Applications

A material or device designed to induce skin or tissue expansion for the purpose of reconstructive and plastic surgeries has been called a tissue expander. Tissue expanders are temporary inflatable implants that are positioned under the skin to facilitate the increase of tissue dimensions for reconstruction [43]. As an example, Figure 9.8 illustrates a schematic diagram of a skin expander using a flatable balloon.

An ideal expander should have several characteristics: easily placed through a small access site, gradually enlarge over a relatively short time, well tolerated over the long term, avoid uncomfortable inflation spikes, and resistant to infection [44]. In 1982, Austad and Rose introduced a self-inflating expander that consisted of a permeable silicone membrane filled with a hypertonic saline solution [45]. However, the expansion of the silicone balloon takes too long (8–14 weeks) and induces tissue necrosis [46].

The use of hydrogels as tissue expanders in reconstructive surgery was first developed in 1992 by Downes *et al*. who exploited the osmotically driven expansion of a biocompatible poly(hydroxylethyl methacrylate) hydrogel [47]. The hydrogels are placed in their dry, contracted states, and expand gradually to their full size with over 10-fold increase in volume. Wiese verified that hydrogels are efficient materials to induce the tissue expansion using vinyl-2-pyrrolidone (VP)/methyl methacrylate (MMA) copolymeric hydrogel and demonstrating their biocompatibility and swelling pressure [46]. Once implanted, the VP/MMA absorbs body fluids that leads to gradual swelling of the device to a 250–300% in volume as shown in Figure 9.9. Wiese *et al*. also introduced the innovative self-filling device, using a hydrogel matrix consisting of MMA/VP by replacing the CH_3 groups in

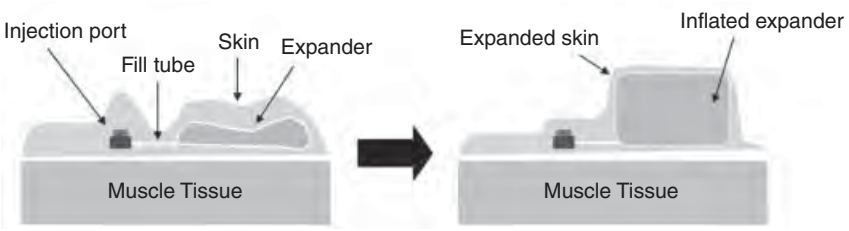


Figure 9.8 Schematic diagram of skin expander using an inflatable balloon.

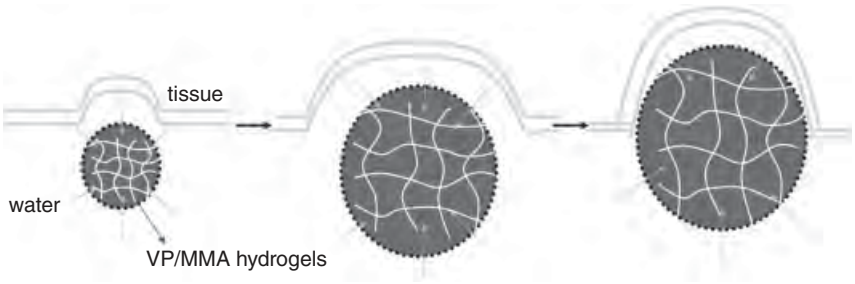


Figure 9.9 Use of VP/MMA copolymeric hydrogels for tissue expansion.

the VP/MMA hydrogel chains with COOH groups, which produced higher swelling than VP/MMA hydrogels [36]. The biocompatibility of VP/MMA hydrogel tissue expander was proved through *in vivo* test using rats; the hydrogels swell and reach their equilibrium swelling rate in 6–8 weeks by absorbing body fluids. This long *in vivo* swelling rates not only avoid tissue necrosis but also allow sufficient time for tissue growth rather than stretching of the skin [48]. Although such attempts use another material for tissue expansion, most clinically used hydrogels are still based on VP and MMA copolymers [43, 48–50].

Recently, Varga *et al.* developed thermosensitive hydrogels consisting of NIPAAm as osmotic tissue expanders. A silicate was added to improve the mechanical behavior of the polymer hydrogel. The rate of hydrogel expansion *in vivo* was highest after 2 weeks without the tissue damage. The hydrogel achieved a 25-fold increase in mass. NIPAAm polymers exhibited the most favorable viscoelastic properties, with the highest tendency to retain their preformed shape [37]. Thus, NPIAAm hydrogels allow the acquisition of more skin for reconstructive interventions. However, the current expanders lack the ability to have their shape and size changed at the time of implantation because these hydrogels are glassy and brittle in the dry state. Therefore, there has been a need to develop flexible and elastic tissue expanders so that they can be easily handled or modified appropriately according to each application. Biodegradable elastic PCL/PEG, PLA–PEG–PLA/PEG, and PLGA–PEG–PLGA/PEG hydrogels were developed for this purpose (Figure 9.10).

All the PLGA–PEG–PLGA/PEG hydrogels were flexible and elastic in dried state, and so they remain intact even after repeated bending or stretching. The hydrogels are able to generate sufficient swelling pressure (more than 400 mmHg) to expand tissue. The actual *in vivo* pressures will be substantially lower than this static condition as the skin and mucosa can stretch reducing the pressure. Furthermore, the PLGA–PEG–PLGA/PEG hydrogels exhibited the lag time before swelling; this will provide sufficient time for the wounded area to heal. The controllable degradation rate makes it possible to apply the hydrogels to various parts of body. The elastic hydrogels with self-inflating behavior, elastic, and delayed swelling properties would be useful as novel hydrogel tissue expanders (Figure 9.11).

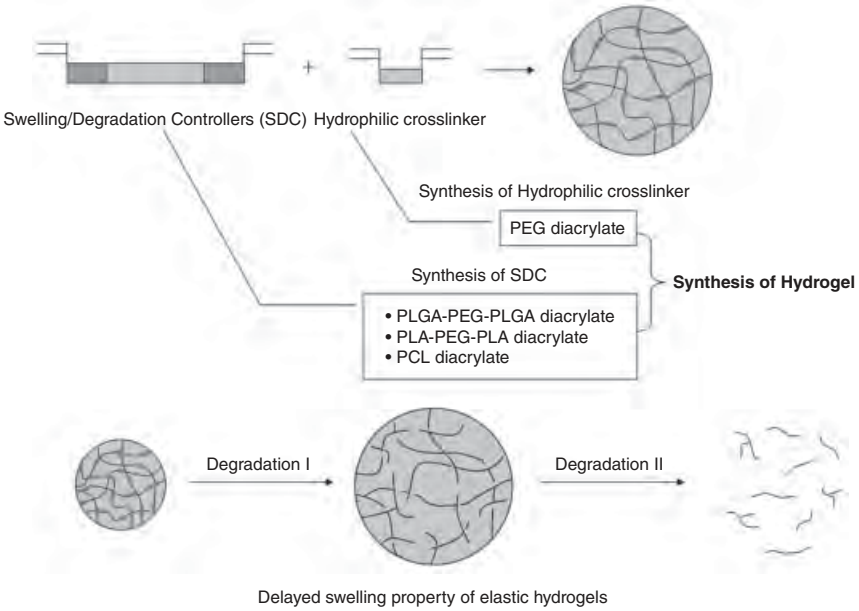


Figure 9.10 Elastic hydrogel tissue expanders with controllable swelling/degradation.

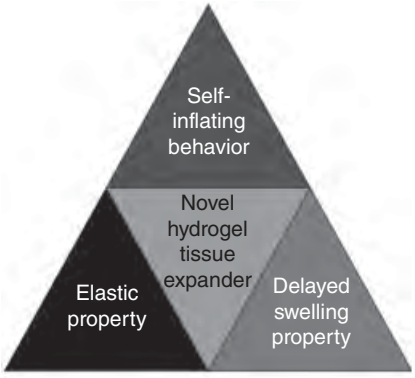


Figure 9.11 A concept of novel hydrogels for tissue expander application.

9.6 Conclusion

Although a number of attractive hydrogel systems are presently available, there are certainly novel systems with improved characteristics. A major concern in the hydrogel development is the mechanical integrity of the systems under modification and processing. Elastic hydrogels can be formed with varying polymer

formulations in three-dimensional patterns. Both chemically and physically crosslinked elastic hydrogels can be rendered biodegradable through the introduction of hydrolytically sensitive groups into the networks. Due to their biocompatibility, biodegradability, and good mechanical properties, biodegradable elastic hydrogels are good candidates as biomaterials for use in medical applications, including tissue engineering. These hydrogels have been used as biodegradable sutures and scaffold materials to engineer various types of tissues in mechanical dynamic environments. Elastic hydrogels with described properties are promising expander candidates which may contribute to more effective harvesting of tissue for reconstructive interventions. However, the synthesis of biodegradable hydrogels with rubberlike elasticity and strength is not easy. Moreover, *in vivo* tests should be done to improve the clinical applicability of elastic hydrogels for tissue expansion as well as other medical applications.

References

1 Hennink, W.E. and Van Nostrum, C.F. (2002) Novel crosslinking methods to design hydrogels. *Adv. Drug Deliv. Rev.*, **54**, 13–36.

2 Kopecek, J. (2007) Hydrogel biomaterials: a smart future. *Biomaterials*, **28**, 5185–5192.

3 Yang, Z., Wang, L., Wang, J., Gao, P., and Xu, B. (2010) Phenyl groups in supramolecular nanofibers confer hydrogels with high elasticity and rapid recovery. *J. Mater. Chem.*, **20**, 2128–2132.

4 Lee, S.H., Kim, B.S., Kim, S.H., Choi, S.W., Jeong, S.I., Kwon, I.K., Kang, S.W., Nikolovski, J., Mooney, D.J., Han, Y.K., and Kim, Y.H. (2003) Elastic biodegradable poly(glycolide-*co*-caprolactone) scaffold for tissue engineering. *J. Biomed. Mater. Res. A*, **66** (**1**), 29–37.

5 Guan, J., Fujimoto, K.L., Sacks, M.S., and Wagner, W.R. (2005) Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. *Biomaterials*, **26**, 3961–3971.

6 Chaterji, S., Kwon, I.K., and Park, K. (2007) Smart polymeric gels: redefining the limits of biomedical devices. *Prog. Polym. Sci.*, **32**, 1083–1122.

7 Kamath, K.R. and Park, K. (1993) Biodegradable hydrogels in drug delivery. *Adv. Drug Deliv. Rev.*, **11**, 59–84.

8 Osada, Y. and Matsuda, A. (1995) Shape memory in hydrogels. *Nature*, **376**, 219.

9 Matsuda, A., Sato, J., Yasunaga, H., and Osada, Y. (1994) Order–disorder transition of a hydrogel containing an *n*-alkyl acrylate. *Macromolecules*, **27**, 7695–7698.

10 Lendlein, A., Schmidt, A.M., and Langer, R. (2001) AB-polymer networks based on oligo(ε-caprolactone) segments showing shape-memory properties. *Proc. Natl. Acad. Sci. USA*, **98** (**3**), 842–847.

11 Lendlein, A. and Langer, R. (2002) Biodegradable, elastic shape-memory polymers for potential biomedical applications. *Science*, **296**, 1673–1676.

12 Choi, N.Y., Kelch, S., and Lendlein, A. (2006) Synthesis, shape-memory functionality and hydrolytical degradation studies on polymer networks from poly(*rac*-lactide)-*b*-poly(propylene oxide)-*b*-poly(*rac*-lactide) dimethacrylate. *Adv. Eng. Mater.*, **8** (**5**), 439–445.

13 Zheng, X., Zhou, S., Li, X., and Weng, J. (2006) Shape memory properties of poly(D,L-lactide)/hydroxyapatite composite. *Biomaterials*, **27**, 4288–4295.

14 Omidian, H., Rocca, J.G., and Park, K. (2006) Elastic, superporous hydrogel hybrids of polyacrylamide and sodium alginate. *Macromol. Biosci.*, **6**, 703–710.

