Polymeric materials represent the largest class of biomaterials. Many types of polymers are widely used in biomedical applications that include dental, soft tissue, orthopedic, cardiovascular implants, contact lenses, artificial skin, artificial pancreas, and drug and gene delivery. In this chapter, the synthesis, properties, characterization, processing, and common bioapplications of polymers will be considered. Even though polymeric nanobiomaterials as a term is not explicitly used or discussed in this chapter, the tools, techniques, and biomedical applications discussed are equally relevant in the development of polymeric nanobiomaterials.

Polymers as the Greek origin of the word dictates (polymers—poly = πολύς = many, mers = μέρη = parts) are long-chain molecules that are composed of a large number of small repeating units (monomers). Because polymers are so much bigger compared to the repeating units, their characteristics are much more complex than those of the repeating units. These characteristics determine their properties and consequentially their properties affect their biomedical applications.
4.1 Structural Characteristics of Polymers: Shape and Size

4.1.1 Molecular Architecture

The most obvious characteristics of a polymer species are the shape of its molecules and its skeletal structure, which is also called molecular architecture (Gedde 1995; Young and Lovell 2002; Temenoff and Mikos 2008). A polymer can be linear, branched, ladder-like, star, or cross-linked (Allcock and Lampe 1990; Gedde 1995; Young and Lovell 2002; Temenoff and Mikos 2008), as shown in Figure 4.1.

Linear polymers are usually randomly coiled, unless they exhibit a strong tendency to crystallize (Remmp and Merrill 1991). In that case, they form crystallites of various sizes that are connected by parts of chains that have not undergone crystallization, since, even in the most favorable cases, polymers will never be crystallized to 100% (Remmp and Merrill 1991).

The molecular architecture is important for many properties (Remmp and Merrill 1991; Gedde 1995; Young and Lovell 2002). Short-chain branches reduce crystallinity while long-chain branches have profound effects on rheological properties. Ladder polymers have high strength and thermal stability (Gedde 1995). Hyperbranched polymers (branched and star polymers) have different viscosity than that of their linear analogue with the same molecular weight (Gedde 1995). Cross-linked macromolecules (also called polymeric networks) cannot be dissolved in a solvent but they may have the ability to swell or absorb liquids and other molecules depending on the cross-link density and hydrophobicity/hydrophilicity (Patrickios and Georgiou 2003). Thermoset polymers are highly cross-linked macromolecules, in fact so tightly cross-linked that they cannot be swollen nor melted, and they retain the shape of the molds in which they are manufactured (Remmp and Merrill 1991).

4.1.2 Homopolymers and Copolymers

A homopolymer is a macromolecule that contains only one single type of repeat unit in its chain (Gedde 1995; Young and Lovell 2002; Temenoff and Mikos 2008). The chemical structure of a polymer is usually represented by that of the repeat unit enclosed by brackets. Thus, the hypothetical homopolymer \( \overset{\text{A A A A A A A A}}{\text{\underbrace{}}} \) is represented by \( \{\text{A}\}_n \) where \( n \) is the number of repeat units linked together.

![FIGURE 4.1 Schematic representation of structures of polymers with different molecular architecture.](image-url)
The naming of polymers is often an area of difficulty. The International Union of Pure and Applied Chemistry (IUPAC) recommended a system of nomenclature based on the structure of the monomer or repeat unit (Hiemenz 1984). A polymer is named polyx, where x is the name of the monomer or the repeat unit, for example, polystyrene. If x is more than one word, then parentheses are used, for example, poly(methyl methacrylate) (Gedde 1995). However, many synthetic polymers of commercial importance like Nylon® and Kevlar® are often widely known by trade names (Gedde 1995).

A copolymer consists of two or more repeating units (A, B, etc.) (Gedde 1995; Young and Lovell 2002; and Temenoff and Mikos 2008). There are several categories of copolymers, each being characterized by a particular arrangement of the repeat units along the polymer chain, as shown in Figure 4.2. Block copolymers (polyA-block-polyB or A_x-B_y, where x and z are the degree of polymerizations of A and B, respectively) are polymers in which the repeat units exist in long sequences or blocks of the same type (Young and Lovell 2002). Statistical copolymers, poly(A-stat-B), are copolymers in which the sequential distribution of repeat units obeys known statistical law (e.g., Markovian) (Gedde 1995). Random copolymers, poly(A-ran-B), are a special type of statistical copolymer in which the distribution of repeat units is truly random (in older textbooks and scientific papers, the term random is often used to describe both random and nonrandom statistical copolymers). Alternating copolymers have only two types of repeat units and these are arranged alternately along the polymer chain (Renn and Merrill 1991; Young and Lovell 2002). Finally, graft copolymers are branched polymers in which the branches have a different chemical structure to that of the main chain. In the simplest form, they consist of a main homopolymer chain with branches of a different homopolymer. It should be noted that a different nomenclature is used for an unspecified copolymer—poly(A-co-B)—and that the copolymers shown in Figure 4.2 consist of only two repeat units. Copolymers can comprise more than two repeat units and this increases the number of different possible ways of distributing the repeat units within the polymer chain and, thus, the molecular architecture. In Figure 4.3, an example of the different architectures triblock copolymers (consisting of three different types of repeating units) can have is shown, while diblock copolymers can only have one (Figure 4.2).

**4.1.3 Tacticity: Stereoisomerism**

Polymers are capable of assuming many conformations through rotation of valence bonds (Callister 2003; Abramson et al. 2004). Thus, different stereoisomers can be observed. Stereoisomerism denotes the situation in which atoms are linked together in the same order but differ in their spatial arrangement...
4.1.4 Molecular Weight: Definition and Distribution

The most important characteristic of polymers that influences all their properties is the molecular weight. Unlike for small molecules, for polymers, there is more than one definition for the molecular weight. This occurs from the fact that when synthesizing a polymer it is usually produced with a distribution of molecular weights; the number of “mers” (structural units) that is defined as the degree of polymerization differs for each polymer (Rempp and Merrill 1991). Therefore, the term molecular weight (or degree of polymerization) cannot be used and the term average molecular weight (or average degree of polymerization) is introduced (Rempp and Merrill 1991).
The number average degree of polymerization \( P_n \) is defined as follows:

\[
P_n = \sum_{i=1}^{\infty} \frac{n_i}{n} = \sum_{i=1}^{\infty} i x_i
\]  

(4.1)

where

- \( n_i \) is the number of molecules with \( i \) monomer units
- \( x_i \) is the mole fraction of molecules with \( i \) monomer units in the chain

If \( M_i \) is the molecular weight of this species, the number average molecular weight is expressed as

\[
M_n = \frac{\sum_{i=1}^{\infty} n_i M_i}{\sum_{i=1}^{\infty} n_i} = \sum_{i=1}^{\infty} x_i M_i = m_0 \sum_{i=1}^{\infty} i x_i
\]

(4.2)

where

- \( m_0 \) is the molecular weight of a repeat unit, hence assumed constant
- \( M_n \) is the total weight of polymer divided by the total number of polymer molecules in the sample

The weight average molecular weight \( M_w \) is defined as the sum of the products of the molecular weight of each fraction multiplied by its weight fraction

\[
M_w = \sum_{i=1}^{\infty} w_i M_i
\]

(4.3)

In terms of the number of molecules, the weight average molecular weight can be expressed as

\[
M_w = \frac{\sum_{i=1}^{\infty} n_i M_i^2}{\sum_{i=1}^{\infty} n_i M_i}
\]

(4.4)

The ratio of the weight average with the number average molecular weight, \( M_w/M_n \), which by definition should be greater or equal to one, is referred to as the polydispersity index (PDI) and provides important information about the width of the molecular distribution of a polymer sample (Rempp and Merrill 1991; Young and Lovell 2002; Abramson et al. 2004). For an ideal, monodisperse polymer, the value of PDI is one, while the less ideal the polymer sample is (contains polymers with a wide range of molecular weights) the higher the value of the PDI.

