Novel Possibilities for
Advanced Molecular Structure Design
of Polymers and Networks

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Om du försöker hitta Hem men istället hittar en grop, kan du försöka att leta efter en grop. Då skulle du med all säkerhet inte hitta en grop, vilket skulle vara bra, för då kanske du hittar någonting du inte letar efter, vilket skulle kunna vara precis det du letar efter.
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ABSTRACT

Synthetic and degradable polymers are an attractive choice in many areas, since it is possible to control the way in which they are manufactured; more specifically, pathways to manipulate the architecture, the mechanical properties and the degradation times have been identified. In this work, L-lactide, 1,5-dioxepan-2-one and ε-caprolactone were used as monomers to synthesize polymers with different architectures by ring-opening polymerization. By using novel initiators, triblock copolymers, functionalized linear macromonomers and star-shaped aliphatic polyesters with well-defined structures have been synthesized. To synthesize triblock copolymers, cyclic germanium initiators were studied. The polymerization proceeded in a controlled manner although the reaction rates were low. To introduce functionality into the polymer backbone, functionalized cyclic tin alkoxides were prepared and used as initiators. During the insertion-coordination polymerization, the initiator fragment consisting mainly of a double bond was incorporated into the polymer backbone. The double bond was also successfully epoxidized and this gave unique possibilities of synthesizing graft polymers with precise spacing. The macromonomer technique is a very effective method for producing well-defined graft polymers. Spirocyclic tin initiators were synthesized and used to construct star-shaped polymers. The star-shaped polymers were subsequently crosslinked in a polycondensation reaction. These crosslinked structures swelled in water, and swelling tests showed that by changing the structure of the hydrogel network, the degree of swelling can be altered. A first evaluation of the surface characteristics of the linear triblock copolymers was also performed. AFM analysis of the heat-treated surfaces revealed nanometer-scale fibers and tests showed that keratinocytes were able to grow and proliferate on these surfaces.

Keywords: ring-opening polymerization, coordination-insertion, germanium, cyclic tin alkoxides, spirocyclic initiators, poly(L-lactide), poly(1,5-dioxepan-2-one), triblock, star-shaped, network, functionalization, morphology, AFM
SVENSK SAMMANFATTNING


Triblock sampolymerer, makromonomerer funktionaliserade med en dubbelbindning samt stjärnformade polymerer har syntetiserats kontrollerat. Dessa har i sin tur använts i efterföljande reaktioner för att skapa mer avancerade strukturer.

- Att använda funktionaliserade makromonomerer där reaktionspunkterna är förutbestämda för att syntetisera nya strukturer är mycket användbart. För att utröna dubbelbindningens reaktivitet i förgrenningsreaktioner utfördes epoxideringstest. Utan att påverka resterande delar av huvudkedjan bildades epoxider, vilka kan användas för att skapa förgrenade polymerer eller nätverk.


- En god interaktion mellan substrat och levande celler är fundamentalt inom tissue engineering. Andra forskargrupper har visat att substratets topografi och morfologi påverkar cellernas utbredning påtagligt och att det går att styra cellernas tillväxtutbildning med hjälp av olika mönster i substratet. För att finna vägar till att stimulera cellers adhesion, spridning och orientering har materialens ytor studerats...
LIST OF PAPERS

This thesis is a summary of the following papers:

I. "Use of Germanium Initiators in Ring-Opening Polymerization of L-Lactide"
   A. Finne, Reema and A. C. Albertsson

II. "L-Lactide Macromonomer Synthesis Initiated by New Cyclic Tin Alkoxides Functionalized for Brushlike Structures"
    M. Ryner, A. Finne, A. C. Albertsson and H. R. Kricheldorf
    *Macromolecules* **2001**, *34*, 7281-7287

III. "New Functionalized Polyesters to Achieve Controlled Architectures"
     A. Finne and A. C. Albertsson

IV. "Controlled Synthesis of Star-Shaped L-Lactide Polymers Using New Spirocyclic Tin Initiators"
    A. Finne and A. C. Albertsson
    *Biomacromolecules* **2002**, *3*, 684-690