15 Qui, Y. and Park, K. (2003) Superporous IPN hydrogels having enhanced

mechanical properties. *AAPS Pharm. Sci. Technol.*, **4** (**4**), 406–412.
16 Zhang, C., Zhang, N., and Wen, X. (2006) Improving the elasticity and cytophilicity of biodegradable polyurethane by changing chain extender. *J. Biomed. Mater. Res. Part B Appl. Biomater.*, **79** (**2**), 335–344.
17 Zhang, C., Zhang, N., and Wen, X. (2007) Synthesis and characterization of biocompatible, degradable, light-curable, polyurethane-based elastic hydrogels. *J. Biomed. Mater. Res. A*, **82** (**3**), 637–650.
18 Zhou, S., Deng, X., and Yang, H. (2003) Biodegradable poly(ε-caprolactone)-poly(ethylene glycol) block copolymers: characterization and their use as drug carriers for a drug carriers for a controlled delivery system. *Biomaterials*, **24**, 3563–3570.
19 Aamer, K.A., Sardinha, H., Bhatia, S.R., and Tew, G.N. (2004) Rheological studies of PLLA. PEO. PLLA triblock copolymer hydrogels. *Biomaterials*, **25**, 1087–1093.
20 Moth, C.G., Drumond, W.S., and Wang, S.H. (2006) Phase behavior of biodegradable amphiphilic poly(*l,l*-lactide)-*b*-poly(ethylene glycol)-*b*-poly(*l,l*-lactide). *Thermochim. Acta*, **445**, 61–66.
21 Chen, S., Pieper, R., Webster, D.C., and Singh, J. (2005) Triblock copolymers: synthesis, characterization, and delivery of a model protein. *Int. J. Pharm.*, **288**, 207–218.
22 Duvvuri, S., Janoria, K.G., and Mitra, A.K. (2005) Development of a novel formulation containing poly(*d,l*-lactide-*co*-glycolide) microspheres dispersed in PLGA. PEG. PLGA gel for sustained delivery of ganciclovir. *J. Control. Release*, **108**, 282–293.
23 Qiao, M., Chen, D., Ma, X., and Liu, Y. (2005) Injectable biodegradable temperature responsive PLGA–PEG–PLGA copolymers: synthesis and effect of copolymer composition on the drug release from the copolymer-based hydrogels. *Int. J. Pharm.*, **294**, 103–112.
24 Im, S.J., Choi, Y.M., Subramanyam, E., and Huh, K.M. (2007) Synthesis and characterization of biodegradable elastic hydrogels based on poly(ethylene glycol) and poly(ε-caprolactone) blocks. *Macromol. Res.*, **15** (**4**), 363–369.
25 Jiang, G., Liu, C., Liu, X., Zhang, G., Yang, M., Chen, Q., and Liu, F. (2010) Self-healing mechanism behavior of hydrophobic association hydrogels with high mechanical strength. *J. Macromol. Sci. Part A: Pure Appl. Chem.*, **47**, 335–342.
26 Bayramov, D.F., Singh, P., Cleary, G.W., Siegel, R.A., Chalykh, A.E., and Feldstein, M.M. (2008) Non-covalently crosslinked hydrogels displaying a unique combination of water-absorbing, elastic and adhesive properties. *Polym. Int.*, **57**, 785–790.
27 Jung, Y., Kim, S.H., You, H.J., Kim, S.H., Kim, Y.H., and Min, B.G. (2008) Application of an elastic biodegradable poly(L-lactide-*co*-ε-caprolactone) scaffold for cartilage tissue regeneration. *J. Biomater. Sci. Polym. Ed.*, **19** (**8**), 1073–1085.
28 Zhu, M., Liu, Y., Sun, B., Zhang, W., Liu, X., Yu, H., Zhang, Y., Kuckling, D., and Adler, H.J.P. (2006) A novel highly resilient nanocomposite hydrogel with low hysteresis and ultrahigh elongation. *Macromol. Rapid Commun.*, **27**, 1023–1028.
29 Park, K., Shalaby, W.S.W., and Park, H. (1993) Physical gels, in *Biodegradable Hydrogels for Drug Delivery*. Technomic Publishing Co., Inc., Lancaster, PA, pp. 99–129.
30 Bae, Y.H., Huh, K.M., Kim, Y., and Park, K.H. (2000) Biodegradable amphiphilic multiblock copolymers and their implication for biomedical applications. *J. Control. Release*, **64**, 3–13.
31 Zhang, X., Guo, X., Yang, S., Tan, S., Li, X., Dai, H., Yu, X., Zhang, X., Weng, N., Jian, B., and Xu, J. (2009) Double-network hydrogel with high mechanical strength prepared from two biocompatible polymers. *J. Appl. Polym. Sci.*, **112**, 3063–3070.
32 Huh, K.M. and Bae, Y.H. (1999) Synthesis and characterization of poly(ethylene glycol)/poly(L-lactic acid) alternating multiblock copolymers. *Polymer*, **40**, 6147–6155.
33 Hoare, T.R. and Kohane, D.S. (2008) Hydrogels in drug delivery: progress and challenges. *Polymer*, **49**, 1993–2007.

34 Van Thienen, T.G., Horkay, F., Braeckmans, K., Stubbe, B.G., Demeester, J., and De Smedt, S.C. (2007) Influence of free chains on the swelling pressure of PEG-HEMA and dex-HEMA hydrogels. *Int. J. Pharm.*, **337**, 31–39.

35 Stubbe, B.G., Hennink, W.E., De Smedt, S.C., and Demeester, J. (2004) Swelling pressure of hydrogels that degrade through different mechanisms. *Macromolecules*, **37**, 8739–8744.

36 Wiese, K.G., Heinemann, D.E.H., Ostermeier, D., and Peters, J.H. (2001) Biomaterial properties and biocompatibility in cell culture of a novel self-inflating hydrogel tissue expander. *J. Biomed. Mater. Res.*, **54** (**2**), 179–188.

37 Varga, J., Janovak, L., Varga, E., Eros, G., Dekany, I., and Kemeny, L. (2009) Acrylamide, acrylic acid and *N*-ispropylacrylamide hydrogels as osmotic tissue expander. *Skin Pharmacol. Physiol.*, **22**, 305–312.

38 Min, Z., Svensson, H., and Svedman, P. (1988) On expander pressure and skin blood flow during tissue expansion in the pig. *Ann. Plast. Surg.*, **21**, 134–139.

39 Park, J.S., Woo, D.G., Sun, B.K., Chung, H.M., Im, S.J., Choi, Y.M., Park, K., Huh, K.M., and Park, K.H. (2007) *In vitro* and *in vivo* test of PEG/PCL-based hydrogel scaffold for cell delivery application. *J. Control. Release*, **124**, 51–59.

40 Kim, B.S., Nikolovski, J., Bonadio, J., and Mooney, D.J. (1999) Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nat. Biotechnol.*, **17**, 979–983.

41 Kim, S.H., Kwon, J.H., Chung, M.S., Chung, E., Jung, Y., Kim, S.H., and Kim, Y.H. (2006) Fabrication of a new tubular fibrous PLCL scaffold for vascular tissue engineering. *J. Biomater. Sci. Polym. Ed.*, **17** (**12**), 1359–1374.

42 Liu, C., Qin, H., and Mather, P.T. (2007) Review of progress in shape-memory polymers. *J. Mater. Chem.*, **17**, 1543–1558.

43 Kobus, K.F. (2007) Cleft palate repair with the use of osmotic expanders: a preliminary report. *J. Plast. Reconstr. Aesthet. Surg.*, **60**, 414–421.

44 Mazzoli, R.A., Raymond, W.R., Ainbinder, D.J., and Hansen, E.A. (2004) Use of self-expanding, hydrophilic osmotic expanders (hydrogel) in the reconstruction of congenital clinical anophthalmos. *Curr. Opin. Ophthalmol.*, **15**, 426–431.

45 Austad, E.D. and Rose, G.L. (1982) A self-inflating tissue expander. *Plast. Reconstr. Surg.*, **70**, 588–593.

46 Wiese, K.G. (1993) Osmotically induced tissue expansion with hydrogels: a new dimension in tissue expansion: a preliminary report. *J. Craniomaxillofac. Surg.*, **21**, 309–313.

47 Downes, R., Lavin, M., and Collin, R. (1992) Hydrophilic expanders for the congenital anophthalmic socket. *Adv. Ophthalmic Plast. Reconstr. Surg.*, **9**, 57–61.

48 Swan, M.C., Tim, E.E., Goodacre, J., Czernuszka, T., and David, G.B. (2008) Cleft palate repair with the use of osmotic expanders: a response. *J. Plast. Reconstr. Aesthet. Surg.*, **61**, 220–221.

49 Ronert, M.A., Hofheinz, H., Manassa, E., Asgarouladi, H., and Olbrisch, R.R. (2004) The beginning of a new era in tissue expansion: self-filling osmotic tissue expander–four-year clinical experience. *Plast. Reconstr. Surg.*, **114** (**5**), 1025–1031.

50 Von See, C., Gellrich, N.C., Jachmann, U., Bormann, H., and Rucker, M. (2010) Bone augmentation after soft-tissue expansion using hydrogel expanders: effects on microcirculation and osseointegration. *Clin. Oral Implants Res.*, **21** (**8**), 842–847.

10
Biodegradable Dendrimers and Dendritic Polymers

Jayant Khandare and Sanjay Kumar

10.1
Introduction

The concept of using a polymer as a carrier for drug delivery system originated from the hypothesis that macromolecules could be used to improve the solubility and half-life of small molecule drugs [1, 2]. Later, it was observed that macromolecules functionalized with a drug in the form of prodrug impart added advantage by increasing accumulation in tumor tissues due to the leaky vasculature, now a concept recognized as *enhanced permeation and retention* effect [3, 4]. It has been clearly demonstrated that the macromolecular carriers have immense potential to enhance pharmacokinetics, leading to enhance the efficacy of small molecule drugs. Several carrier systems have been studied (viz., linear polymers, micellar assemblies, liposomes, polymersomes, and dendrimers) and are observed to have most of the properties required for ideal drug carrier [5]. Thus, it is not surprising that the ideal drug carrier would facilitate long blood circulation time, high accumulation in tumor tissue, high drug loading, lower toxicity, and simplicity in preparation. Within the milieu of nanocarriers, dendrimers represent a fascinating platform because of their nanosize, monodisperisty, and degree of branching to facilitate the multiple attachments of both drugs and solubilizing groups [6].

Dendrimers are excellent candidates for providing a well-defined molecular architecture, which is a result of a stepwise synthetic procedure consisting of coupling and activation steps [7]. They consist of branched, wedge-like structures called dendrons that are attached to a multivalent core, and emerge readily toward the periphery. The architecture and synthetic routes result in highly defined dendritic structure with polydispersity index near 1.00, as opposed to the much higher polydispersity of linear or hyperbranched structures [5]. The flexibility to tailor both the core and surface of these systems create them innovative *nanovehicle*, since different groups can be provided so as to optimize the properties of drug carrier. For instance, the functional periphery is one of the intriguing properties of dendritic architecture with extensive number of end groups that may be modified to afford dendrimers with tailored chemical and physical properties [8, 9]. The general

Handbook of Biodegradable Polymers: Synthesis, Characterization and Applications, First Edition. Edited by Andreas Lendlein, Adam Sisson.

methods of synthesizing dendrimers are classified into (i) convergent and (ii) divergent approaches. The synthesis process involves repetitive coupling and activation steps, which makes it difficult to obtain dendrimers in high yield, at reasonable cost. These barriers have definitely limited the application of dendrimers primarily in biomedical field [7].

Dendrimers differentiate themselves largely from hyperbranched polymers in terms of their controlled size and shapes as well as narrow polydispersity [9]. Conversely, in linear polymers, the influence of end groups on physical properties such as solubility and thermal behavior is negligible at infinite molecular weight. However, in dendritic polymers, the situation is quite different. The fraction of end groups approaches a final and constant high value at infinite molecular weight, and therefore, the nature of the end groups is expected to strongly influence both the solution and the thermal properties of a dendrimer [10]. An explosion of interest has been fueled due to chemicophysical properties in dendritic macromolecules to be versatile nanomaterials, such as peripheral reactive end groups, viscosity, or thermal behavior, and differ significantly from those of linear polymers [11]. Till date, a variety of hyperbranched dendrimers and their polymeric architectures (e.g., polyglycerol (PG) dendrimers) have been implicated for diverse applications in the form of drug encapsulation, catalysis, and polymerization initiators [12–14].

This chapter highlights an overview on biodegradable dendrimers. More specifically, design of biodegradable dendritic architectures has been discussed keeping focus on challenges in designing such dendrimers; their relation of biodegradability and biocompatibility, and its biological implications.

Tomalia and Newkome *et al*. introduced well-defined and highly branched dendrimers [5, 15], and almost a decade later, the first form of biodegradable dendrimer was simultaneously published by various groups [16–18]. Groot *et al*. reported a biodegradable form of dendrimers that have been built to completely and rapidly dissociate into separate building blocks upon a single triggering event in the dendritic core [17]. These dendrimers collapse into their separate monomeric building blocks after single (chemical or biological) activation step that triggers a cascade of self-elimination reactions, thereby releasing the entire end groups from the periphery of the "exploding" cascade-release dendrimer. Thus, such multiple-releasing dendritic systems have been termed as "cascade-releasing dendrimers." The degrading dendritic system possesses two major advantages over the conventional dendrimers: (i) multiple covalently bound drug molecules can be site-specifically released from the targeting moiety by a single cleaving step, and (ii) they are selectively as well as completely degraded and therefore can be easily drained from the body [17].

Fascinatingly, Suzlai *et al*. demonstrated that the linear dendrimer could undergo *self-fragmentation* through a cascade of cleavage reactions initiated by a single triggering event [18]. The degradation of dendrimer cleavage eventually leads to two subsequent fragmentations per subunit, or geometric dendrimer disassembly. Overall, the concept of "dendritic amplification" was disclosed, in which an initial stimulus triggers the efficient disassembly of a dendrimer resulting in the ampli-

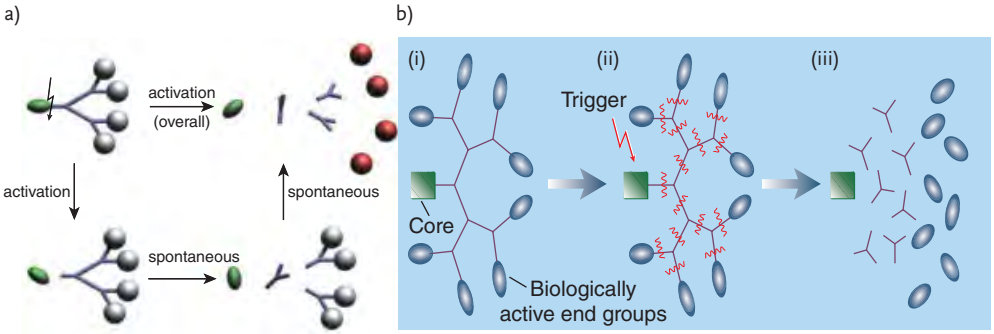


Figure 10.1 (a) Single activation of a second-generation cascade-releasing dendrimer triggers a cascade of self-eliminations and induces release of all end groups. Covalently bound end groups are depicted in gray, branched self-elimination linkers in blue, and the specified in green. The released end groups are depicted in red [17]. (b) Schematic of simultaneous release of biologically active end groups from trigger-tuned dendrimer: (i) dendrimer consists of two-dimensional part of a sphere, (ii) dendrimer is triggered with a specific signal so that the dendrimer scaffold falls apart in a chain of reactions, and (iii) the net result is observed with release of all molecules, including the end groups. In the experiments of de Groot *et al.*, the end groups represented are the anticancer drug paclitaxel [17, 19].

fication of a certain property or quality of a system due to the large increase in molecular species (dendrimer fragments) [18].