### 4.2 Synthesis

Polymerization reactions can be, in simple terms, classified into two main types: step-growth polymerizations and chain-growth polymerizations (Gedde 1995; Young and Lovell 2002; Abramson et al. 2004). In step-growth polymerization, the polymer chains grow stepwise by reactions that occur between two molecular species, while in chain-growth polymerizations, the polymer chains grow only by reaction of monomer with the reactive end-group on the growing chains (Young and Lovell 2002).

A typical example of step-growth polymerization, also called condensation polymerization, is the synthesis of Nylon 6,6 (shown in Figure 4.5) (Gedde 1995; Abramson et al. 2004). Two monomers react
to form a covalent bond, usually with elimination of a small molecule such as water, hydrochloric acid, methanol, or carbon dioxide. Step-growth polymerization is involved in the formation of polyesters and polyamides. Different techniques are available for obtaining a high yield and high molar mass (Gedde 1995). Moreover, polymers with different molecular architectures can be made using monomers of different functionality—trifunctional monomers yield branched and ultimately cross-linked polymers (Gedde 1995). Common biomaterials prepared with this polymerization method are nylon and polyurethanes (Rempp and Merrill 1991; Gedde 1995; Abramson et al. 2004).

Chain-growth polymerization with the exception of ring opening polymerization involves the polymerization of unsaturated monomers (Gedde 1995; Abramson et al. 2004). It usually requires an initial reaction between the monomer and an initiator to start the growth of the chain and thus involves several consecutive stages: initiation, propagation, and termination (Rempp and Merrill 1991; Gedde 1995; Abramson et al. 2004). Each chain is individually initiated and grows until its growth is terminated. The initiators can be free radical, cations, anions, or stereospecific catalysts. The initiator opens the double bond of the monomer, creating another initiation site on the opposite side of the monomer bond for continuing growth. Rapid chain growth ensues during the propagation step until the reaction is terminated by reaction with a radical or a molecule, depending on the polymerization technique. Chain-growth polymerization can be divided into several subgroups depending on the mechanism: radical, anionic, cationic, or coordination polymerization (Rempp and Merrill 1991; Abramson et al. 2004). Commonly used biomaterials that are prepared by step-growth polymerizations are polymethacrylates like poly(methyl methacrylate), PMMA, poly(2-hydroxyethyl methacrylate), PHEMA, and poly[2-(dimethylamino)ethyl methacrylate], PDMAEMA, shown in Figure 4.6. It should be noted that some polymerization techniques called “living” or “controlled” polymerization techniques enable the synthesis of well-defined polymers with narrow molecular weight distributions, and the synthesis of polymers with different architectures like block copolymers and star polymers (Webster 1991; Matyjaszewski and Müller 2006). Examples of these techniques include the conventional “living” anionic polymerization (Szwarc 1956; Szwarc et al. 1956; Hadjichristidis et al. 2001), group transfer polymerization (GTP) (Webster et al. 1983; Webster 2000, 2004), ring-opening polymerization (Hashimoto 2000), quasi-living carbocationic polymerization (Kennedy and Iván 1992), and more recently developed polymerization techniques like reversible addition–fragmentation chain transfer (RAFT) polymerization (Moad et al. 2006) and atom transfer radical polymerization (ATRP) (Patten and Matyjaszewski 1998).

### 4.3 Properties

#### 4.3.1 Crystallinity

Polymers can be divided into fully amorphous and semicrystalline (Gedde 1995; Callister 2003; Abramson et al. 2004). The fully amorphous polymers show no sharp crystalline Bragg reflection in the x-ray diffractograms taken at any temperature (Allcock and Lampe 1990). The reason why these polymers are unable to crystallize is commonly their irregular chain structure and their small side groups (Gedde 1995; Abramson et al. 2004). Atactic polymers, statistical copolymers, and highly branched polymers belong to this class of polymers (Gedde 1995). The semicrystalline polymers show crystalline Bragg reflections superimposed on an amorphous background because they always consist of

![Figure 4.5](https://example.com/4-5.png)  
**Figure 4.5** Nylon 6,6 synthesis by condensation polymerization.
two components differing in the degree of order: a component composed of crystals and an amorphous component (Gedde 1995; Callister 2003). The degree of crystallinity can be as high as 90% for certain low molecular weight polyethylenes and as low as 5% for polyvinylchloride (Gedde 1995).

To some extent the physical properties of polymeric materials are influenced by the degree of crystallinity. The presence of crystallites in the polymer usually leads to enhanced mechanical properties, unique thermal behavior, and increased fatigue strength (Callister 2003; Abramson et al. 2004).
4.3.2 Mechanical Properties

The tensile properties of polymers can be characterized by their stress–strain response and their deformation behavior (Callister 2003; Abramson et al. 2004). Three typically different types of stress–strain behavior are found in polymeric materials (Figure 4.7). Curve I illustrates the stress–strain character of a brittle polymer; Curve II is typical for a plastic material and the initial deformation is elastic, which is followed by yielding and a region of plastic deformation; and Curve III illustrates a totally elastic polymer—a class of polymers that are called elastomers, which have a rubber-like elasticity (Callister 2003; Abramson et al. 2004).

The mechanical property is influenced by the freedom of motion of the polymer chain. The freedom of motion is retained at a local level while a network structure resulting from chemical cross-links and/or chain entanglements prevents large-scale movements or flow. Rubbery polymers tend to exhibit a lower modulus, or stiffness and extensibilities of several hundred percent (Callister 2003; Abramson et al. 2004). Glassy and semicrystalline polymers have higher moduli and lower extensibilities (Callister 2003; Abramson et al. 2004).

The ultimate mechanical properties of polymeric materials at large deformations are important in selecting particular polymers for biomedical applications (Abramson et al. 2004). For example, a rigid, strong material is more suitable for a hip implant, whereas a flexible, less strong material would be sufficient for a vascular graft. Furthermore, the ultimate strength of polymer (the stress at or near failure) is also very important since failure for many biomaterials is catastrophic. Finally, the fatigue behavior of polymers is also important in evaluating materials for applications where dynamic stress is applied, for example, cardiovascular implants that must be able to withstand many cycles of pulsating motion.

4.3.3 Thermal Properties

Unlike small molecules, most polymers exhibit another transition upon decreasing the temperature. The temperature point that this transition happens is called the glass transition temperature, \( T_g \). The glass transition occurs in amorphous (or glassy) and semicrystalline polymers and is due to a reduction of motion of large segments of molecules with decreasing temperature (Callister 2003). The long segments of the polymer before the \( T_g \) have enough thermal energy to randomly move, but after the \( T_g \) the segment motion ceases (Abramson et al. 2004).

Upon cooling a polymer, it gradually transforms from a liquid to a rubbery material, and finally to a rigid solid (Callister 2003). The latter change, from rubbery to solid, corresponds to the \( T_g \) (Callister 2003) and it is different for every polymer (Abramson et al. 2004). In addition, abrupt changes in other physical properties accompany this glass transition, for example, stiffness, heat capacity, and coefficient of thermal expansion (Abramson et al. 2004). Even so, the glass transition is not considered a true thermodynamic phase transition like melting of a crystal (Gedde 1995) and it also takes place over
a range of temperatures, usually in a 5°C–10°C temperature span (Callister 2003; Abramson et al. 2004). The $T_g$, therefore, is an important parameter that has to be taken into consideration for the polymers’ applications. For most biomedical applications, depending on the temperature applied, polymers that are within their rubbery region are targeted (Abramson et al. 2004).