V. "Polyester Hydrogels with Swelling Properties Controlled by the Polymer Architecture, Molecular weight, and Crosslinking Agent"
   A. Finne and A. C. Albertsson

VI. "Well-Organized Phase-Separated Nanostructured Surfaces of Hydrophilic/Hydrophobic ABA Triblock Copolymers"
    A. Finne, N. Andronova, A. C. Albertsson
    *Biomacromolecules* **2003**, *4*, 1451-1456
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**ABBREVIATIONS**

- **AFM**: atomic force microscopy
- **DS**: degree of swelling [%]
- **DSC**: differential scanning calorimetry
- **DXO**: 1,5-dioxepan-2-one
- **ε-CL**: ε-caprolactone
- **ECM**: extracellular matrix
- **ESEM**: environmental scanning electron microscope
- **HOMO**: highest occupied molecular orbital
- **LLA**: L-lactide
- **LUMO**: lowest unoccupied molecular orbital
- **MWD**: molecular weight dispersity
- **NMR**: nuclear magnetic resonance
- **PLLA**: poly(L-lactide)
- **PEG**: poly(ethylene glycol)
- **ROP**: ring-opening polymerization
- **SEC**: size exclusion chromatography
- **SEM**: scanning electron microscope
- **mCPBA**: m-chloroperoxybenzoic acid

**SYMBOLS**

- **DP**: degree of polymerization
- **[I]**: initiator concentration [M]
- **[M]**: monomer concentration [M]
- **[M]₀**: initial monomer concentration [M]
- **Mₐ**: number-average molecular weight [g/mol]
- **Tₐ**: crystallization temperature [°C]
- **Tₓ**: glass transition temperature [°C]
- **Tₘ**: melting temperature [°C]
- **W**: final weight [g]
- **W₀**: initial weight [g]
1. **PURPOSE OF THE STUDY**

The present work discusses the research reported in the appended publications. The work was designed to provide a broader experimental basis for a final conclusion as to whether or not new initiators can be used to synthesize well-defined usable polymers with different architectures.

Synthetic polymers have found an enormous amount of different applications during recent decades. Every application requires specific properties. One major problem is that the synthetic polymers are relatively simple compared to the polymers built by nature. An objective of molecular architecture, and also of this work, is to design new specialized polymers in a controlled way. Studies have been made to assess the use of new initiators for obtaining functionalized polymers as well as polymers with advanced and controlled architectures. Three different types of initiators have been used:

- Germanium initiators
- Functionalized cyclic tin alkoxide initiators
- Spirocyclic tin alkoxide initiators

Subsequent reactions of the synthesized macromonomers and polymers were studied in order to determine their usefulness. Epoxidation and crosslinking reactions were carried out using the functionalized macromonomers and the star-shaped polymers respectively.

The potential application of polymers synthesized in this work is in the biomedical field. The morphology and topography of the material are therefore decisive since cell adhesion and spreading are influenced by the physico-chemical characteristics of the underlying substrate. Some surface characterization and cell growth studies have been performed as a part of this work.
2. **INTRODUCTION**