Degradable dendritic architectures mainly consist of the following classes:

1) dendrimers with degradable backbones (pH labile, enzymatic hydrolysis, etc.),
2) dendritic cores with cleavable shells (pH environment), and
3) cleavable dendritic prodrug forms.

Typically, the dendritic skeleton can be degraded or hydrolyzed based on environmental or external stimuli, for example, pH, hydrolysis, or by enzymatic degradation. Meijer and van Genderen reported that the dendrimer skeleton can be constructed in such a way that it can disintegrate into known molecular fragments once the disintegration process has been initiated (Figure 10.1a and b) [17, 19]. The dendrimers scaffold can fall apart in several steps in a chain reaction, releasing all of its constituent molecules by a single trigger. This has been demonstrated by de Groot *et al.* to achieve the release of the anticancer drug paclitaxel. Furthermore, the by-products of dendrimers degradation have proven to be noncytotoxic except for the drug paclitaxel itself [17]. The simultaneous release of biologically active end groups from a trigger-tuned dendrimer is represented in Figure 10.1. With single activation of a second generation, cascade-releasing dendrimer can trigger a cascade of self-eliminations and induces release of all end groups (Figure 10.1a). On the other hand, other forms of dendrimers can be triggered by a specific signal, and the dendrimer scaffold can fall apart in a chain of reactions. Notably, the first reaction activates the dendrimer's core, thereby

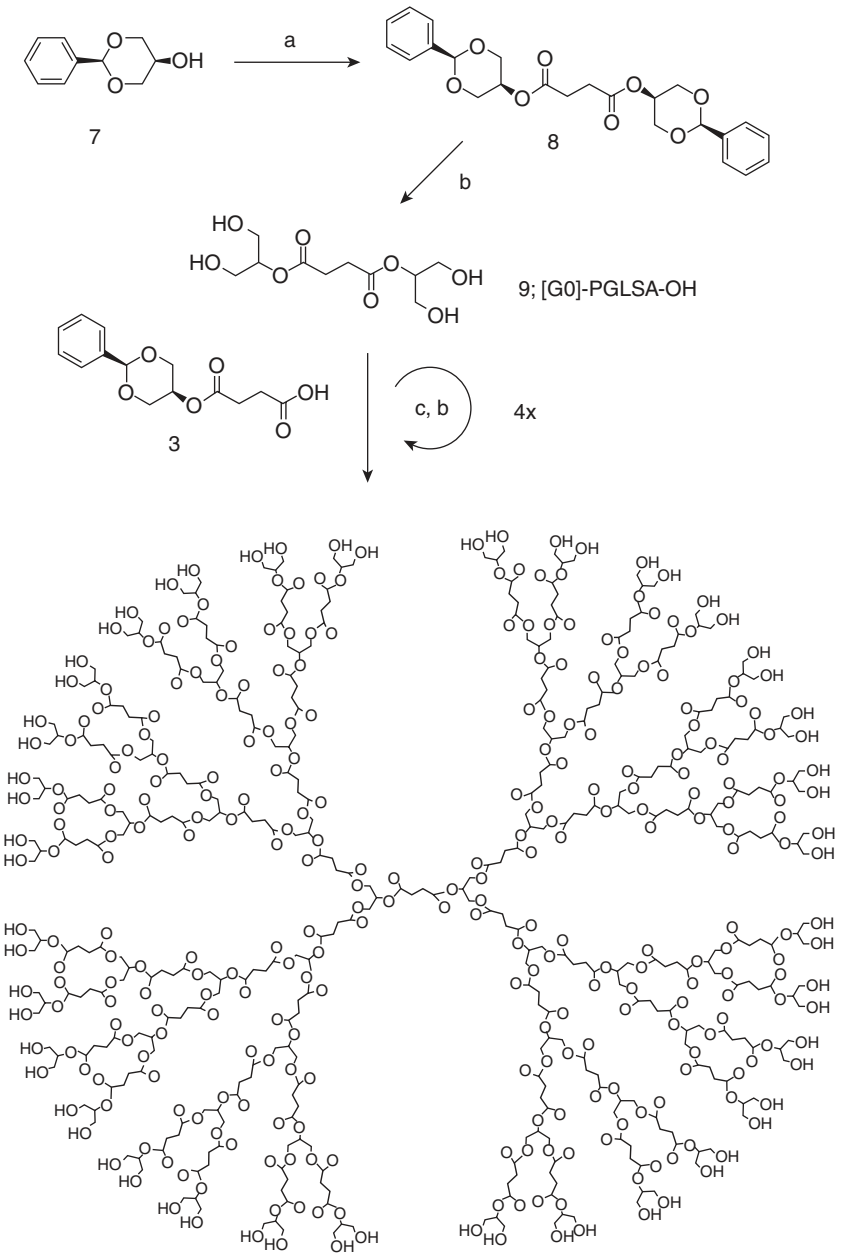
initiating a cascade of "elimination" reactions leading to release of drug molecules (Figure 10.1b). The dendritic forms with many identical units mean that amplification can be achieved as a kind of explosion. However, there could be a possible drawback since if such a system is activated at the wrong time or place, the result could be devastating [17]. The details of design and synthesis of such degradable scaffolds have been discussed in the text below.

Several biodegradable polymers, dendrimers, and their prodrugs have been widely used as drug carriers [20, 21]. Recently, dendrimer carriers based on polyethers, polyesters, polyamides, melanamines or triazines, and several polyamides have been explored extensively [13, 22, 23]. Other forms, for example, dendritic polyglycerols (dPGs) are structurally defined, consisting of an aliphatic polyether backbone, and possessing multiple functional end groups [14, 24]. Since dPGs are synthesized in a controlled manner to obtain definite molecular weight and narrow molecular polydispersity, they have been evaluated for a variety of biomedical applications [25]. Hyperbranched PG analogs have similar properties as perfect dendritic structures with the added advantage of defined mono- and multifunctionalization [13, 14]. Additionally, Sisson *et al*. demonstrated PGs functionalized by emulsification method to create larger micogel structures emphasized for drug delivery [26]. Among plethora of dendritic carriers, polyester dendrimers represent an attractive class of nanomaterials due to their biodegradability trait; however, the synthesis of these nanocarriers is challenging because of the hydrolytic susceptibility of the ester bond [27, 28]. In contrast, polyamide- and polyamine-based dendrimers could withstand much wider selection of synthetic manipulations, but they do not degrade as easily in the body and thus they may be more prone to long-term accumulation *in vivo*.

Grinstaff recently described biodendrimers comprising biocompatible monomers [21] using natural metabolites, chemical intermediates, and monomers of medical-grade linear polymers. Interestingly, these dendritic macromolecules (e.g., poly(glycerol-succinic acid) dendrimer) (PGLSA) are foreseen to degrade *in vivo* (Scheme 10.1). Furthermore, these dendrimers have been tuned for degradation rate and the degradation mechanism for future *in vivo* applications.

10.2
Challenges for Designing Biodegradable Dendrimers

Biological applications of dendrimers have paved far ahead, comparatively over to its newer forms of core designs-exhibiting biodegradability. As a consequence to obtain a universal biodegradable, yet highly aqueous soluble and unimolecular dendrtic carrier capable of achieving high drug pay loading remains to be an unmet challenge. The greater aspect is to limit the early hydrolysis of the polymeric chains at the core compared to the periphery. Therefore, the prime objective remains to design biodegradable dendrimers having precise branching, molecular weight, monodispersity, and stable multiple functional appendages for covalent attachment of the bioactives.



Scheme 10.1 Divergent synthetic method for G4-PGLSA-OH biodendrimer (**10**): (a) succinic acid, DPTS, DCC, CH_2Cl_2, 25 °C, 14 h; (b) 50 psi H_2, $Pd(OH)_2/C$, THF, 25 °C, 10 h; (c) 3, DPTS, DCC, THF, 25 °C, 14 h. 3 (2-(*cis*-1,3-*O*-benzylidene-glycerol) succinic acid mono ester) *cis*-1,3-*O*-benzylideneglycerol (**7**), 4-(dimethylamino) pyridinium 4-toluenesulfonate (DPTS) (**8**) [21].

It has been realized that the hydrolysis rate of polyester dendrimers dramatically depends on the hydrophobicity of the monomer, repeating units, steric environment, and the reactivity of the functional groups located within the dendrimer. Independently of one another, teams led by de Groot, Shabat, and McGrath have explored a much more advanced concept – the simultaneous release of all of dendrimer's functional groups by a single chemical trigger [16–18]. All three researchers presented that the dendrimer skeleton can be constructed to disintegrate into the known molecular fragments, once the disintegration process has been initiated. Now they have been variously termed as "cascade-release dendrimers," "dendrimer disassembly," and colorfully "self-immolative dendrimers" (SIDs), effective to perform chemical amplification reactions. Triggered by a specific chemical signal, the dendrimer scaffold can fall apart in several steps in a chain reaction, releasing all of the constituent molecules [16–18].

Szalai *et al*. [18] have reported a small dendrimer that can be disassembled geometrically by a single chemical trigger leading to two subsequent fragmentations in each subunit and completely reducing the polymer back to its monomers. The authors described dendrimers with 2,4-bis(hydroxymethyl)phenol repeat units capable of geometric disassembly of the corresponding anionic phenoxide species having labile vinylogous hemiacetals. With removal of the trigger group from 2,4-bis-(hydroxymethyl)phenol-based dendrimer subunit resulted in the formation of an *o*,*p*-bis(benzyl ether)phenoxide. The phenoxide – a bis(vinylogous hemiacetal) anion – cleaves to liberate alkoxide and *p*-quinone methide, which are trapped by an appropriate nucleophile under the reaction conditions, consistent with the electrophilic nature of quinine methides. The resulting phenoxide further cleaves to liberate a second equivalent of alkoxide and *o*-quinone methide, in turn trapped by the nucleophile to yield a fully cleaved phenoxide. The authors suggest that if alkoxide was analogous in structure to phenoxide, then the subsequent cleavages could occur, resulting in a geometric fragmentation through a dendrimer. Such unique dendrons are build with a core of 2,4-bis(hydroxymethyl)phenol units. The removal of a carbocation creates a phenoxide that could be cleaved and liberates two alkoxide groups in the presence of a suitable nucleophile. Small dendrimers with nitrophenoxy reporter groups and a single "trigger" group exhibit that second-generation dendrimers can be disassembled in under a minute time. If such process can be extended to higher generation dendrimers, it could be widely used to release drug molecules, in a complex form between the arms of the dendrimer vehicle [29].

The focus on biodegradable dendrimers could offer numerous advantages in biology compared to its nondegradable counterparts. Toward this direction, different biodegradable dendritic architectures have been designed. For example, SIDs have been designed possessing the capability to release all of their tail units through a self-immolative chain fragmentation. The trigger is initiated by a single cleavage event at the dendrimer's core [29]. The authors have hypothesized that by incorporation of drug molecules as tail units and an enzyme substrate as the trigger, multiprodrug units can be generated that could be activated on a single enzymatic cleavage. Such kind of biodegradable dendritic forms can be used to

achieve targeted drug delivery. Another key challenge with polymeric and dendritic prodrug forms has been to achieve the complete elimination of these macromolecules from the body. More precisely, SIDs are reported to be excreted easily from the body due to their complete biodegradability [29]. Furthermore, the advantage of cleavage effect in SIDs with tumor-associated enzyme or a targeted one could be amplified and therefore may increase the number of active drug molecules in targeted tumor tissues.

The conventional method has been to attach covalently bioactive molecules to dendritic scaffolds by controlling the loading and release of active species. Chemical conjugation to a dendritic scaffold allows covalent attachment of different kinds of active molecules (imaging agents, drugs, targeting moieties, or biocompatible molecules) in a controlled ratio [14, 21, 23]. The loading as well as the release can be tuned by incorporating cleavable bonds that can be degraded under specific conditions present at the site of action (endogeneous stimuli, e.g., acidic pH, overexpression of specific enzymes, or reductive conditions as well as exogeneous stimuli, e.g., light, salt concentration, or electrochemical potential). In a recent report, Calderon *et al*. reported the use of the thiolated PG scaffold for conjugation to maleimide-bearing prodrugs of doxorubicin (DOX) or methotrexate (MTX) which incorporate either a self-immolative para-aminobenzyloxycarbonyl spacer coupled to dipeptide Phe–Lys or the tripeptide D-Ala–Phe–Lys as the protease substrate [30]. Both prodrugs were cleaved by cathepsin B, an enzyme overexpressed by several solid tumors, to release DOX or an MTX lysine derivate. An effective cleavage of PG–Phe–Lys–DOX and PG–D-Ala–Phe–Lys–Lys–MTX and release of DOX and MTX–lysine in the presence of the enzyme was observed.