The $T_g$ is affected by the molecular weight and the degree of branching of a polymer. Specifically, the increase of the molecular weight tends to raise the $T_g$ (Callister 2003), while a small amount of branching will tend to lower the $T_g$ (Callister 2003). On the other hand, a high density of branches reduces chain mobility and elevates the $T_g$ (Callister 2003). It has been observed that when some amorphous polymers are cross-linked, the $T_g$ is elevated since cross-links restrict molecular motion (Callister 2003). Since the cross-links inhibit flow at all temperatures, chemically cross-linked polymers do not display flow behavior and, thus, cannot be melt processed like linear polymers (Abramson et al. 2004). Instead these materials can be machined to be formed into useful shapes like, for example, PHEMA (Figure 4.6), the polymeric material used for soft lenses (Abramson et al. 2004).

The $T_g$ is also affected by the polymers’ composition and architecture. A copolymer can exhibit two different $T_g$s or one, depending on its composition and architecture. In particular, a random copolymer will exhibit a $T_g$ that approximates the weighted average of the $T_g$ values of the two homo-polymers (Abramson et al. 2004). Block copolymers of sufficient size and incompatible block types will exhibit two individual transitions, each one characteristic of the homopolymer of one of the component blocks (in addition to other thermal transitions), but slightly shifted, owing to incomplete phase separation (Abramson et al. 2004). Even segmented copolymer networks, polymer networks with one type of repeating unit are placed in different segments (blocks) (Patrickios and Georgiou 2003), may exhibit two individual transitions depending on the cross-linking density and the size (Guan et al. 2000).

4.3.4 Aqueous Solution Properties

In solution, the properties of the polymers depend on their compatibility with the solvent; if they are thermodynamically compatible with the solvent. The Flory–Huggins theory describes the thermodynamics of polymer solutions and provides a useful parameter that describes the compatibility of the polymer chain in the solvent, the Flory–Huggins interaction parameter, $\chi$.

This parameter $\chi$ is proportional to the square of the difference of the Hildebrand solubility parameters of the solvent, $\delta_1$, and the polymer, $\delta_2$: $\chi \propto (\delta_1 - \delta_2)^2$ (Gedde 1995; Young and Lovell 2002; Rubinstein and Colby 2003) and depending on its value the solvent is considered either a good or a bad solvent for the polymer. In particular, when $\chi > \frac{1}{2}$ the solvent is a poor solvent for the polymer and the polymer is precipitated (phase separation) or adopts a collapsed conformation so it will interact as little as possible with the solvent. When $\chi < \frac{1}{2}$ the polymer is in a “good” solvent and the polymer chain is extended (Gedde 1995; Rubinstein and Colby 2003). Finally, when $\chi = \frac{1}{2}$ the borderline between the good and poor solvent conditions apply and these conditions are called theta, $\theta$ (Gedde 1995; Rubinstein and Colby 2003). At $\theta$ conditions the polymer has no preference in interacting with itself or the solvent and it adopts a random coil conformation (Gedde 1995; Rubinstein and Colby 2003). The $\chi$ parameter is also affected by the temperature as is illustrated in the following equation:

$$\chi = \frac{1}{2} \cdot \frac{C}{\theta} \left(1 - \frac{\theta}{T}\right)$$  \hspace{1cm} (4.5)

where

$C$ is a constant

$\theta$ is a number, a temperature characteristic for each polymer
When the temperature $T$ is equal to $\theta$, it is called the theta temperature or Flory temperature and $\theta$ conditions apply (Gedde 1995), while depending on the polymer above $\theta$ temperature, the polymer phase separates or is soluble in the solvent. In particular, some polymers exhibit an upper critical solution temperature (UCST), while some other polymers exhibit a lower critical solution temperature (LCST) (Gedde 1995; Young and Lovell 2002; Rubinstein and Colby 2003). This temperature limit is important for many applications since many common polymeric biomaterials are based on polymers that exhibit LCST, like poly(ethylene glycol), PEG, also called poly(ethylene oxide), and poly($N$-isopropylacrylamide), PNIPAAm (Figure 4.6).

For copolymers, the interaction parameters and the compatibility of the polymer and of a segment of the polymer with a solvent are very important. For block copolymers or segmented polymers these parameters become crucial. If the block copolymer is in a solvent that is specific; the solvent will interact more with one block of the copolymer than the other. This will force the block copolymer to form aggregates or micelles. Specifically, if one block copolymer is amphiphilic, it consists of a block that is hydrophilic (from Greek, it means “friend” with the water) and a block that is hydrophobic (from Greek, it means “fears” the water), then the block copolymer is self-assembled in water in such a manner than the hydrophobic part is in contact with the water as little as possible. Since the hydrophobic block of the polymer is not thermodynamically compatible with the solvent that is water, the water compels the polymer chains in solution to form micelles (Hadjichristidis et al. 2003). Micelles are of great importance in biomedical applications since these functional nanomaterials are used for drug and gene delivery. In Figure 4.8 a schematic representation of a micelle (formed by amphiphilic block copolymers) that encapsulates a hydrophobic drug is shown.

### 4.3.5 Degradation

Many polymers that are commonly used in biomedical applications are degradable. The degradation of the polymer has a crucial rule for the materials applicability. In particular, the material should have the appropriate mechanical properties for the indicated application, and the variation in mechanical properties with degradation should be compatible with the healing or regeneration process (Nair and Laurencin 2007). Other properties that a degradable polymer should have in order to be bioapplicable are biocompatibility, degradation time that matches the healing or regeneration process, degradation products that are nontoxic, and the ability to get metabolized and cleared from the body (Nair and Laurencin 2007). The most common degradable functional groups that biodegradable polymers bear are
esters, orthoesters, anhydrides, carbonates, amides, β-amino esters, and urethanes (Nair and Laurencin 2007). The degradation of the polymer or the polymeric material can be studied by monitoring the weight loss (if it is a cross-linked polymer material such as a scaffold for tissue engineering), the molecular weight of the polymer (with techniques that determine the molecular weight), or the breaking of specific chemical bonds (with techniques that analyze the chemical structure of the polymer).

4.4 Characterization

4.4.1 Molecular Weight Analysis

The most important characterization technique of polymers is gel permeation chromatography (GPC), also called size exclusion chromatography (SEC). With this technique, the molecular weight and the molecular weight distribution of a polymer can be determined (Hiemenz 1984; Allcock and Lampe 1990; Remmp and Merrill 1991; Young and Lovell 2002; Callister 2003; Abramson et al. 2004; Temenoff and Mikos 2008). A typical GPC setup is shown in Figure 4.9. A pump pumps the solvent from the solvent reservoir to the collector flask, while it passes through the set of columns and the detector. The sample is injected in the injection port and then it passes through the set of columns. In the columns, the polymer molecules are separated in terms of their size. The smaller polymer molecules enter the smaller pores of the columns and delay, while the bigger polymer molecules do not, and elute faster (Figure 4.9). After passing through the column, the polymer solution passes through the detector (Allcock and Lampe 1990) and then it is collected in the collector flask. Common detectors include a differential refractometer, absorption spectrophotometric detection (such as ultraviolet and infrared), light scattering photometer, and viscometer (Rubinstein and Colby 2003). The most common one is the differential refractometer.

![Figure 4.9](image_url) A typical GPC setup. The sample is injected at the injection port and it passes through the column where the molecules are separated in terms of their size. The bigger molecules do not enter the pores of the polymer beads that the columns are packed with, while the smaller molecules enter the pores and delay, thus eluting later than the bigger molecules.
The detector monitors the concentration of the polymer that is eluted and the chromatograph obtained is a plot of concentration against elution volume, which provides a qualitative indication of the molecular weight distribution. In order to convert a GPC chromatogram into a molecular weight distribution \( \frac{M_w}{M_n} \) and also calculate the average molecular weights, it is necessary to know the relationship between the molecular weight and the elution volume, \( V_e \). A calibration curve is usually obtained with the use of polystyrene or PMMA standards for GPC systems in organic solvents and PEG standards for aqueous GPC systems.