2.1 Background

Synthetic polymers are nowadays used all around us. Their applications range from electrical conductors to scaffolds in tissue engineering. Properties such as hydrophilicity, degradation rate and mechanical properties have to be optimized in relation to the envisioned application. There are various ways to influence these properties; the most common being by copolymerization or by synthesizing functionalized polymers with specific architectures. The ability to control and reproduce the reactions is extremely important in every method. Different characterization methods make it possible to obtain the exact structure of the material and to relate the material properties to this structure. Thus it is possible to design the synthetic approach for achieving the best material properties for the desired application. In the medical field, the need for specialized materials with controlled properties is high. Loss or failure of organs as a result of an injury or other type of damage is a growing human health problem. The health care costs in North America for tissue loss or end-stage organ failure exceed $400 billion per year.¹ There are not enough donors and the need for synthetic alternatives is growing. Tissues and organs consist of living cells arranged within a framework called the extracellular matrix (ECM). The ECM is a gel composed of proteins and polysaccharides, and it plays an important role during growth and wound repair. It meshes the cells together and serves as a reservoir for the signaling molecules that control the migrating, proliferating, and differentiating cells. It also provides strength, rigidity, cellular communication, cellular protection and transport of nutrients and hormones. The ECM acts as the communication highway between cells and other extracellular fluids. Because of the local differences in the composition and organization of the ECM tendons resist tension and cartilage resist compression. Degeneration of ECM affects, for example, the ability to effectively assimilate nutrients into the cell, it will result in a slowed repair of damaged tissue with increased scar tissue deposition. Artificial substitutes for ECM are called scaffolds, and tissue engineering is the development of these artificial scaffolds. Laboratory-grown tissues, cells and/or molecules are cultured in a temporary three-dimensional scaffold to form the new organ or tissue. The function of a scaffold is to act as a guide and to direct the growth of the cells that migrate from the surrounding tissue or the cells that have been seeded within the scaffold prior to implantation. This method provides opportunities to solve the organ donor deficiency problem. The demands of the scaffolds are many; they must provide a suitable substrate for cell attachment, proliferation, and cell migration. In addition, there are several design criteria:
Introduction

- The material should have the correct pore size, pore orientation, porosity and fiber structure
- The surface should permit cell adhesion
- The scaffolds should be biocompatible and degradable
- The material should maintain its form stability, be reproducibly processable into a three-dimensional structure and mechanically strong

Resorbable polymers are preferred in medical applications, because polymers that do not degrade carry the permanent risk of giving unwanted tissue responses. It is also advantageous in self-repair processes to have a device that can be used as an implant but does not require a second surgical intervention for removal. Polymers, both degradable and non-degradable, are already used in the body today to assist and replace the function of organs and tissues. The applications using "biomedical" polymers range from the long-term, as with a pacemaker casing, to the short-term like a suture. Because of the wide spectra of applications the rate and extent of degradability of a polymeric biomaterial must be predetermi ned for each assigned function. Factors influencing the degradability are, for example, chemical structure, copolymer composition, architecture, molecular weight, morphology, surface area and medium character. Tailoring an implant for controlled degradation and transfer of stress to the surrounding tissue as it heals at an appropriate rate is one of the greatest challenges facing researchers today.

2.2 Monomers

Among the various families of degradable polymers, aliphatic polyesters have a leading position. They are most effectively derived from ring-opening polymerization and they have long been considered as degradable materials for medical applications. The interest has been high since the hydrolytic and/or enzymatic chain cleavage yields \( \omega \)-hydroxyacids, which are in most cases ultimately metabolized.

2.2.1 L-Lactide (LLA)

\[
\begin{align*}
\text{L-lactide} & \quad \text{poly(L-lactide)} \\
(\text{dimer of lactic acid}) & \quad \text{seamcrystalline} \\
T_m \text{ PLLA} & \quad 170-190^\circ C \\
T_m \text{ LLA} & \quad 97^\circ C \\
T_g \text{ PLLA} & \quad 55-60^\circ C \\
\end{align*}
\]

*Figure 2.1 Structure and properties of L-lactide (LLA) and poly(L-lactide) (PLLA).*
Poly(L-lactide) (PLLA), Figure 2.1, is a semicrystalline polymer able to form spherulites and lamellar crystals.11 The polymer is degradable having low immunogenicity. It is considered to be biocompatible and is often utilized as a medical material.12-14 PLLA belongs to the group of poly(α-hydroxy acids), and the hydrolysis of PLLA yields lactic acid. Lactic acid is also a by-product of the anaerobic metabolism in the human body. It is incorporated into the tricarboxylic acid cycle and finally excreted by the body as carbon dioxide and water. PLLA exhibits high tensile strength and low elongation, and consequently it has a high modulus making it suitable for load-bearing applications. However, PLLA is a hydrophobic polymer having no reactive chain group and the use of PLLA is therefore limited. It is difficult to chemically attach active molecules like drugs and recognition agents onto these and other polyesters. The rather low hydrophilicity, due to its non-polar pendant methyl substituents, results in a limited water uptake. This in turn results in a slow hydrolytic degradation rate and the long-term biocompatibility can be affected. The degradation kinetics of the implant are important for its biocompatibility. It is well accepted that the degradation by-products are responsible for tissue reactions and if large quantities of by-products are released per time unit, they cannot be adequately handled by the clearing capacity of the surrounding tissue.15-17