Another challenge in dendritic or polymeric platforms is to tune the pharmacokinteics and extend the ability of a macromolecule to carry multiple copies of bioactive compounds [31]. This can be achieved by designing PEGylated dendrimers, which can circumvent the synthetic and biological limitations [27]. The polymeric architecture can be designed to avoid the destructive side reactions during dendrimer preparation while maintaining the biodegradability. Here, in this chapter, we highlight dendrimers with biodegradable characteristic in the presence of a suitable environment (e.g., pH). Chemical synthetic approaches have been discussed in detail, limited for their biodegradation and their biological implications.

10.2.1
Is Biodegradation a Critical Measure of Biocompatibility?

In the past, many polymers have been proven clinically safe. For example, PEG and PLGA polymers are being routinely used in delivering anticancer bioactives [23]. However, newer polymeric forms, which are currently being used in the biomedical field, are inherently heterogeneous in their structures, wherein the individual molecules have different chain lengths, due to their intrinsic polydispersed nature [8]. Therefore, their biodegradation profile is a crucial measure since the heterogeneous traits can substantially increase undesired effects on the

biological activities, since it is not clear which part of the polymers with heterogeneous molecular weights is predominantly responsible for producing the undesired effect [32]. In order to minimize the heterogeneity, novel synthetic methods have to be employed for the preparation of polymers, and dendrimers for overcoming this heterogeneity, with the potential advantages of unimolecular homogeneity and defined chemical structures [33].

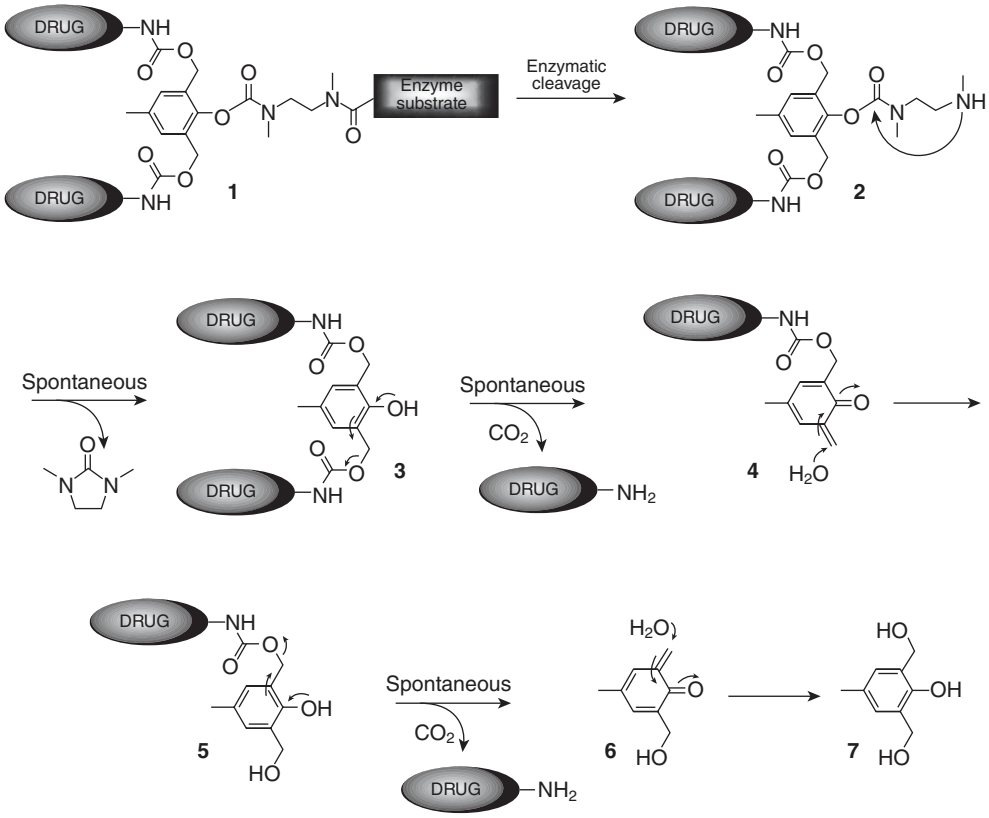
There have been numerous limitations to use poly(amidoamine) (PAMAM) dendrimers for biomedical applications due to their nonbiodegrading traits. Nevertheless, these polymers have shown to be biocompatible and can be easily prepared with various surface functionalities, such as $-NH_2$, –COOH, and –OH groups, and are commercially available up to generation 10 (G10) [7]. Even though most applications of PAMAM are studied *in vitro*, a wide range of biomedical applications has been proposed in the fields of gene delivery [34], anticancer chemotherapy [35], diagnostics [36], and drug delivery [37, 38]. The cytotoxicity of PAMAM dendrimers is difficult to generalize and depends on their surface functionality, dose, and the generation of the dendrimers; however, the nonbiodegradable nature of PAMAM is one of the reasons for its toxicity [39]. Toward this end, more insights were recently described by Khandare *et al*. with respect to the structure–biocompatibility relationship of dPG derivatives possessing neutral, cationic, and anionic charges [40]. *In vitro* toxicity for various forms of dPGs was reported and compared with PAMAM dendrimers, polyethyleneimine (PEI), dextran, and linear polyethylene glycol (PEG) using human hematopoietic cell line U-937. It has been reported that dPGs possess greater cell compatibility similar to linear PEG polymers and dextran, and is therefore suitable for developing sysmetic formulation in therapeutics [40].

Polymeric and dendritic carrier systems are expected to possess suitable physicochemical properties for improved bioavailability, cellular dynamics, and targetability [23]. This is particularly true if the polymeric architectures have high surface charge, molecular weight, and a tendency to interact with biomacromolecules in blood due to their surface properties [40]. Most of the hyperbranched polymeric architectures consisting of bioactive therapeutic agents are administered by a systemic route. Therefore, their fate in blood and interactions with the plasma proteins and immune response are very critical to establish the overall biocompatibility. Studies in this direction have established the molecular and physiological interactions of the dendritic polymers with plasma components [41].

Conclusively, biodegradable dendrimers and its other architectures ideally should possess the following traits: (i) nontoxic, (ii) nonimmunogenic, and (iii) preferably be biocompatible and biodegradable. In this last instance, one of the potential virtues of dendrimers other than biodegradability comes under the heading of "multivalency"–the enhanced effect that stems from lots of identical molecules being present at the same time and place. Such simultaneous combination of multivalency and biodegradability with precision architectures can make dendrimers a greater versatile platform with many interesting biomedical applications, not least for the drug delivery [42].

10.3
Design of Self-Immolative Biodegradable Dendrimers

Polymeric forms of prodrugs have been designed and synthesized for achieving targetability in malignant tissues, due to overexpression of specific molecular receptive targets [43, 44]. The release of the free drug by a specific enzyme is very crucial for the cleavage of a prodrug-protecting group. Although many dendritic prodrugs have been designed to target the cancer, only few biodegradable approaches have been explored till date [16–19, 27, 45]. Toward this end, SIDs have been lately synthesized, which may open new opportunities for targeted drug delivery. In contrast to conventional dendrimers, SIDs are fully degradable and can be excreted easily from the body [29]. Since the dendrimer are multi-immolative, this effect may increase the number of active drug molecules in targeted tumor tissues. SID dendritic building units are conceptualized on 2,6-bis-(hydroxymethyl)-*p*-cresol (**7**), which has three functional groups (Scheme 10.2).



Scheme 10.2 Mechanism of dimeric prodrug activation by a single enzymatic cleavage [29].

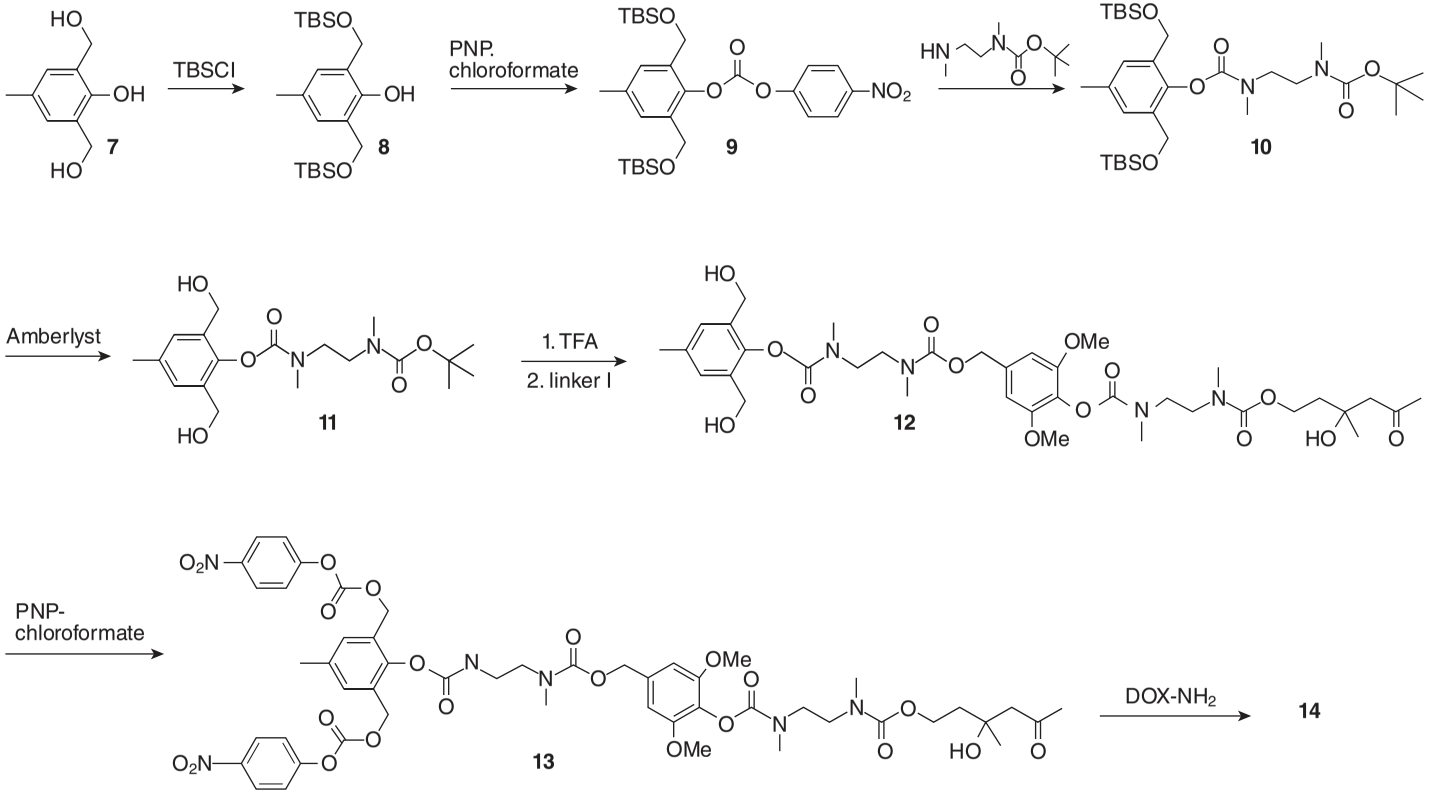
Two hydroxybenzyl groups were attached through a carbamate linkage to drug molecules, and a phenol functionality was conjugated to a trigger by using *N,N*-dimethylethylenediamine (compound **1**) as a short spacer molecule. The cleavage of the trigger is initiative for the self-immolative reaction, starting with a spontaneous cyclization of amine intermediate **2**, to form an *N,N′*-dimethylurea derivative. On the other hand, the generated phenol **3** undergoes a 1,4-quinone methide rearrangement followed by a spontaneous decarboxylation to liberate one of the drug molecules. Similarly, the quinone methide species **4** is rapidly trapped by a water molecule to form a phenol (compound **5**), which further undergoes an 1,4-quinone methide rearrangement to liberate the second drug entity. Furthermore, the quinone methide-generated species **6** is once again trapped by a water molecule to form **7**. Thus, compound **7** is reacted with **2** equivalent of (TBS)Cl to afford phenol **8**, which is acylated with *p*-nitrophenyl (PNP) chloroformate to form carbonate **9** (Scheme 10.3). The latter is reacted with mono-Boc-*N,N′*-dimethylethylenediamine to generate compound **10**, which is deprotected in the presence of Amberlyst-15 to give diol **11**. Later, the deprotection with trifluroacetic acid (TFA) afforded an amine salt, which is reacted *in situ* with linker I (activated form of antibody 38C2 substrate) to generate compound **12** [29].

Thereafter, the latter was reacted with **2** equivalent of DOX to obtain a prodrug **14**. Acylation of diol **11** with **2** equivalent of PNP chloroformate resulted in compound dicarbonate **15**, which is reacted with **2** equivalent of camptothecin amine units to give compound **16** (Scheme 10.4). Deprotection with TFA resulted in an amine salt, which is reacted *in situ* with linker II to yield prodrug **17**. The authors selected the anticancer drug DOX and catalytic antibody 38C2 [46] as the activating enzyme. Antibody 38C2 catalyzes a sequence of retro-aldol retro-Michael cleavage reactions, using substrates that are not recognized by human enzymes.