It should be noted that it is very common to present the chromatograph with respect to the elution time and not the elution volume. What is important to remember is that the higher the molecular weight the smaller the elution volume (or the shorter the elution time) (Allcock and Lampe 1990; Remm and Merrill 1991). A typical GPC chromatogram is shown in Figure 4.10. The first peak from the right corresponds to the precursor to a diblock copolymer, a homopolymer, while the peak on the left corresponds to the diblock copolymer. The fact that the peak of the diblock copolymer is at a shorter time confirms that the molecular weight of the diblock copolymer is of course bigger than the molecular weight of its precursor. Other useful information that can be obtained from the chromatogram is the full conversion of the homopolymer to the diblock copolymer due the lack of any extra peak in addition to the diblock copolymer curve.

There are other conventional techniques that also determine the molecular weight of polymers but not the molecular weight distribution. Specifically, with static light scattering (SLS) and osmometry, the \( M_w \) and \( M_n \) can also be determined, respectively (Abramson et al. 2004).

### 4.4.2 Determination of the Structure

Nuclear magnetic resonance (NMR) spectroscopy is commonly used for determination and confirmation of the chemical structure of polymers (Drobsny et al. 2003; Abramson et al. 2004). NMR can provide both qualitative and quantitative information with respect to the comonomer composition and the stereochemical configuration of the polymeric molecules (Drobsny et al. 2003). This is due to the fact that there is a proportional relation between the observed peak intensity in the NMR spectrum and the number of nuclei that produce the signal. Both conventional solution and solid-state (particularly for nonsoluble materials) NMR techniques are used for the characterization of polymeric materials (Mathur and Scranton 1996; Drobsny et al. 2003; Abramson et al. 2004; Zhang et al. 2005). Many types of nuclei can be observed, but the most frequently used for polymers are proton, \(^1\)H NMR and carbon-13, \(^{13}\)C NMR (Drobsny et al. 2003).
Synthesis, Properties, Characterization, and Processing of Polymeric

$^1$H NMR is widely used in order to provide information on the monomeric species used in the preparation of polymers (confirm the chemical structure), the average composition (for copolymers), tacticity, and configuration of polymeric chain (Mathur and Scranton 1996; Droby et al. 2003; Abramson et al. 2004; Zhang et al. 2005). These studies are done in solution and a disadvantage is that polymer spectra are frequently poorly resolved with broad overlapping lines (Droby et al. 2003). On the other hand, $^{13}$C NMR is more revealing than $^1$H NMR in polymer work because of the inherently wider spectra separation of the carbon chemical shifts that makes these spectra more interpretable (Droby et al. 2003; Zhang et al. 2005). NMR can also be used to study micellar solutions and investigate the phenomena within micelles (Droby et al. 2003), thus provide important information for the biomedical applications of micelles. Solid-state NMR, which is not as conventional, is very useful, since it provides information about secondary structure of polymers, proteins, and peptides (Mathur and Scranton 1996).

Infrared (IR) absorption spectroscopy is also used to provide information on the chemical, structural, and conformational aspects of polymeric chains (Abramson et al. 2004; Kasaal 2008). In IR spectroscopy, absorption of energies corresponding to transitions between vibrational or rotational energy states gives rise to characteristic patterns (Droby et al. 2003). These characteristic patterns can be translated into qualitative and quantitative information regarding the presence of functional groups, thus identifying the monomer types and their concentration within the polymer chain (Droby et al. 2003). IR spectroscopy is often used to monitor the degradation and modification of polymeric biomaterials like chitosan and polyurethane (Griesser 1991; Kasaal 2008).

Wide-angle x-ray scattering (WAXS) is a technique useful for providing the local structure of semicrystalline polymeric solid or polymeric networks (Gedde 1995; Callister 2003; Abramson et al. 2004; Matyjaszewski and Muller 2006). Under appropriate conditions, crystalline materials diffract x-rays, giving rise to spots or rings, and these, according to Bragg’s laws, can be interpreted as interplanar spacings. By using the appropriate model to fit the data, the crystalline chain conformation and atomic placements can be inferred, for example, if the chain is extended or it has the form of a helix (Abramson et al. 2004). WAXS is used for assessing structure with repeating distances typically less than 1 nm, whereas small angle x-ray scattering (SAXS) is useful for assessing bigger structures (Gedde 1995).

In particular, SAXS is used to determine the structure of many multiphase materials (Gedde 1995; Abramson et al. 2004) and it has been applied to study polymer systems for more than 30 years (Hadjichristidis et al. 2003). This technique requires an electron density difference to be present between two components (Abramson et al. 2004). It has been widely applied to morphological studies of copolymers and ionomers since it can provide information about the molecular weight, overall size, and internal structure of individual micelles (Abramson et al. 2004; Nair and Laurencin 2007). It can probe features of 1–100 nm in size. With appropriate modeling of the data, SAXS can provide detailed structural information like the dimensions of a micellar core (Abramson et al. 2004; Nair and Laurencin 2007).

Small angle neutron scattering (SANS) is in a way very similar to SANS and it is also used to provide information about the dimensions of nanophases of polymer samples of 1–100 nm. This technique is based on scattering neutrons and it also requires the two components to have different scattering densities. It is very common for deuterated analogues of the solvent or one part of the polymer to be used (Droby et al. 2003). It is commonly used to characterize polymers (especially block copolymers), proteins, DNA, polymeric networks (Seymour et al. 1998; Harada and Kataoka 2006; Melnichenko and Wignall 2007), and their interactions (complexes) (Galant et al. 2005; Melnichenko and Wignall 2007; Horkay and Hammouda 2008). The latter makes SANS a very useful technique to characterize polymer-DNA complexes that are used in gene delivery. Unfortunately, however, SANS instruments are only available at a few places around the world.

4.4.3 Mechanical and Thermal Properties Studies

Dynamic mechanical analysis (DMA) can be used to study the mechanical properties and the deformation behavior of polymers (Abramson et al. 2004; Menard 2008). It can be simply described as applying an oscillating force to the sample and analyzing the material’s response to that force (Menard 2008).
Therefore, properties like the tendency to flow (viscosity) and the stiffness (modulus) can be calculated from the phase lag and the sample recovery, respectively (Menard 2008). These properties are often described as the ability to lose energy as heat (damping) and the ability to recover from deformation (elasticity) (Menard 2008), which are of great importance for the biomedical applicability of polymers. What is usually measured with DMA is the sample modulus, which, of course, depends on the temperature, for example, glass at low temperatures has a high modulus, while a rubber at high temperatures has a low modulus. The $T_g$ of the polymers can also be determined by DMA (Abramson et al. 2004).

Differential scanning calorimetry (DSC) is another method that provides information about the thermal properties of the polymers. Specifically by DSC, the crystallization temperature, $T_c$, the melting temperature, $T_m$, as well as the $T_g$ of a polymer can be determined (Abramson et al. 2004; Kasaal 2008). DSC can also provide useful information about the degradation of a material since many polymeric materials used for bioapplications can be thermolyzed.

### 4.4.4 Surface Characterization

The surface characteristics of polymeric biomaterials are critically important since it is the surface of the material that will be in contact with the body, and the surface properties and composition are different from the bulk (Abramson et al. 2004). Atomic force microscopy (AFM) and scanning electron microscopy (SEM) are commonly used to characterize the surface of a polymeric biomaterial. SEM provides images of surfaces by focusing an electron beam on it, while AFM provides images of surfaces by applying force on it (Abramson et al. 2004).

Specifically, in AFM a sharp tip attached to a cantilever is scanned across a surface. As the tip moves over the material’s surface, changes in surface topography change the interatomic attractive or repulsive forces between the surface and the tip (Dee et al. 2002). The height adjustments or changes in interatomic force are recorded and used to construct images of surface topography (Dee et al. 2002). The resolution of AFM depends on the size of the tip (Dee et al. 2002)—the sharper the tip, the better the resolution. Under the proper conditions, images showing individual atoms can be obtained. Thus, a major feature of AFM is the ability to acquire three-dimensional images with Å or nm level resolution (Dee et al. 2002). One of AFM’s advantages is that imaging can be conducted without scanning, coating, or other preparation and under physiological conditions (Dee et al. 2002).