2.2.2 ε-Caprolactone (ε-CL)

![Structure of ε-caprolactone](image)

Figure 2.2 Structure and properties of ε-caprolactone (ε-CL) and poly(ε-caprolactone) (PCL).

The caprolactone monomer, Figure 2.2, is a colorless liquid. Poly(ε-caprolactone) (PCL) is a partially crystalline degradable thermoplastic polymer with ε-hydroxy-caproic acid as the major degradation product. The field of application is wide and includes, for example, resins for surface coatings, adhesives and fabrics. It also finds a use as stiffener for orthopedic splints, compostable bags and sutures.18 The degradation rate is slower than that of PLLA, and it is designed for use in long-term implantable systems. Table 2.1 compares the mechanical properties of PLLA and PCL.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Tensile strength [Pa \cdot 10^5]</th>
<th>Elongation (%)</th>
<th>Modulus [Pa \cdot 10^5]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>500-800</td>
<td>5 - 10</td>
<td>27-40 \cdot 10^3</td>
</tr>
<tr>
<td>PCL</td>
<td>200-300</td>
<td>300 - 500</td>
<td>2-3 \cdot 10^3</td>
</tr>
</tbody>
</table>

Table 2.1 Comparison of the mechanical properties of commercial PLLA and PCL.19
2.2.3 1,5-Dioxepan-2-one (DXO)

![Structure of DXO]

The synthetic route to obtain 1,5-dioxepan-2-one (DXO) was published for the first time in 1972. Poly(1,5-dioxepan-2-one) (PDXO) is an amorphous polymer, and amorphous thermoplastics without chemical and physical crosslinks do not have any form stability, which is a drawback in many applications. PDXO copolymers of different kinds have therefore been investigated. Copolymerization with PLLA has the advantage that the crystallinity, brittleness and high melting point of PLLA is decreased. The copolymers show a low stiffness and high elasticity compared to PLLA. The DXO/LA copolymers are interesting materials, with possible applications in e.g. the biomedical field. The degradation has been studied and the copolymer is hydrolyzed mainly by ester bond cleavage. Degradation studies of triblock copolymers, PLLA-PDXO-PLLA, revealed that the degradation rate was influenced by the original molecular weight and not by the composition. Microspheres of the copolymers have been prepared and the drug release pattern investigated, by altering the components in the polymer could the degradation and erosion be varied. Another alternative way of improving the stability of PDXO is by chemical crosslinking, either by using tetrafunctional bis(e-caprolactone) as a crosslinking agent or by photocrosslinking. The crosslinked films have a high degree of swelling in chloroform, are elastic without crystallinity and have a high glass transition temperature (Tg). Crosslinked PDXO has been used as substrate for grafting acrylamide in attempts to design new degradable systems.

2.3 Ring-opening polymerization

Aliphatic polyesters can be synthesized through either polycondensation of acids and alcohols or ring-opening polymerization (ROP) of cyclic esters. In contrast to the traditional step-polycondensation method, the ROP of a cyclic ester is an effective method of preparation of an aliphatic polyester. Under rather mild conditions, high molecular weight aliphatic polyesters can be prepared in short periods of time. The development of ROP of lactones, anhydrides and carbonates started around 1930. ROP of lactones can be carried out in the melt, in the bulk or in solution and by, for example, cationic, anionic, free radical, active enzymatic or coordination-insertion mechanisms depending on the monomer and the catalyst. During a typical ROP
reaction, the chain end reacts with a new monomer during the propagation step and the kinetics during the polymerizations then follow the typical pattern of a chain-growth polymerization.