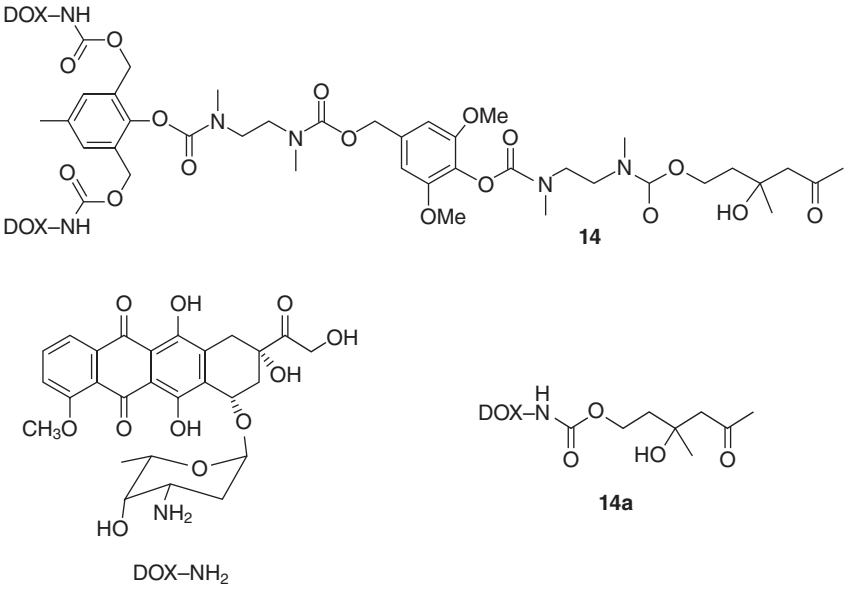
Prodrugs of this kind can demonstrate slight toxicity increased over activation of monomeric prodrugs. Both monomeric and dimeric prodrugs showed chemical stability in the cell medium. *In vitro* and *in vivo* efficacy of the dendritic conjugates was demonstrated by activating several prodrugs. Figures 10.2 and 10.3 represent *in vitro* activity of these polymers and have been detailed in later section.

10.3.1
Clevable Shells – Multivalent PEGylated Dendrimer for Prolonged Circulation

The unique structural properties of dendrimers increasingly entice scientists to use them for many biomedical applications [9–11, 14, 19, 47]. In particular, biodegradable and disassembled dendritic molecules have been attracting growing attention [16–19]. Toward this direction, anticancer prodrugs of DOX PEGylated dendrimers have been designed for the selective activation in malignant tissues by a specific enzyme, which is targeted or secreted near tumor cells [48]. In recent studies, a family of polyestercore dendrimers based on a 2,2-bis(hydroxymethyl) propanoic acid (bis-HMPA) monomer unit, functionalized in the form of shells with eight 5 kDa PEG chains [27], was shown to be biocompatible and capable of high drug loading while facilitating high tumor accumulation through its long



Scheme 10.3 Synthesis of the prodrug of DOX with a trigger which can be activated with catalytic antibody 38C2 [29].



Scheme 10.4 Chemical structure of DOX prodrugs **14** and **14a** [29].

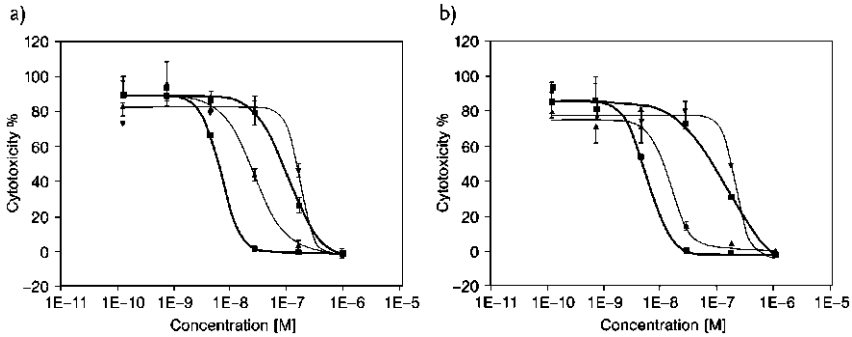


Figure 10.2 Growth inhibition assay of the human Molt-3 leukemia cell line, with addition of prodrugs in the presence and absence of catalytic antibody 38C2 (cells were incubated for 72 h): (a) (9) DOX, (b) pro-DOX **14**a, (2) pro-DOX **14**a + 1 μM 38C2, (1) solvent control; (b) (9) DOX, (b) pro-DOX **14**, (2) pro-DOX **14** + 1 μM 38C2, (1) solvent control [29].

circulation half-life. Polyester dendrimers based on bis(HMPA) monomer units have attracted a lot of attention as they are nonimmunogenic, biodegradable, and nontoxic in nature. Scheme 10.5 describes the synthesis of a core-functionalized PEGylated dendrimer [27]. In brief, the tetrafunctional pentaerythritol core **1** was tailored by benzylidene-protected bis(HMPA) monomer **2** to yield generation **1** dendrimer **3**. The protecting groups were removed by hydrogenolysis, and periph-

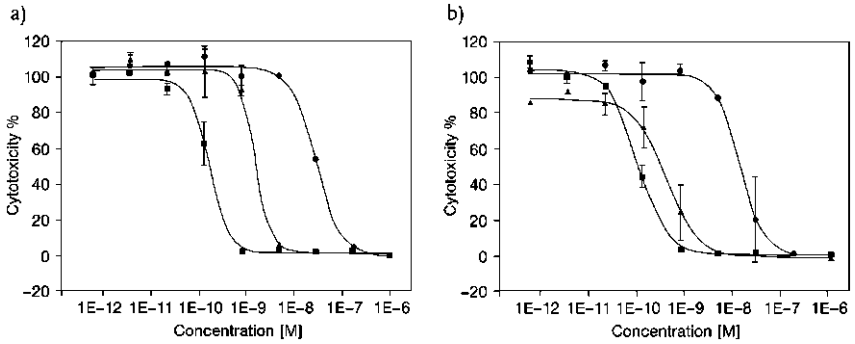
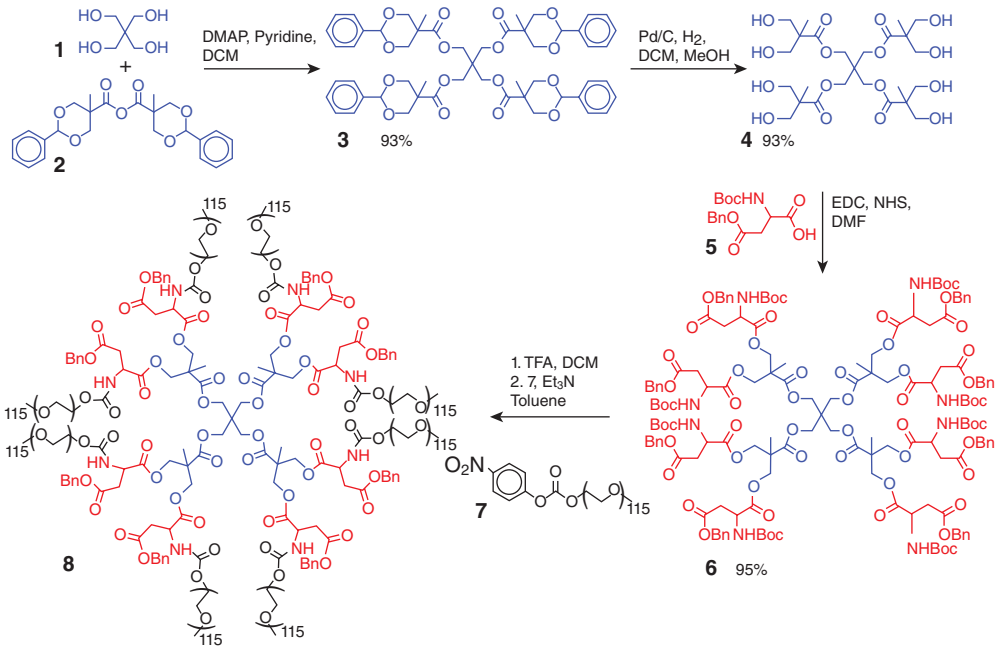


Figure 10.3 Growth inhibition assay of the human Molt-3 leukemia cell line, with prodrugs in the presence and absence of catalytic antibody 38C2 (cells were incubated for 72 h): (A) (9) CPT, (b) pro-CPT **17a**, (2) pro-CPT **17a** + 1 μM 38C2; (B) (9) CPT, (b) pro-CPT **17**, (2) pro-CPT **17** + 1 μM 38C2 [29].



Scheme 10.5 Synthesis of symmetrically PEGylated dendrimer [27].

eral hydroxyl groups (as shown in Scheme 10.5) were functionalized using orthogonally protected aspartic acid to obtain compound **6**. Amino groups were subsequently deprotected and PEGylation was carried out with 5 kDa PEG electrophiles to obtain dendrimer **8**.

The protecting groups in benzyl ester **8** were removed by hydrogenolysis and dendrimer **9** was afforded using carboxylic acids moieties which is further

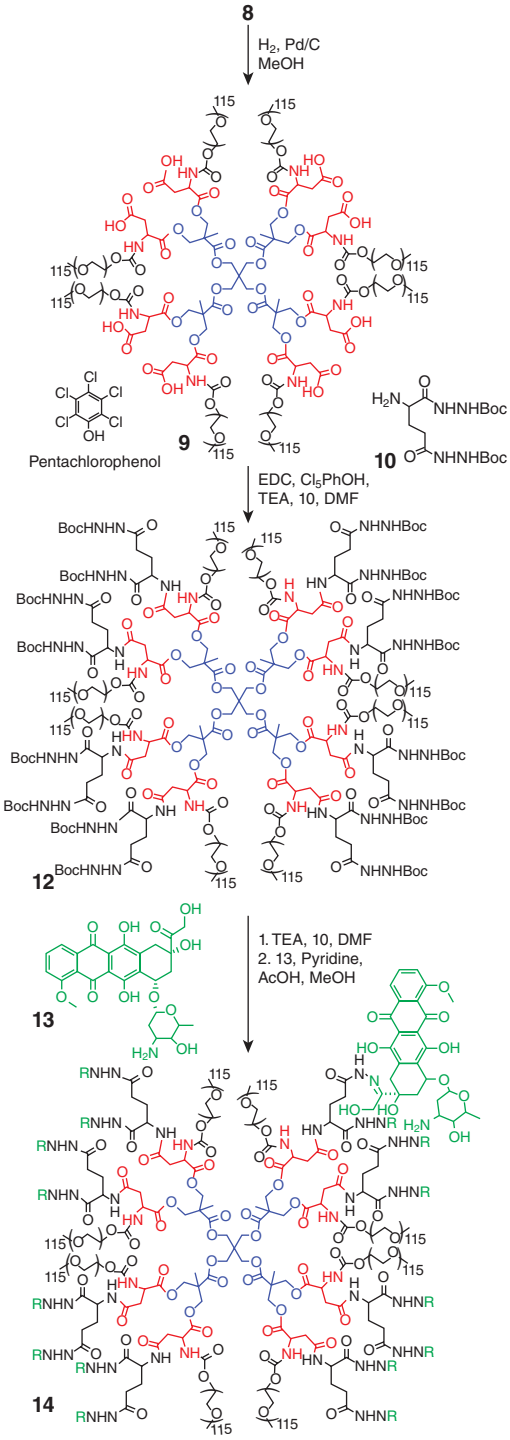
envisioned for attaching drug molecules of choice. The functionalization of this dendrimer with *t*-butyl carbazate or glutamic acid derivative **10** was not successful as degradation of the dendrimer was occurring during the reaction. For further insight with respect to the degradation pathway, the authors designed dendrimer **11** and further functionalized with aspartic acid chain ends. Scaffold **11** was used since the progress of its reaction was monitored by MALDI-TOF over to PEGylated dendrimer **9** (Scheme 10.6).

Due to degradation side reaction, only a small amount of the target moiety was only reported, thereby leading to the appearance of lower molecular weight products as a result of intramolecular cyclization reactions as proposed in Scheme 10.7. This kind of cyclization reactions with benzyl ester-protected aspartic acid residues are reported in the literature [49]. Earlier, pentachlorophenol (PCP) has been used to decrease the formation of the aminosuccinyl by-product by inhibiting amide deprotonation. While, in buffered conditions, the primary amines are favorable to react with PNP carbonates and other electrophiles. It has been reported that the use of PCP was beneficial since it allows the functionalization of the carboxylic acid side chains of dendrimer **9** with protected nucleophile **10** to give dendrimer **12** (Scheme 10.6). Furthermore, DOX hydrazone conjugate **14** was synthesized by removing Boc groups from the hydrazide linkers in **12** and by condensation of the resulting amines with the ketone group of DOX **13** (Scheme 10.6). The degradation of polyester architecture was evaluated in physiological conditions. Therefore, **12** was incubated in phosphate-buffered saline buffer at 37 °C and the molecular weight with time was monitored by SEC. Due to rapid degradation, alternative dendrimer scaffolds based on robust polyamide core were explored.