In SEM, an electron beam is scanned across the sample’s surface (Dee et al. 2002; Abramson et al. 2004). The primary electrons penetrate the surface and transfer energy to the material (Dee et al. 2002). In this way, sufficient energy is transferred to the sample and thus electrons (secondary electrons) are emitted from the sample. The intensity of the secondary electrons primarily depends on the topography of the surface (Dee et al. 2002); thus, by scanning the electron beam across the surface and determining the current generated from secondary electrons, images of the surface are obtained (Dee et al. 2002; Abramson et al. 2004). Some chemical information can be obtained from SEM but it is not specific (Dee et al. 2002); brighter and darker images reflect higher atomic number and lower atomic number, respectively (Dee et al. 2002). The disadvantages of SEM is that nonconductive samples, like most polymers and biological materials, must be coated with a conductive film, and that is conducted in a high-vacuum environment, which prevents biological samples from being investigated in their native state (Dee et al. 2002).

X-ray photoelectron spectroscopy (XPS) also known as electron spectroscopy for chemical analysis is based on the process of photoemission and provides chemical information about the surface (identification of the elements of the surface, determination of approximate atomic concentrations, and information about the chemical bonding) (Dee et al. 2002; Abramson et al. 2004).

Finally, contact angle measurements are used to characterize polymeric materials (Dee et al. 2002; Abramson et al. 2004) and are significant, since the adhesion of a number of cells types, including bacteria, granulocytes, and erythrocytes, has been shown, under certain conditions to correlate with solid–vapor surface tension (Abramson et al. 2004).
4.4.5 Characterization in Solution

In previous sections of this chapter, some techniques that characterize the bulk phase of polymers as well as their size in solution were described (SANS and SAXS). There are other techniques that can analyze the polymers in solution. SLS as mentioned before can give information about the $M_w$ of the polymer. It can also provide information about the size of the polymer—specifically, the radius of gyration ($R_g$) of a polymer (Hiemenz 1984; Allcock and Lampe 1990; Gedde 1995; Young and Lovell 2002; Hadjichristidis et al. 2003). $R_g$ is the root mean square distance of every point of the macromolecular chain from its center of mass. In order to obtain this information, a Zimm plot must be made that follows the equation

$$
\frac{KC}{R_\theta} = \left( \frac{1}{M_w} + 2A_2C + \cdots \right) \left( 1 + \frac{16\pi^2}{3\lambda^2} R_g^2 \sin^2 \left( \frac{\theta}{2} \right) + \cdots \right)
$$

(4.6)

where
- $\lambda$ is the wavelength of the laser of the equipment
- $\theta$ is the angle at which the detector is located with respect to the transmitted beam
- $A_2$ is the second virial coefficient (a measure of solvent–solute interactions)
- $K$ is the material constant
- $R_\theta$ is the Rayleigh ratio (contains information about the refractive index of the material)
- $C$ is the concentration of the polymer solution

However, in order to obtain a Zimm plot (an example of which is shown in Figure 4.11), light scattering measurements of polymer solution of different concentrations at different angles must be made, and that requires a considerable amount of sample. Moreover, these measurements can often prove to be time consuming and tricky.

It should be noted that unlike GPC, SLS does not require a calibration curve in order to determine the molecular weight of the polymer. However, the refractive index of the polymer that is being analyzed must be known in order to obtain the Zimm plot.

![Zimm plot obtained from SLS measurements. A number of solution of varying concentrations are measured at different angles and the data are extrapolated to zero concentration and angle to determine the molecular weight and the radius of the polymer.](image-url)
Dynamic light scattering (DLS) also called quasi-elastic light scattering or photon correlation spectroscopy provides information about the hydrodynamic radius, $R_h$, of the polymer in solution (Hiemenz 1984; Gedde 1995; Young and Lovell 2002; Hadjichristidis et al. 2003). DLS measures the correlation of the scattering intensity. From the correlation graph obtained, the diffusion coefficient ($D$) can be determined. Consecutively, $D$ can be related to the hydrodynamic radius through the Stokes–Einstein relation

$$R_h = \frac{k_BT}{6\pi\eta D} \tag{4.7}$$

where
- $k_B$ is the Boltzmann constant
- $D$ is the diffusion coefficient
- $T$ is the temperature
- $\eta$ is the solvent viscosity

It is important to point out that the Stokes–Einstein equation assumes that the sample has a spherical shape. DLS is commonly used to determine the size of micelle and aggregates in solution (Hadjichristidis et al. 2003; Harada and Kataoka 2006), an example of which is shown in Figure 4.12. A bimodal distribution of the hydrodynamic diameter of a block copolymer in aqueous solution is shown that corresponds to the unimers (block copolymer) and the micelles. Moreover, DLS is significant for the determination of the size of drug-polymer and DNA-polymer complexes. Another measurement that is useful for the characterization of the polymer complexes is zeta potential measurement, to determine the charge of complexes.

### 4.5 Processing

Depending on their biomedical application, polymers may need to be processed to produce the right material. Specifically, in order for a polymer to be employed in a medical device, the polymeric material must be manipulated physically, thermally, or mechanically into the desired shape (Allcock and Lampe 1990; Abramson et al. 2004). Polymers can be fabricated into shaped objects by casting, compression molding, injected molding, blow molding (to make hollow objects), thermofusion and thermoforming, and rotational molding (Allcock and Lampe 1990). They can also be expanded and then be stabilized in the expanded structure, for example, to make polyurethane foams (Allcock and Lampe 1990).
In addition, polymers can be coated on a surface using dipping, calendar coating, electrostatic coating, knife coating, roll coating, fluidized-bed coating and powder molding, and radiation-cured coatings (Allcock and Lampe 1990). Moreover, they can be fabricated into sheets or fibers by wet spinning, dry spinning, or electrospinning (Allcock and Lampe 1990; Abramson et al. 2004; Pham et al. 2006; Yoon and Fisher 2007; Sill and von Recum 2008).

For tissue engineering that is one of the most common biomedical applications of polymers, there are two basic strategies of polymeric scaffold fabrication: prefabrication and \textit{in situ} fabrication (Yoon and Fisher 2007). Prefabrication structures are cured before implantation and are often preferred since the polymeric scaffolds are formed outside the body allowing the removal of cytotoxic and non-bio-compatible component prior to implantation (Yoon and Fisher 2007). However, the scaffold may not properly fit in a tissue defect site causing gaps between the engineered graft and the host tissue, leading to undesirable results (Yoon and Fisher 2007). Therefore, \textit{in situ} scaffold are also being investigated, which involves curing of a polymeric matrix within the tissue defect itself (Yoon and Fisher 2007), like injectable polymeric gels (Kretlow et al. 2007; Klouda and Mikos 2008). This strategy has two main advantages: the deformability of an \textit{in situ} fabricated matrix creates an interface between the scaffold and the surrounding tissue, facilitating tissue integration, and it allows minimally invasive surgery techniques to be used since it may require a little as a narrow path for injection of the liquid scaffold (Yoon and Fisher 2007).

In terms of fabricating the polymeric scaffold, two methods are used: polymer entanglement and polymer cross-linking. Entanglement usually involves intertwining long, linear polymer chains to form a loosely bound polymer network (Yoon and Fisher 2007), a physical gel (not a covalent linked gel) (Patrickios and Georgiou 2003) (see Figure 4.13), while cross-linking involves the formation of covalent or ionic bond between individual polymer chains (Yoon and Fisher 2007). Caution should be made when referring to a polymeric network since it can be chemically cross-linked or be a physical gel where no chemical cross-links exist, only physical entanglements of the polymer chains (shown in Figure 4.13). These physical gels will solubilize in a solvent if given enough space and time to unravel, unlike the covalent linked networks.