Numerous publications during the past years have shown that it is possible to use ROP in the living and controlled polymerizations of cyclic esters. Living polymerization means that the initiated species maintain their activity until all monomers have reacted. There is no irreversible deactivation (termination) or irreversible transfer. Kinetic studies can be carried out to elucidate whether the reaction is living. If there is no termination during the polymerization, 

\[ -\ln \left( \frac{[M]_t}{[M]_0} \right) = \ln([\text{monomer concentration}]) \]

should be a linear function of time. Where \([M]_0\) is the initial monomer concentration and \([M]_t\) the concentration at a given reaction time. Without any transfer, the degree of polymerization (DP) should be a linear function of monomer conversion. It is correct to say that the polymerization is living if both these plots are linear.

2.3.1 Coordination-insertion mechanism

When metal alkoxides containing free p- or d-orbitals of a favorable energy (Mg, Sn, Ti, Zr, Fe, Zn, Al, Sm, Zn-alkoxides) are used as initiators, a "coordination-insertion" mechanism is proposed, Figure 2.4. This mechanism involves a rearrangement of polarized covalences and no ionic species. This is an active research area where new compounds are continuously being tested as initiators.
2.3.2 Ring-opening polycondensation

Ring-opening polycondensation reactions can be used for the synthesis of new molecular architectures. One example is presented in Scheme 2.1, were the initiator is cyclic and has two reactive bonds. The different functional groups can react with each other and the kinetics follows the step-growth polymerization. Polymers with different end groups as well as networks have been synthesized by this method.\textsuperscript{46, 47}

\textit{Scheme 2.1 Schematic presentation of ring-opening polycondensation.}

2.4 Macromolecular design

ROP enables the polymer molecular weight and backbone stereochemistry to be controlled and it can yield macromolecular samples with narrow molecular weight distributions. Such a tuning of polymerization reactions is important because of the close relationship between molecular characteristics and material properties. A representative example of the correlation between structure and properties is the differences between PCL and 2-oxepan-1,5-dione. PCL cannot be used as a packaging material because its melting temperature ($T_m$) is too low. By synthesizing 2-oxepane-1,5-dione instead, which has the same structure as CL except that the central methylene group is replaced by a carbonyl group, a semicrystalline polymer with a high $T_m$ (147°C) is obtained.\textsuperscript{48}

2.4.1 Copolymerization

The idea behind a resorbable material in medical applications is that the scaffold should act as a temporary replacement while the tissue is regenerating. A major problem is to find a material that starts to degrade and lose its mechanical properties in a predetermined way while the damaged tissue is regenerating and then disappears afterwards without a trace. The material needs good mechanical properties to be able to stand up to all the complex forces and maintain its form stability, and for this reason crystalline materials are often used. The drawback with crystalline materials is that they can cause adverse tissue responses. The material has hard edges, which cause
irritation and inflammation. A crystalline material also becomes brittle during degradation, the surface cracks and pieces from the surface are loosened.49-51 Amorphous polymers are much better materials in this sense, but instead they may suffer from poor mechanical properties. To meet all the demands, degradable block copolymers have been found to be promising biomaterials because of the potential to manipulate their amphiphilic behavior, and their mechanical and physical properties by adjusting the ratio of the building blocks or by adding new blocks of desired properties. This is one of the simplest and most widely used ways of modifying polymer properties to meet specific requirements, and it involves random, alternating copolymers and segmental block block copolymers. The research in this area has been going on for many years and there are many publications regarding block copolymers and their properties.