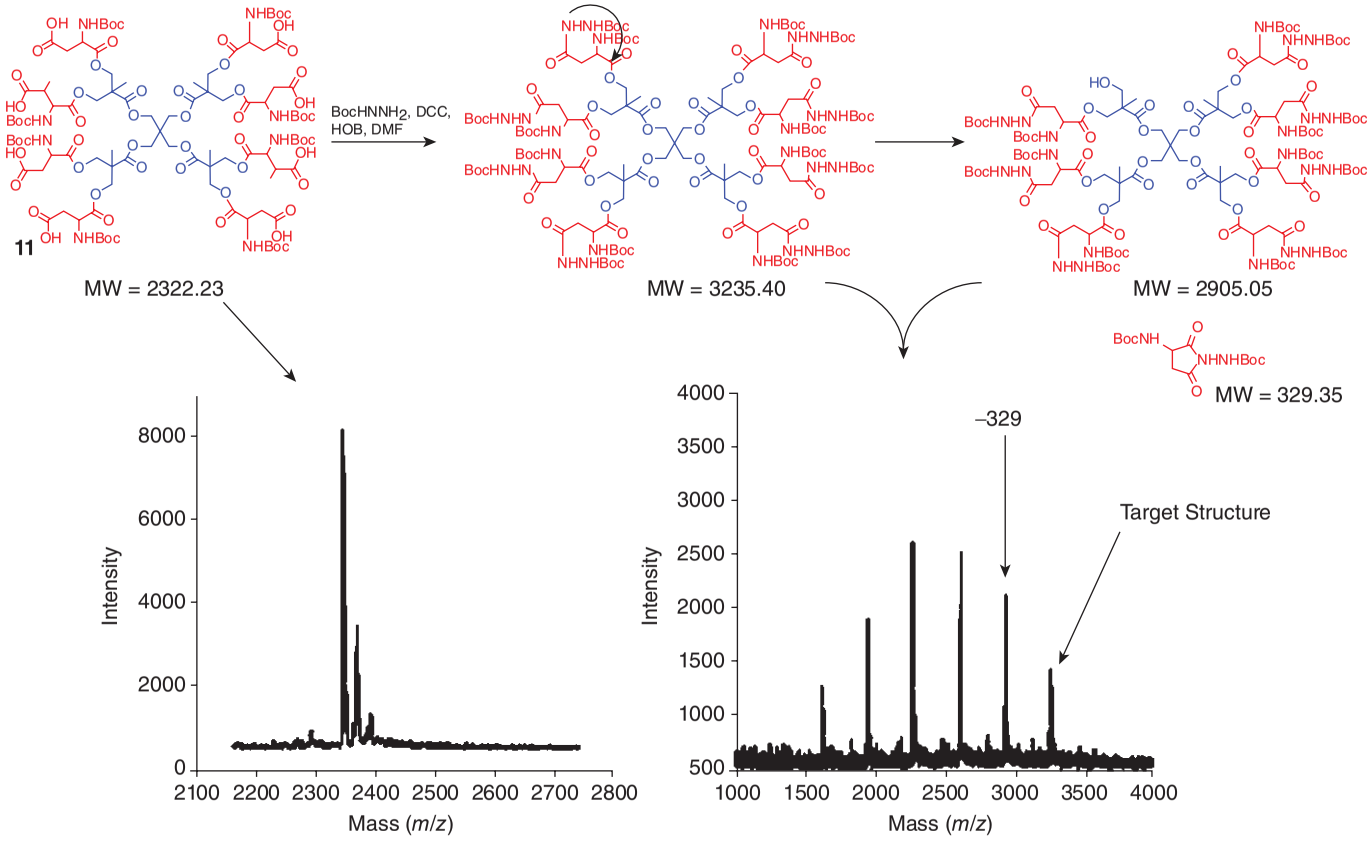
10.3.1.1 Polylysine-Core Biodegradable Dendrimer Prodrug

Compared to polyester dendrimers, polyamide dendrimers are less susceptible to hydrolysis; however, due to increased stability, it may affect *in vivo* pharmacokinetics Recently, Fox *et al.* reported PEGylated polylysine consisting of camptothecin with 100% survival in transgenic mice using HT-29 human colon carcinoma for a period of 70 days (Scheme 10.8) [50]. It is to be noted that the very slow or incomplete degradation of the polymeric carrier's by-product may lead to toxicity [51]. Delivery of DOX using polylysine carrier is reported [50] in the form of dendrimer **18** having protected hydrazide molecules (Scheme 10.8).

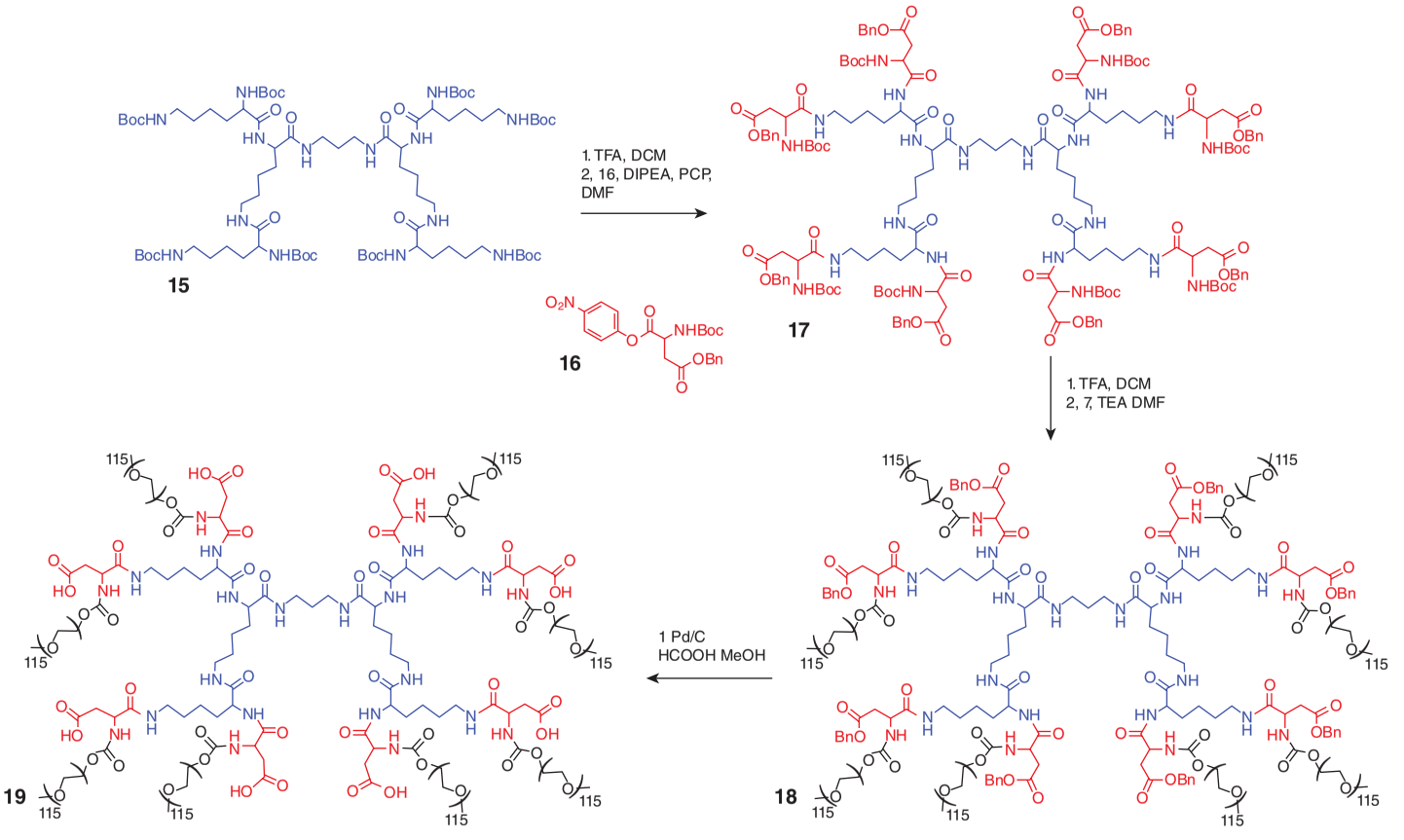
As shown in Scheme 10.8, lysine dendrimer **15** was used as the starting material. Its peripheral amines were acylated with PNP-Asp(Bn)Boc to afford dendrimer **17**. The authors pointed out that the PCP additive is critical for conjugating aspartic acid to G2 lysine periphery; if not, a five-membered amino succinyl by-product may be formed via amidolysis of the benzyl ester-protected side chain. By deprotecting of amino groups of the aspartate and PEGylation with PEG-*p*-nitrophenyl, carbonate yields **18**. However, coupling *t*-butyl carbazate to the deprotected side chain of carboxylic acid terminal moieties (**19**) leads to degradation by-products such as **20** (Scheme 10.8). Furthermore, the drug was conjugated using PEGylated ester amide dendrimers (**25–27**) as represented in Scheme 10.10.



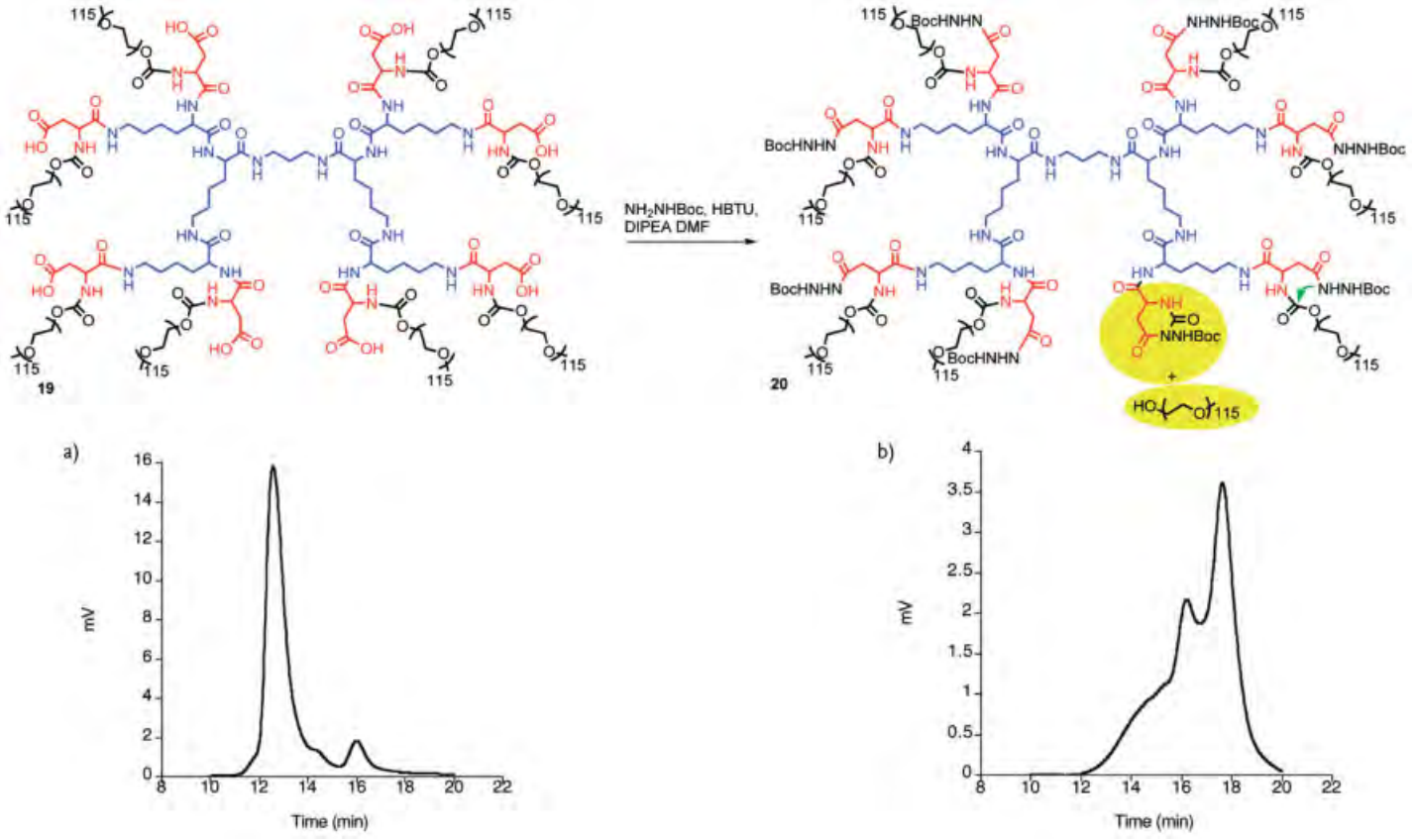
Scheme 10.6 Linker attachment and conjugation of DOX [27].