The first fabrication method of a polymeric scaffold, polymer entanglement, is simple, allowing the polymer to be molded into a bulk material using heat, pressure, or both. However, the material often lacks mechanical strength, something that the second method has as its advantage. Chemical cross-linking enhances the mechanical strength. However, with the cross-linking method, a radical or ion is needed to promote cross-linking along with an initiator, such as heat, light, chemical accelerant, or time while leading to increased cytotoxicity, especially if the cross-linking takes place in situ (Yoon and Fisher 2007).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.13.png}
\caption{A covalently linked polymeric network (gel) and a physical gel.}
\end{figure}
A successful in situ fabrication of a polymer is PMMA when it is used for dental or bone cement (Abramson et al. 2004). The final polymerization step is carried out once the precursors (monomer or low molecular weight prepolymers) are in a casting or a mold device, yielding a solid, shaped end product (Abramson et al. 2004).

### 4.6 Bioapplications

#### 4.6.1 Polymeric Materials for Tissue Engineering

Tissue engineering as it was defined by Langer and Vacanti is “an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore or improve tissue function” (Langer and Vacanti 1993). It aims to regenerate or replace biological damaged or diseased tissue or generate replacement organs for a wide range of medical conditions such as heart diseases, diabetes, cirrhosis, osteoarthritis, spinal cord injury, and disfiguration (Langer and Vacanti 1993; Calafiore 2001; Matthew 2001; Grigorescu and Hunkeler 2003; Hoerstup et al. 2004; Salem and Leong 2005; Kretlow et al. 2007; Reddi 2007; Vert 2007; Yoon and Fisher 2007; Klouda and Mikos 2008; Place et al. 2009).

A typical scaffold is a biocompatible polymer in a porous configuration in the desired geometry for the engineered tissue, often modified to facilitate selective adhesion, while in some cases it is selective for a specific circulating cell population (Matthew 2001; Hoerstup et al. 2004). The first phase is the *in vitro* formation of a tissue construct by placing the cells and scaffold in an environment with growth media (in a *bioreactor*), in which the cells proliferate and elaborate extracellular matrix (Hoerstup et al. 2004; Place et al. 2009). In the second phase, the construct is implanted *in vivo* and remodeled to recapitulate the normal functional architecture of an organ or tissue (Hoerstup et al. 2004; Place et al. 2009). The key processes occurring during the *in vitro* and *in vivo* phase of tissue formation and maturation are (1) cell proliferation, sorting, and differentiation; (2) extracellular matrix production and organization; (3) degradation of the scaffold (for most applications); and (4) remodeling and potential growth of the tissue (Hoerstup et al. 2004). In Figure 4.14, a general paradigm of tissue engineering is illustrated.

---

**FIGURE 4.14** A general paradigm of tissue engineering is illustrated: (1) *in vitro* formation of a tissue construct by placing the cells and scaffold in an environment with growth media (in a *bioreactor*), in which the cells proliferate and elaborate extracellular matrix; (2) the construct is implanted *in vivo* and remodeled to recapitulate the normal functional architecture of an organ or tissue.
An ideal tissue engineering polymeric scaffold should combine many properties in order to provide a metabolically and mechanically supportive environment to facilitate tissue growth:

1. The first essential criterion is biocompatibility (Matthew 2001; Hoerstup et al. 2004; Place et al. 2009). Some important factors that determine biocompatibility, such as the chemistry, structure, and morphology, can be affected by polymer synthesis, scaffold processing, and sterilization.

2. It must also have a porous structure that will allow cellular ingrowth (Vert 2007). Depending on the application some scaffolds may require to have a porous morphology to help orient cells or surface properties to facilitate selective cell adhesion and/or migration, while in some other applications inhibition of cell adhesion is required (Matthew 2001; Hoerstup et al. 2004). Porosity, pore size, and pore structure are important factors to be considered with respect to nutrient supply to transplanted or regenerated cells (Hoerstup et al. 2004; Salem and Leong 2005; Vert 2007), while the hydrophobicity/hydrophilicity of the polymer should be considered for the enhancement or inhibition of cell adhesion and for the scaffolds wettability.

3. It should be mechanically strong enough to support the structural integrity of the implant. The mechanical stability needed depends on the application and may also vary with time. The mechanical stability is affected by many factors like the polymer chemistry (composition, structure, morphology, molecular weight, and molecular weight distribution) and the scaffold structure (density, shape, size, mass, pore size, and pore structure).

4. Similarly, depending on the application, it could be essential for the polymeric scaffold to be degradable. For most applications, a biodegradable polymeric scaffold that will allow its gradual and orderly replacement with functional tissue is needed (Matthew 2001; Grigorescu and Hunkeler 2003; Hoerstup et al. 2004; Salem and Leong 2005). The degradation, similarly to the mechanical stability, can be affected by many factors like the polymer chemistry, the scaffold structure, the pH and the ionic strength of the medium, enzymes, and the type and density of the cultured cells (Hoerstup et al. 2004).

5. Finally, since the polymeric scaffolds should be designed to mimic the body, it is desirable for them to have chemically modifyable functional groups onto which sugars, proteins, or peptides can be attached (Matthew 2001; Hoerstup et al. 2004). Moreover, in many citations the scaffold may be required to be modified in order to release, in a controlled manner, tissue-specific growth factors to enhance the process of organ or tissue regeneration (Salem and Leong 2005; Place et al. 2009).

Natural materials like polypeptides (collagen, gelatin, and silk) and polysaccharides (alginate, agarose, chitosan, and hyaluronic acid) are commonly used to fabricate tissue engineering scaffolds (Mathur and Scranton 1996; Seymour et al. 1998; Galant et al. 2005). Natural polymers are biocompatible and enzymatically biodegradable and their main advantage is that they contain biofunctional molecules that aid attachment, proliferation, and differentiation of cells. Their disadvantage arises from the fact that they are enzymatically degradable such that their degradation cannot be easily controlled in vivo and may not be desirable, depending on the application (Matthew 2001; Yoon and Fisher 2007). Furthermore, natural polymers are often weak in terms of mechanical strength, but cross-linking these polymers has been shown to enhance their structural stability (Yoon and Fisher 2007).

On the other hand synthetic polymers have the advantage that they can be easily moderated to change their structural stability, depending on the application. In general, it is easier to tailor the mechanical and chemical properties of synthetic polymers (Yoon and Fisher 2007; Place et al. 2009). Furthermore, since many synthetic polymers undergo hydrolytic degradation, a scaffold’s degradation rate should not vary significantly between hosts (Yoon and Fisher 2007) and should be easier to control. Finally, synthetic polymers must be nontoxic, readily available, and relatively inexpensive to produce, and in many cases should be able to be processed under mild conditions that are compatible with cells (Place et al. 2009). A significant disadvantage for using synthetic polymers is that some degrade into unfavorable products, often acids that can change the local pH and result in adverse responses (Yoon and Fisher 2007).
Synthetic polymers used in tissue engineering are usually polyesters, but polyanhydrides, polycarbonates, and polyphosphazenes are also used (Matthew 2001; Salem and Leong 2005; Yoon and Fisher 2007; Place et al. 2009). Polysters include poly(α-hydroxy acids) like poly(lactic acid) (PLLA) poly(glycolic acid) (PGA) and their copolymer poly[(lactic acid)-co-{glycolic acid}] (PLGA) that are the most widely used synthetic polymers in tissue engineering (Matthew 2001; Hoenstup et al. 2004; Klouda and Mikos 2008; Place et al. 2009). Other polyester, used in tissue engineering are poly(ε-c-caprolactone) (PCL), poly(propylene fumarate) (PPF), and poly(orthoesters) (Matthew 2001; Hoenstup et al. 2004; Yoon and Fisher 2007; Klouda and Mikos 2008). Moreover, PMMA, polyanhydrides, polyphosphates, polyphosphazenes, polycarbonates, and polyurathenes have also been used (Matthew 2001; Hoenstup et al. 2004; Yoon and Fisher 2007; Klouda and Mikos 2008; Place et al. 2009). Most of these polymers with the exception of the poly(α-hydroxy acids) are considered to be hydrophobic. The most common hydrophilic component of tissue engineering scaffolds is PEG. PEG is a hydrophilic, FDA approved, biocompatible polymer, which is mainly used in hydrogels (water absorbing polymeric networks) due to its ability to imbibe water (Grigorescu and Hunkeler 2003). Furthermore, thanks to its protein repellent effect, it can be useful as a noninterfering background upon which specific biological cues can be built up (Hoenstup et al. 2004). Other hydrophilic polymers like poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), and PHEMA have also been applied (Hoenstup et al. 2004).