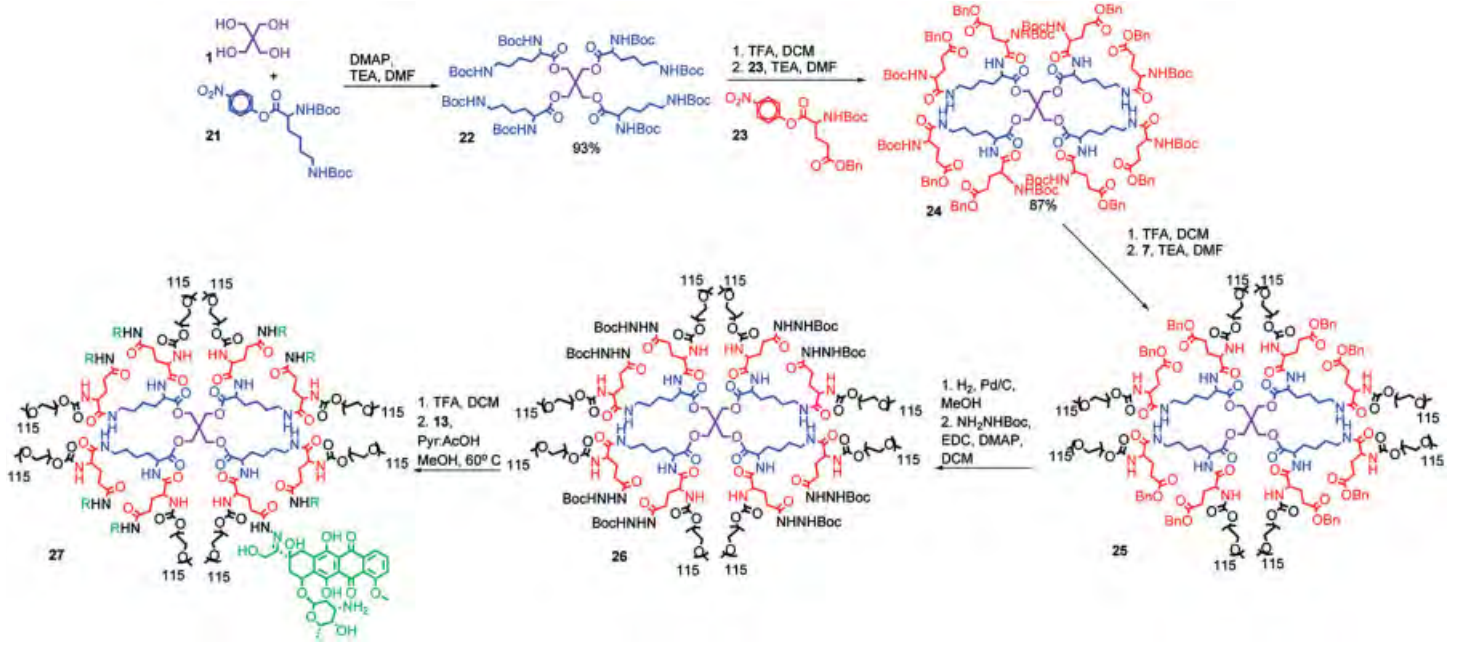
Scheme 10.7 Proposed degradation pathway for polyester dendrimer [27].



Scheme 10.8 PEGylated polylysine synthesis [27].



Scheme 10.9 PEGylated polylysine degradation: (a) SEC of compound **19** and (b) SEC of reaction mixture with by-product 20 [27].



Scheme 10.10 Synthesis of drug-loaded PEGylated ester amide dendrimer [27].

Table 10.1 *In vitro* efficacies of ester amide dendrimer conjugate and controls against balb/C mice with C26 carcinoma [27].

Treatment group	No. mice	Dose (mg/kg)	Mean TGD[a)] (%)	Median survival time (days)	TRD[b)]	LTS
PBS[c)]	10			20	0	0
Doxil	10	20	245[b)]	60[b)]	2	8
27	10	20	229[b)]	60[b)]	0	9
27	10	15	175[b)]	60[b)]	0	6
27	10	10	74[c)]	33[b)]	0	1

a) TGD, tumor growth delay, calculated from time of growth to 400 mm^3.
b) TRD, treatment-related death; LTS, long-term survivors. Compared to PBS, $P \leq 0.0001$.
c) Compared to PBS, $P = 0.004$. Compound **27** is represented in Scheme 10.10.

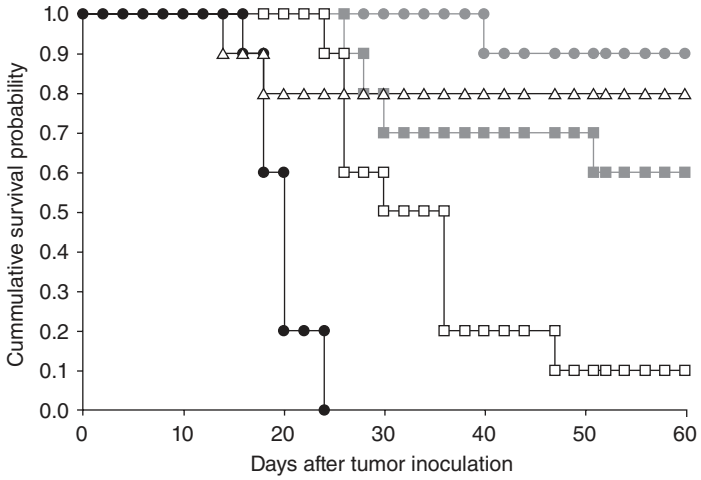


Figure 10.4 Representative survival probability versus time in Balb/C mice bearing subcutaneous C26 colon carcinoma after a single injection of PEGylated poly(ester amide) Dox conjugate or control. After tumor generation, mice were treated for 8 days. [**27** (20 mg Dox equivalent/kg); 9, **27** (15 mg Dox equivalent/kg); 0, **27** (10 mg Dox equivalent/kg); Δ, Doxil (20 mg Dox equivalent/kg); •, PBS [27].]

The *in vitro* evaluation and *in vivo* tumor efficacy of drug-loaded PEGylated ester amide dendrimer have been described in details in Section 1.4 (Table 10.1 and Figure 10.4).

10.4 Biological Implications of Biodegradable Dendrimers

After years of seeking to synthesize polymers in the form of long linear chains, the attention has been focused on highly defined polymeric topologies, especially

in the form of highly branched dendritic architectures [52]. The interesting application of these intriguing and well-defined materials seems to be branching out. Especially, they offer water solubility due to enriched hydrophilic groups at the surface, ester linkages to make them biodegradable, and appropriate amounts of drug can be added so that a uniform supply of the drug is delivered when the polymer degrades. Dendrimers find implications in biology for delivering drugs, bioactives (e.g., SiRNA and peptides), diagnostics (dyes), and in targeted delivery systems (use of LHRH peptide, folic acid, antibodies, etc.) [14, 21, 22, 37, 43, 44].

Among many other crucial characteristics of cellular internalization of dendritic architectures, the ability of dendrimers to cross cell membrane is of much interest particularly for their application in drug and gene delivery. A recent study has demonstrated that dendrimers are capable to enter cells by endocytosis, but the intracellular pathway following their internalization remains controversial [53]. The intracellular trafficking property of PAMAM dendrimers was observed using confocal fluorescence microscopy with high spatial and temporal resolution in living HeLa cells. Macromolecules of different chemical functionality (neutral, cationic, and lipidated), size (from G2 up to G6), and surface charge are investigated and their internalization properties correlated with the molecular structure. So far, not many strategies have been reported to synthesize biodegradable dendrimers and among them very few suggest their biomedical applications. Section 10.5 highlights biological perspectives of biodegradable dendrimers.

Interestingly, SIDs have been developed and are introduced as a potential platform for a multiprodrug (synthesis section – Schemes 10.2–10.4 [29]). The reported dendrimers release all of their tail units, through a self-immolative chain fragmentation, which is initiated by a single cleavage at the dendrimer's core. Incorporation of drug molecules as the tail units and an enzyme substrate as the trigger generates a multiprodrug unit, which can be activated with a single enzymatic cleavage. The authors evaluated bioactivation of the dendritic prodrugs by cell-growth inhibition assay with the Molt-3 leukemia cell line in the presence and the absence of antibody 38C2 (Figures 10.2 and 10.3). The dendritic unit can be considered as a platform to develop a heterodimeric prodrug approach for remarkable increase in toxicity with its bioactivation.

When catalytic antibody 38C2 was incubated with the prodrugs, it was observed that the IC_{50} of the dimeric prodrug had shifted closer to the IC_{50} of the free DOX (Figure 10.2). The IC_{50} values of the monomeric and the dimeric prodrugs were found to be almost the same, and the prodrugs were about 200-fold less toxic than free CPT (Figure 10.3). On the other hand, catalytic antibody 38C2 was added, and both the prodrugs were activated. However, while the activity of the monomeric prodrug had shifted to a 10-fold difference from that of free CPT, the dimeric prodrug was shown to be about four times more active upon addition of 38C2, meaning that more toxicity was achieved using the dimeric prodrug and 38C2 in comparison to monomeric prodrug and the same concentration of antibody. For **27** compound administered at 15 and 10 mg DOX/kg, the treatment groups had 175% and 74% tumor growth inhibition with a medium survival time of 60 and 33 days, respectively. A dramatic 75% decrease in subcutaneous tumor size has been observed in mice that received a combination of intratumoral

injections of antibody 38C2 and systemic treatments with an etoposide prodrug [54].

Tansey *et al*. described synthesis and characterization of branched poly(L-glutamic acid) (PG) containing multiple PG chains centered on a PAMAM dendrimer or PEI cores [55]. The branched PG polymers were obtained by ring-opening polymerization of benzyl ester of L-glutamic acid *N*-carboxyanhydride using PAMAM or PEI as the initiator. The polymers were degradable in the presence of the lysosomal enzyme cathepsin B, albeit more slowly than linear PG. Unlike conventional linear PG, each branched PG possessed multiple terminal amino groups. This made it possible to attach multiple targeting moieties selectively to the termini of branched PG. Conjugation of monofunctional or heterodifunctional PEG to the chain ends of branched PG demonstrated in the presence of side-chain carboxyl groups. Furthermore, folic acid, a model-targeting moiety, and the near-infrared dye indocyanine green, a model diagnostic agent, were successfully conjugated to the terminal amino groups and the side-chain carboxyl groups of branched PG, respectively. The resulting conjugate had reduced nonspecific interaction and bound selectively to tumor cells expressing folate receptors. Thus, branched PG may be useful as a polymeric carrier for targeted drug delivery.

Degradable dendrimer architectures can be conjugated with linearly branched polymers (PEG) to improve biological application due to the enhanced pharmacokinetic ability [27]. For example, van der Poll reported efficient synthesis of a robust and biodegradable PEGylated dendrimer based on a polyester–polyamide hybrid core (synthesis section, Schemes 10.8–10.10 [27]). The architecture has been designed to avoid destructive side reactions during dendrimer preparation while maintaining biodegradability. Dendrimer functionalized with DOX was also prepared from commercial starting materials in nine, high-yielding linear steps. Both the dendrimer and Doxil were evaluated in parallel using equimolar dosage in the treatment of C26 murine colon carcinoma, leading to statistically equivalent results with the most mice tumor-free at the end of the 60-day experiment. The attractive features of this dendritic drug carrier are its simple synthesis, biodegradability, and capability to deliver high payload of drugs.

Similarly, many glycol-dendrimers have been reported for applications in biology. For example, glycopeptide dendrimers containing ω-amino acids (Gly, β-Ala, γ-abu, and ε-aminohexanoic acid) are of interest for immunological studies [56]. Interestingly, biodegradable forms of dendrimers have been used as pH-sensing biodegradable near-infrared nanoprobe capable of providing complementary information through both fluorescence lifetime measurements and signal amplification in acidic environments *in vivo* [57]. Such tools may find extensive role in drug delivery systems, and such noninvasive approach may shed light on the kinetics of such drug delivery strategies *in vivo* in a cost-effective and more accurate manner.

It has been realized that in many cases the prepared dendrimeric structures are more a result of an intellectual capability to prepare some unusual compounds with new cores, branches, etc., than an exact approach based on the knowledge of

size, shape, polarity, and other parameters that must be fulfilled to satisfy the strict demands of the given receptor [58].

10.5
Future Perspectives of Biodegradable Dendrimers

Now, it is possible to precisely manipulate dendrimers for their molecular weight and chemical composition, thereby to allow predictable tuning of its biocompatibility and pharmacokinetics [40].

Degradable polymeric systems have wide perspective in several established and emerging technologies such as controlled-release systems for drug delivery and photoresist methodology for microlithography among other applications [59–61] As the demand increases, the higher levels of control over the structure, properties, and performance of degradable materials would be desired [18, 20]. So far few groups have addressed the strategies for the controlled degradation of dendritic structures or the implications of the development of such systems. Researchers have now shown the perspectives of biodegradable dendrimers and for sure such forms will find immense implications in biology.

Advances in realizing the role of molecular weight and architecture on the *in vivo* behavior of dendrimers, together with recent progress in the design of biodegradable chemistries, have enabled the application of these branched polymers as antiviral drugs, tissue repair scaffolds, and targeted carriers of chemotherapeutics. It is expected that the products could reach the market soon and therefore the field must address the long-term human and environmental health consequences of dendrimer exposure *in vivo*.

The synergy due to biodegradation, multivalency, and size in nanoscale has a range of options to impart chemical "smartness" along their molecular scaffold to achieve environment-sensitive modalities; such materials are envisioned to revolutionize the existing therapeutic practices [14]. Therefore, biodegradable dendritic architectures are expected to lead to new strategies for *nanomedicine* as well as *regenerative medicine*.

10.6
Concluding Remarks

The diverse dendritic structures with multiple functional groups at the periphery for chemical modifications render dendrimers to tune biological properties. The potential virtue of dendrimers comes under the heading of "multivalancy": the enhanced effect that stems from lots of identical molecules being present at the same time and place [14, 19]. Dendrimers have shown to enter into the cells remarkably easily, with a potential to deliver drugs at the targeted site. Furthermore, there has been great emphasis to achieve the release of a drug at various pH environments. However, most demanding aspect of dendrimers is to construct

them into self-disintegrating forms termed as SIDs [16, 17, 19]. Therefore, the combination of multivalancy and self-destruction characteristics of biodegradable dendrimers will have increasing interest for biomedical implications.

References

1 Kopecek, J. (1977) Soluble biomedical polymers. *Polym. Med.*, **7**, 191–221.
2 Bader, H., Ringsdorf, H., and Schmidt, B. (1984) Water-soluble polymers in medicine. *Angew. Makromol. Chem.*, **123**, 457–485.
3 Seymour, L.W. (1992) Passive tumor targeting of soluble macromolecules and drug conjugates. *Crit. Rev. Ther. Drug*, **9**, 135–187.
4 Matsumura, Y. and Maeda, H. (1986) A new concept for macromolecular therapeutics in cancer-chemotherapy-mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Res.*, **46**, 6387–6392.
5 Tomalia, D.A., *et al.* (1985) A new class of polymers: starburst-dendritic macromolecules. *Polym. J.*, **17**, 117–132.
6 Grayson, S.M. and Fréchet, J.M.J. (2001) Convergent dendrons and dendrimers: from synthesis to applications. *Chem. Rev.*, **101**, 3819–3868.
7 Esfand, R. and Tomalia, D.A. (2001) Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discov. Today*, **6**, 427–436.
8 Lee, J-S., Huh, J., Ahn, C-H., Lee, M., and Park, T.G. (2006) Synthesis of novel biodegradable cationic dendrimers. *Macromol. Rapid Commun.*, **27**, 1608–1614.
9 Fréchet, J.M.J. and Tomalia, D. (2001) Introduction to the dendritic state editors, in *Dendrimers and Other Dendritic Polymers* (eds J.M.J. Fréchet and D. Tomalia), John Wiley & Sons, Ltd, Chichester, UK, pp. 1–44.
10 Ihre, H.R., Padilla De Jesus, O.L., Szoka, F.C. Jr., and Frechet, J.M. (2002) Polyester dendritic systems for drug delivery applications: design, synthesis, and characterization. *Bioconjug. Chem.*, **13**, 443–452.
11 Jevprasesphant, R., Jalal, R., Attwood, D., McKeown, N.B., and D'Emanuele, A. (2003) The influence of surface modification on the cytotoxicity of PAMAM dendrimers. *Int. J. Pharm.*, **252**, 263–266.
12 Quadir, M.A., Radowski, M.R., Kratz, F., Licha, K., Hauff, P., and Haag, R. (2008) Dendritic multishell architectures for drug and dye transport. *J. Control. Release*, **132**, 289–294.
13 Haag, R., Sunder, A., and Stumb'e, J.F. (2000) An approach to glycerol dendrimers and pseudo-dendritic polyglycerols. *J. Am. Chem. Soc.*, **122**, 2954–2955.
14 Caldero'n, M., Quadir, M.A., Sharma, S.K., and Haag, R. (2010) Dendritic polyglycerols for biomedical applications. *Adv. Mater.*, **22**, 190–218.
15 Newkome, G.R., Moorefield, C.N., and Vögtle, F. (1996) *Dendritic Macromolecules: Concepts, Synthesis, Perspectives*, Wiley-VCH, Weinheim, Germany.
16 Amir, R.J., Pessah, N., Shamis, M., and Shabat, D. (2003) Self-immolative dendrimers. *Angew. Chem. Int. Ed.*, (**42**), 4494–4499.
17 de Groot, F.M., Albrecht, C., Koekkoek, R., Beusker, P.H., and Scheeren, H.W. (2003) "Cascade-release dendrimers" liberate all end groups upon a single triggering event in the dendritic core. *Angew. Chem. Int. Ed.*, (**42**), 4490–4494.
18 Szalai, M.L., Kevwitch, R.M., and McGrath, D.V. (2003) Geometric disassembly of dendrimers: dendritic amplification. *J. Am. Chem. Soc.*, **125**, 15688–15689.
19 Meijer, E.W. and van Genderen, M.H.P. (2003) Dendrimers set to self-destruct. *Nature*, **426**, 128–129.
20 Li, S., Szalai, M.L., Kevwitch, R.M., and McGrath, D.M. (2003) Dendrimer disassembly by benzyl ether depolymerization. *J. Am. Chem. Soc.*, **125**, 10516–10517.

21 Grinstaff, M. (2002) Biodendrimers: new polymeric biomaterials for tissue engineering. *Chem. Eur. J.*, **8**, 2838–2846.
22 Haag, R. (2004) Supramolecular drug-delivery systems based on polymeric core–shell architectures. *Angew. Chem.*, **116**, 280–284.
23 Duncan, R. (2006) Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer*, **6**, 688–701.
24 Sunder, A., Hanselmann, R., Frey, H., and Mulhaupt, R. (1999) Controlled synthesis of hyperbranched polyglycerols by ring-opening multibranching polymerization. *Macromolecules*, **32**, 4240–4246.
25 Stiriba, S.E., Frey, H., and Haag, R. (2002) Dendritische polymere fur medizinische anwendungen: auf dem weg zum einsatz in diagnostik und therapie. *Angew. Chem. Int. Ed. Eng.*, **114**, 1385–1390.
26 Sisson, A.L., Steinhilber, D., Rossow, T., Welker, P., Licha, K., and Haag, R. (2009) Biocompatible functionalized polyglycerol microgels with cell penetrating properties. *Angew. Chem. Int. Ed. Engl.*, **48**, 7540–7545.
27 van der Poll, D.G., Kieler-Ferguson, H.M., Floyd, W.C., Guillaudeu, S.J., Jerger, K., Szoka, F.C., and Fréchet, J.M. (2010) Design, synthesis, and biological evaluation of a robust, biodegradable dendrimer. *Bioconjug. Chem.*, **21**, 764–773.
28 Szuromi, P.D. (2003) Triggering polymer destruction. *Science*, **302**, 1863.
29 Shamis, M., Lode, H.N., and Shabat, D. (2004) Bioactivation of self-immolative dendritic prodrugs by catalytic antibody 38C2. *J. Am. Chem. Soc.*, **126**, 1726–1731.
30 Calderón, M., Graeser, R., Kratz, F., and Haag, R. (2009) Development of enzymatically cleavable prodrugs derived from dendritic polyglycerol. *Bioorg. Med. Chem. Lett.*, **14**, 3725–3728.
31 Khandare, J.J., Jayant, S., Singh, A., Chandna, P., Wang, Y., Vorsa, N., and Minko, T. (2006) Dendrimer versus linear conjugate: influence of polymeric architecture on the delivery and anticancer effect of paclitaxel. *Bioconjug. Chem.*, **17**, 1464–1472.
32 Svenson, S. (ed.) (2004) *"Carrier-Based Drug Delivery", ACS Symposium Series*, American Chemical Society, Washington, DC, p. 879.
33 Svenson, S. and Tomalia, D.A. (2005) Dendrimers in biomedical applications – reflections on the field. *Adv. Drug Deliv. Rev.*, **57**, 2106–2129.
34 Kukowska-Latallo, J.F., Bielinska, A.U., Johnson, J., Spindler, R., Tomalia, D., and Baker, A.J.R. Jr. (1996) Efficient transfer of genetic material into mammalian cells using starburst polyamidoamine dendrimers. *Proc. Natl. Acad. Sci. USA*, **93**, 4897–4902.
35 Malik, N., Evagorou, E.G., and Duncan, R. (1999) Dendrimer-platinate: a novel approach to cancer chemotherapy. *Anticancer Drugs*, **10**, 767–776.
36 Kobayashi, H. and Brechbiel, M.W. (2005) Nano-sized MRI contrast agents with dendrimer cores. *Adv. Drug Deliv. Rev.*, **57**, 2271–2286.
37 Patri, A.K., Majoros, I.J., and Baker, J.R. Jr. (2002) Dendritic polymer macromolecular carriers for drug delivery. *Curr. Opin. Chem. Biol.*, **6**, 466–471.
38 Khandare, J., Kolhe, P., Pillai, O., Kannan, S., Lieh-Lai, M., and Kannan, R.M. (2005) Synthesis, cellular transport, and activity of polyamidoamine dendrimer–methylprednisolone conjugates. *Bioconjug. Chem.*, **16**, 330–337.
39 Lee, C.C., MacKay, J.A., Fréchet, J.M.J., and Szoka, F.C. (2005) Designing dendrimers for biological applications. *Nat. Biotechnol.*, **23**, 1517–1526.
40 Khandare, J., Mohr, A., Calderon, M., Welker, P., Licha, K., and Haag, R. (2010) Design, synthesis, and cytotoxicity evaluation of dendritic polyglycerol architectures. *Biomaterials*, **31**, 4268–4277.
41 Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J.W., *et al.* (2000) Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of 125i-labelled polyamidoamine dendrimers *in vivo*. *J. Control. Release*, **65**, 133–148.
42 Khandare, J. and Minko, T. (2006) Polymer–drug conjugates: progress in polymeric prodrugs. *Prog. Polym. Sci.*, **31**, 359–397.

43 Dharap, S.S., Wang, Y., Chandna, P., Khandare, J.J., Qiu, B., Gunaseelan, S., Stein, S., Farmanfarmaian, A., and Minko, T. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 12962–12967.

44 Zhang, Y., Thomas, T.P., Desai, A., Zong, H., Leroueil, P.R., Majoros, I.J., and Baker, J.R. Jr. (2010) Targeted dendrimeric anticancer prodrug: a methotrexate-folic acid-poly(amidoamine) conjugate and a novel, rapid, "one pot" synthetic approach. *Bioconjug. Chem.*, **21**, 489–495.

45 de Groot, F.M., Damen, E.W., and Scheeren, H.W. (2001) Anticancer prodrugs for application in monotherapy: targeting hypoxia, tumor-associated enzymes, and receptors. *Curr. Med. Chem.*, **8**, 1093–1122.

46 Wagner, J., Lerner, R.A., and Barbas, C.F. III (1995) Efficient aldolase catalytic antibodies that use the enamine mechanism of natural enzymes. *Science*, **270**, 1797–1800.

47 Jensen, A.W., Maru, B.S., Zhang, X., Mohanty, D.K., Fahlman, B.D., Swanson, D.R., and Tomalia, D.A. (2005) Preparation of fullerene–shell dendrimer–core nanoconjugates. *Nano Lett.*, **5**, 1171–1173.

48 Padilla De Jesus, O.L., Ihre, H.R., Gagne, L., Frechet, J.M., and Szoka, F.C. Jr. (2002) Polyester dendritic systems for drug delivery applications: *in vitro* and *in vivo* evaluation. *Bioconjug. Chem.*, **13**, 453–461.

49 Martinez, J. and Bodanszky, M. (1978) Side reactions in peptide-synthesis, suppression of the formation of aminosuccinyl peptides with additives. *Int. J. Pept. Protein Res.*, **12**, 277–283.

50 Fox, M.E., Guillaudeu, S., Fre'chet, J.M.J., Jerger, K., Macaraeg, N., and Szoka, F.C. (2009) Synthesis and *in vivo* antitumor efficacy of PEGylated poly(l-lysine) dendrimer–camptothecin conjugates. *Mol. Pharmaceut.*, **6**, 1562–1572.

51 Kaminskas, L.M., Boyd, B.J., Karellas, P., Krippner, G.Y., Lessene, R., Kelly, B., and Porter, C.J.H. (2008) The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of PEGylated poly L-lysine dendrimers. *Mol. Pharmaceut.*, **5**, 449–463.

52 Hodge, P. (1993) Polymer science branches out. *Nature*, **362**, 18–19.

53 Albertazzi, L., Serresi, M., Albanese, A., and Beltram, F. (2010) Dendrimer internalization and intracellular trafficking in living cells. *Mol. Pharmaceutics*, **7**, 680–688.

54 Shabat, D., Lode, H., Pertl, U., Reisfeld, R.A., Rader, C., Lerner, R.A., and Barbas, C.F. III (2001) *In vivo* activity in a catalytic antibody-prodrug system: antibody catalyzed etoposide prodrug activation for selective chemotherapy. *Proc. Natl. Acad. Sci. USA*, **98**, 7528–7533.

55 Tansey, W., Ke, S., Cao, X.-Y., Pasuelo, M.J., Wallace, S., and Li, C. (2004) Synthesis and characterization of branched poly(L-glutamic acid) as a biodegradable drug carrier. *J. Control. Release*, **94**, 39–51.

56 Sato, K., Hada, N., and Takeda, T. (2006) Syntheses of new peptidic glycoclusters derived from β-alanine: di- and trimerized glycoclusters and glycocluster-custers. *Carbohydr. Res.*, **341**, 836–845.

57 Almutairi, A., Guillaudeu, S.J., Berezin, M.Y., Achilefu, S., and Fréchet, J.M.J. (2008) Biodegradable pH-sensing dendritic nanoprobes for near-infrared fluorescence lifetime and intensity imaging. *J. Am. Chem. Soc.*, **130**, 444–445.

58 Niederhafner, P., Sebest'ik, J., and Jězek, J. (2008) Glycopeptide dendrimers. Part II. *J. Pept. Sci.*, **14**, 44–65.

59 Sawada, H. (1985) *Encyclopedia of Polymer Science and Engineering*, vol. 4 (eds H.F. Mark, N.M. Bikales, C.G. Overberger, G. Menges, and J.I. Kroschwitz), John Wiley & Sons, Inc., New York, pp. 719–745.

60 Seebach, D., Herrmann, G.F., Lengweiler, U.D., Bachmann, B.M., and Amrein, W. (1996) Enzyme-triggered disassembly of dendrimer-based amphiphilic nanocontainers. *Angew. Chem. Int. Ed. Engl.*, **35**, 2795–2797.

61 Tully, D.C., Wilder, K., Fréchet, J.M.J., Trimble, A.R., and Quate, C.F. (1999) Dendrimer-based self assembled monolayers as resists for scanning probe lithography. *Adv. Mater.*, **11**, 314–318.