### 4.6.2 Polymeric Gene Delivery Systems

Gene delivery is a term used when referring to the delivery of genetic material like DNA and siRNA into cells (also called transfection) (Merdan et al. 2002; Jordan 2003; Langer 2005; Wong et al. 2007; Luten et al. 2008). Gene delivery is essential in gene therapy that aims to treat or cure many diseases (Geddes and Alton 1998; Hersh and Stepeck 1998; Langer 2005), in tissue engineering and is also used to study gene function.

A gene delivery vector is essential in order to carry the hydrophilic, negatively charged DNA through the hydrophobic and negatively charged cell membrane. The first vectors used for gene delivery were viruses, but due to their disadvantages—limitations on the size of DNA that they can carry, their high production cost, and, most importantly, their safety risks (immunogenicity and potential oncogenicity)—nonviral vectors have been developed (Merdan et al. 2002; Jordan 2003; Mrsny 2005; Wong et al. 2007; Luten et al. 2008). Nonviral vectors are divided into two main categories: lipid- and polymer-based, with the polymeric nonviral vectors having the advantage that their properties are easier to customize.

The main steps of polymeric gene delivery (see Figure 4.15) are (Merdan et al. 2002; Jordan 2003; Wong et al. 2007; Luten et al. 2008) as follows: (1) DNA/polymer complexation. The cationic polymer neutralizes the charged phosphate backbone of DNA to prevent charge repulsion with the negatively charged cell membrane and condenses the bulky structure of the DNA to form nanosize complexes. (2) DNA/polymer complex (also called polyplex) passes through the cell membrane. The complex is transported into the cell, through the cell membrane, by a nonspecific or receptor-mediated endocytosis. (3) The complex enters the cytoplasm usually in an endosome (depending on the cell type and the type of entry). The complex is later released from the endosome into the cytoplasm. (4) Cytosolic transport to the nucleus. The complex or the DNA, if it is already released from the complex, passes through the cytoplasm close to the nucleus. (5) The transfer of the genetic material into the nucleus where it is free to be encoded into a therapeutic protein or be inserted into the genome.

The most important property that a polymeric vector should have is to be nontoxic (biocompatible). It is also desirable to be biodegradable. If the biodegradability of the polymer is modulated correctly, with respect to the application, it could decrease the toxicity of the vector and also help the DNA release from the complex into the cytoplasm. A polymeric vector must be able to condensate the genetic material. This is usually done through electrostatic interactions by using cationic polymeric vectors. However, studies on noncondensing polymeric systems have also been done (Kabanov et al. 2005; Nicolaou et al. 2005). Polymeric vectors with permanent cationic charges are not preferred since they will condensate the DNA so strongly that it will prevent its release into the cell. Thus, ionizable cationic polymer vectors
are used, usually with a pK between 5 and 7. Finally, the polymeric vector should be hydrophilic in order to be mobile in the aqueous medium and the body; the vector may be composed of both hydrophobic and hydrophilic components and be stabilized in an aqueous solution by forming micelles or/and aggregates.

One of the first polymers used as a nonviral gene delivery agent polye(ethylenimine), PEI (chemical formula shown in Figure 4.6) (Boussif et al. 1995; Godbey et al. 1999a,b; Godbey and Mikos 2001; Orgis 2005). PEI has a high positive charged density; every third atom of PEI is a protonatable amino nitrogen atom (Boussif et al. 1995; Orgis 2005) and, thus, condensates the DNA effectively and delivers it into cells. It has been used for both in vitro and in vivo applications (Orgis 2005) and it is often used as the golden standard to which many novel synthetic polymer vectors are compared. In fact, since 1995 that PEI was first trialed in transfection (Boussif et al. 1995), over 800 publications (as of June 2009) have appeared that use PEI-based polymers as transfection agents. Many of these investigations use copolymers (Merdan et al. 2002; Wong et al. 2007) and degradable (Luten et al. 2008) PEI-based polymers.

PDMAEMA is also one of the first polymers to be investigated that is still commonly used in gene delivery since it has shown great potential (Verbaan et al. 2005). Several in vitro studies on DMAEMA and its derivatives—copolymers (van de Wetering et al. 1997, 1998, 1999a,b, 2000; van Dijk-Wolthuis et al. 1999; Georgiou et al. 2004; Georgiou et al. 2005; Verbaan et al. 2005; Georgiou et al. 2006; Xu et al. 2009) and degradable (Luten et al. 2003; de Wolf et al. 2007; Luten et al. 2008) DMAEMA-based polymers—have been reported in the literature. In vivo evaluation of PDMAEMA-based polymers has also been made (van de Wetering 1999b; Verbaan et al. 2005). PDMAEMA homopolymers like PEI homopolymers are also often used to compare the newly synthesized novel polymer vectors that are being investigated.

PEI and PDMAEMA homopolymers are nondegradable polymers. Other common nondegradable polymers that were used in gene delivery are chitosans (Borchard and Bivas-Benita 2005), poly(1-lysine)s (Lee and Kim 2005), cyclodextrin-containing polymers (Pun and Davis 2005), and dendrimers (Tang et al. 1996; Hudde et al. 1999; Cloninger 2002; Kubasiak and Tomalia 2005; Luten et al. 2008). The latter are spherical, highly branched polymers prepared either by divergent (starting from a central core molecule) or by convergent (starting with what will become the periphery of the molecule building inwards) synthesis strategies (Cloninger 2002; Merdan et al. 2002). The degree of branching is expressed in the
generation of the dendrimer (Merdan et al. 2002). Commonly used dendrimers are poly(amideamines), one of which is commercially available, called SuperFect®. Interestingly, the gene delivery performance is improved with the “activated” dendrimers that are, in fact, fractured dendrimers that show 50-fold enhanced transfection levels compared to the intact polymer (Tang et al. 1996; Cloninger 2002; Merdan et al. 2002). This is may be attributed to a better binding of the DNA, better ability of the polymer to complex the DNA due to the increased flexibility of the fractured polymer (Tang et al. 1996). It should be mentioned that SuperFect is also commonly used as a standard to compare to in gene delivery studies of novel cationic polymers (Georgiou et al. 2004, 2005, 2006).

Degradable polymers used for delivery of genetic material into cells are often polyesters (Lim et al. 2005; Lynn et al. 2005; Luten et al. 2008), especially poly(β-amino ester)s (Lynn et al. 2005), polysaccharides (Azzam and Domb 2005), polyurathenes (Luten et al. 2008), phosphor-containing polymers (Luten et al. 2008), and derivatives of the cationic nondegradable polymers (Luten et al. 2008). The potential advantage of biodegradable carriers as compared to their nondegradable counterparts is their reduced toxicity (provided their degradation products are nontoxic) and the avoidance of accumulation of the polymer in the cells after repeated administration (Luten et al. 2008). Furthermore, the degradation of the polymer can be used as a tool to release the plasmid DNA into the cytoplasm (Luten et al. 2008).

Other important points to consider when engineering a polymeric gene delivery vector, besides its toxicity and chemical structure, is the molecular weight, the molecular structure, and the composition of the polymer. The effect of the molecular weight on the transfection efficiency of the polymer has been studied with contradicting results (Godbey et al. 1999b; Georgiou et al. 2004; de Wolf et al. 2007), probably due to the range of the molecular weight tried, and the difference of the polymers’ chemical structure and cell types used. What can be safely concluded from these studies is that by increasing the polymer’s molecular weight, its toxicity is also increased (Georgiou et al. 2004; de Wolf et al. 2007). Polymers of different molecular structure such as linear (van de Wetering et al. 1997, 1998, 1999a,b, 2000; van Dijk-Wolthuis et al. 1999; Godbey et al. 1999a,b; Godbey and Mikos 2001; Verbaan et al. 2005), branched, stars (Georgiou et al. 2004, 2005, 2006; Xu et al. 2009), and dendrimers (Tang et al. 1996; Hudde et al. 1999; Cloninger 2002; Kubasiak and Tomalia 2005) have also been studied and, as mentioned before, the molecular structure has shown an important effect on the vectors’ ability to transfer genes into cells (Tang et al. 1996; Merdan et al. 2002; Georgiou et al. 2004). Moreover, the introduction of a second monomer, a comonomer, into the polymer’s structure influences the polymers transfection efficiency and cytotoxicity (van de Wetering et al. 1998, 1999b, 2000; van Dijk-Wolthuis et al. 1999; Georgiou et al. 2005, 2006; Verbaan et al. 2005; Xu et al. 2009). In general, copolymers with PEG-containing groups have reduced toxicity compared to their homopolymer counterparts (van de Wetering et al. 1998, 2000; Georgiou et al. 2005). Finally, note that, in general, direct comparison of different published studies should be avoided since transfection protocols, reported genes used, molecular weights, and polydispersities of the polymer and cell types used may vary.

It should be stated that the human body is a very complex environment. So naturally this was taken into account and many studies that aim at a specific organ or a specific type of cells, like cancer cells, have been reported (Hersh and Stopeck 1998; Kircheis and Wagner 2001; Ouyang et al. 2001; Merdan et al. 2002; Kinsey et al. 2005; Mrsny 2005). Commonly, studies target cancer cells (Hersh and Stopeck 1998; Merdan et al. 2002; Mrsny 2005) or aim to deliver genetic material into the lung (Kinsey et al. 2005) or liver (Ouyang et al. 2001). In order to achieve this, a targeting moiety, enabling uptake into a specific cell type is incorporated onto the polymer (Merdan et al. 2002).

### 4.6.3 Polymers for Drug Delivery

The selective and controlled delivery of drugs to malignant cells is essential for a successful treatment. There are many factors that influence the delivery of the drug to the intended target (Bae and Kwon 1998; Yokoyama 1998; Barrat et al. 2001; Hadjichristidis et al. 2003; Harada and Kataoka 2006; Qiu and
Bae 2006; Kabanov and Gendelman 2007; Liu et al. 2009). Specifically, many drugs encounter solubility and stability problems when administered into the body because they are hydrophobic while the body and the bloodstream in particular consist mostly of water (Yokoyama 1998; Hadjichristidis et al. 2003; Harada and Kataoka 2006). Moreover, factors like the drug’s absorption, distribution, and elimination influence its delivery to the target site (Barrat et al. 2001). Thus, drug delivery systems, also called controlled released systems, have been designed in order to deliver the drug to a specific site, at a specific time scheme, and in a specific release pattern (Bae and Kwon 1998; Barrat et al. 2001). Extensive research has been done on polymer-based drug delivery systems since polymers are easy to modulate and modify to encapsulate the drug, to be target specific, and to be stimuli-responsive to release the drug.

Important characteristics that a polymer-based drug delivery system should have is as follows: to be biocompatible, to have small and uniform size (in the nanometer scale <200 nm), to have long circulation in bloodstream, to be easily sterilized (usually by filtration), not to have long-term accumulation, to be applicable to various drugs, to be able to encapsulate a high drug content but maintain water solubility, to have the right microenvironment for drug preservation (not inhibit the drug’s activity), and to release the drug in a target- and time-controlled manner (Bae and Kwon 1998; Yokoyama 1998; Kabanov and Gendelman 2007).

Many polymeric drug delivery systems have been fabricated in the last few decades (Bae and Kwon 1998; Yang and Robinson 1998; Yokoyama 1998; Barrat et al. 2001; Brown 2001; Domb et al. 2001; Haeverdings et al. 2001; Felt et al. 2001; Kratz et al. 2001; Maysinger et al. 2001; Michniak and El-Kattan 2001; Wollfson et al. 2001; Worakul and Robinson 2001; Harada and Kataoka 2006; Qiu and Bae 2006; Kabanov and Gendelman 2007; Rapoport 2007; Liu et al. 2009); biodegradable polymeric systems (Domb et al. 2001), for a specific organ or tissue (Felt et al. 2001; Haeverdings et al. 2001; Maysinger et al. 2001; Kabanov and Gendelman 2007), for a specific disease (Kratz et al. 2001; Rapoport 2007), for drug delivery via a specific route (Michniak and El-Kattan 2001; Wollfson et al. 2001; Worakul and Robinson 2001), and for specific drug such as insulin (Brown 2001).

It is not very easy to name the most common polymers used for drug delivery. Most polymeric drug delivery systems form micelles or are a part of nanoparticles (Kabanov and Gendelman 2007; Rapoport 2007). As mentioned before, a micelle is formed by amphiphilic block copolymer (Figure 4.8). The inner part of the micelle, called the micelle core, is composed of the hydrophobic core that is usually poly(propylene glycol), poly(D,L-lactide), PCL, while the outer part of the micelle, called the micelle shell is composed of hydrophilic block, which is often PEG (Harada and Kataoka 2006; Kabanov and Gendelman 2007; Rapoport 2007). On the other hand, nanoparticles are often composed of insoluble polymer(s). During their formulation drug is captured within the precipitating polymer, forming nanoparticles, and then released upon degradation of a polymer in the biological environment (Kabanov and Gendelman 2007).

In order for the drug to be released from the polymeric micelle or nanoparticle, often a stimuli response of the polymeric material is required (Bae and Kwon 1998; Liu et al. 2009). Thus, polymeric biomaterials that are responsive to temperature, pH, ionic strength, enzymatic conversion, or magnetic field have been developed (Bae and Kwon 1998; Liu et al. 2009). Alternatively, drug release can be initiated by polymer degradation, thus eliminating the need to remove the scaffold after drug release (Liu et al. 2009).

### 4.6.4 Other Biomedical Applications of Polymers

Tissue engineering, gene, and drug delivery are just three of a number of biomedical applications of polymers. Just to name these applications in order to demonstrate this wide range of usages, polymers are used in diagnostics (imaging) (Borovetz et al. 2004; Kim et al. 2007; Wolinsky and Grinstaff 2008; Khentong et al. 2009) in cardiovascular devices and heart valves (Alcock and Lampe 1990; Bhuveneshwar et al. 2001; El-Zaim and Heggers 2001; Abramson et al. 2004; Borovetz et al. 2004; Venkatraman et al. 2008; Kidane et al. 2009), in surgery and as orthopedic implants (El-Zaim and Heggers 2001; Rokkanen 2001;
Tomita et al. 2001; Borovetz et al. 2004; Tran et al. 2009), as contact lenses (Allcock and Lampe 1990; Abramson et al. 2004), in dressings for burns and wounds (Sheridan et al. 2001; Borovetz et al. 2004), in dental applications (Bascones et al. 2001), and for cosmetic implants (El-Zaim and Heggers 2001).

4.7 Conclusions

Polymers represent a broad family of materials, which are cost-effective and easy to modulate, and have properties that make them useful in a variety of biomedical applications. In this chapter, the main synthetic methodologies, properties, characterization and processing methods, and examples of biomedical applications of polymers were summarized.

References