Efforts to increase and vary the hydrophilicity of PLLA using copolymerization are well documented because PLLA is often used as a polymer in medical applications and its high hydrophobicity is a limiting factor. Changing the hydrophilicity also changes the degradation rate and profile of the PLLA. New possibilities for the drug delivery industry and others are created in cases where both the hydrophilicity and degradation profile are controlled. Copolymerization can also be used to solve the second problem associated with PLLA, namely the absence of reactive sites. Sites which can be tuned and that allow the selective attachment of substances like bioactive molecules have been synthesized by copolymerization.52 Most interesting in this context is the copolymerization of PLLA and poly(ethylene glycol) (PEG). There are numerous possibilities to influence the properties and optimize them for medical applications. It has been shown that adjusting the block lengths of the components can modulate the crystallinity and that the hydrophilicity is improved compared with that of the PLLA homopolymers.53-56 The melting points of PLLA-PEG copolymers are lower than that of PLLA and, with the incorporation of PEG as the center block in the PLLA homopolymer, the elasticity and toughness of the resultant copolymer are higher than those of PLLA.57 Cytotoxicity tests for the triblock have been performed, and these showed a high level of cytobiocompatibility.58 Positive results regarding micro-domain structure and drug-release properties using block copolymers of PLLA and poly(ethylene oxide) have also been presented.59, 60 An interesting system in this context are also the block copolymers of PLLA and PDXO. PDXO is an amorphous polymer and, even in this case, the hydrophilicity and mechanical properties of the polymer can be tuned by using different compositions of the monomers.61, 62

2.4.2 Functionalized macromonomers

Another way of synthesizing new advanced and controlled molecular structures with specific properties is by using macromonomers.63-65 Depending on the functional group and modification method, different kinds of architectures can be obtained, Figure 2.5. All architectures will possess unique properties.
The functional groups can be inserted into the main chain during polymerization or already into the monomer. One example of the usefulness of functionalized molecules is to be found in the brominated polyesters. The bromide can be converted into an unsaturated group using tertiary amines or dehydrohalogenating reagents. The unsaturated bonds make the polymer suitable for crosslinking, and this is useful for the synthesis of degradable networks. The unsaturated units can also be converted into other functional groups, such as epoxy, carboxylic acid, and hydroxyl groups. Epoxidation with, for example, peroxy acids is one of the most important reactions for the introduction of oxygen atoms into organic molecules and is often used in syntheses. The polymers can also be functionalized during polymerization. For example, acrylic macromonomers of PLLA have been synthesized using functionalized aluminum alkoxides as initiators. The macromonomers obtained are suitable for graft copolymerization. Basically, for the synthesis of graft copolymers, the macromonomers can be end-functionalized (grafting onto), and the macromonomer backbone can be functionalized (grafting from). The three main strategies commonly used are:

1) Copolymerization of a macromonomer with vinyl or acrylic comonomers
2) Grafting-onto

![Diagram of Grafting-onto](image1)

3) Grafting-from

![Diagram of Grafting-from](image2)

Each option can be used in copolymerization in order to achieve the desired properties.

The material properties can be varied and in some cases even controlled by changing the architecture. The thermal properties will of course be affected when branching points are introduced into the polymer. The chain length will decrease and the number of chain ends increase. Both these give a lower melting point because of the less ordered fold pattern of the crystal. It has been concluded with three- and four-armed star-shaped PCL that it is the arm length that affects the melting point rather than the total weight. The lower melting points and lower melt viscosities will be a major advantage for the melt processing of polylactides for e.g. sutures. Long-chain branches predominantly affect the viscoelasticity, decreasing the viscosity and increasing the elasticity, and short-chain branches mostly affect the crystallinity. This offers possibilities to adjust the crystallinity by variation of the chain length and their number, and this can be utilized and optimized for each application. By manipulating parameters such as chain length, composition and molecular weight, both the three-dimensional structure and the hydrophilicity of polyesters can be varied. Grafting short hydrophobic PLA chains to a hydrophilic backbone generates polyesters with a more rapid water uptake and faster degradation rates. Since aliphatic polyesters are thought to degrade by random hydrolytic cleavage of the ester bonds, crystallinity and water uptake are the key factors determining the rate of polymer degradation. The degradation rate can thus be controlled by not only the by crystallinity but also by the molecular architecture.
2.4.3 Star-shaped polymers

Star-shaped polymers are branched polymers with more than two linear polymeric arms attached to a central core. These polymers provide a lot of end groups which are used in subsequent derivatization in, for example, surface functionalization. Star-shaped degradable aliphatic polyesters have a great potential in biomedical applications due to their high polymer mass and high functionality per unit volume. They are also interesting since using different number of arms varies the physical properties as well as the degradation rates and the properties are different from those of linear polymers. There is a challenge in producing well-defined stars in terms of the dispersity of arm number and arm length. Among various attempt to synthesize polyesters with this architecture, there are two methods that are most often used and can be classified and distinguished. The methods are similar to the ones described in the last section:

- Core first, living polymerization with a multifunctional initiator
- Arm first, coupling reaction of linear living polymers with a multifunctional coupling agent

The method most often described in the literature for the synthesis of star-shaped polyesters is one in which stannous octoate (SnOct$_2$) is used as a catalyst together with a multifunctional alcohol. SnOct$_2$ is widely used because it is commercially available, soluble in common organic solvents and cyclic ester monomers, and a permitted food additive in numerous countries. The drawback is that it is difficult to achieve the architecture without any imperfections. There is a need for new methods for the synthesis of well-defined star-shaped polymer. Kricheldorf and coworkers were the first to introduce spirocyclic tin initiators, which were supposed to be used to synthesize star-shaped polymers and networks in a precise way. The initiator was synthesized from dibutyltin oxide and pentaerythritol, but it did not fulfil all expectations. The initiator had a low solubility in organic solvents and it was difficult to use. The continuation of this work showed promising results with well-defined structures as a result.

In this connection, the hyperbranched and dendritic architectures must also be mentioned. The polymers are highly branched, resulting in many end-groups and an amorphous material with low melt-viscosity and high solubility. There has been a tremendous development in this area during recent years, and controlled architectures can now be synthesized and the conformation of the polymer can be controlled by self-assembly.

2.5 Networks

Another class of interesting synthetic materials with many possible applications is that of polymer networks in which several linear polymer chains are interconnected.
Aliphatic polyesters sometimes present strength limitations and the use of networks represents one way of providing the necessary strength and rigidity. Different methods have been used to construct such networks. In many of these methods, it is not possible to control the chain lengths between the crosslinks of the polymer network. If the network cannot be synthesized in a controlled and predetermined manner, the properties are difficult to tailor. The molecular structure of the crosslinked polymers has been shown to affect the dynamic mechanical and swelling properties. The crosslinking of homo- and copolymers provides further possibilities for modifying the physical and mechanical properties of materials.

2.5.1 Hydrogels

Hydrogels, water-swellable networks, are three-dimensional, hydrophilic, polymeric networks able to capture large amounts of water. The network is composed of homopolymers or copolymers, and is insoluble due to the presence of chemical crosslinks or physical crosslinks, such as entanglements or crystallites. Hydrogels are useful as biocompatible synthetic materials, especially in short- and intermediate-term applications. They are used as super-absorbents, tissue-engineering scaffolds, sensors, chemical memories, molecular separation systems, drug delivery systems and other biomaterials. Hydrogels are preferred and are useful in medical applications because of their similarity to natural living tissue due to their high water contents and soft consistency. The properties that make hydrogels useful in medical applications are:

- The hydrodynamic properties of the hydrogels are similar to those of the tissue
- The frictional irritation due to the presence of the hydrogel is low because of its soft and rubbery nature

Polymer hydrogels containing both hydrophobic and hydrophilic units are called amphiphilic polymer hydrogels. Compared to simple homopolymer hydrogels, they have improved mechanical properties because of the presence of the hydrophobic units. This reduces the water content and produces a more coherent material. The presence of both hydrophilic and hydrophobic segments enables the materials to be used in the release of both hydrophilic and hydrophobic drugs. The side-chain length, degree of crosslinking, swelling kinetics and composition strongly affect the release behavior and other mechanical properties of the hydrogel. For example, when the hydrodynamic radius of the solute is much larger than the mesh size of the network, the solute-release behavior is controlled by the degradation. Another growing field were hydrogels are preferred is that of molecular imprinting. Hydrogels can bind analytes and also choose between different molecules. In the future, an exact and well-defined network structure seems to be necessary. It is therefore important to develop methods for the synthesis of well-defined hydrogels.
2.6 Biomedically adapted surfaces

Integrins are cell-surface receptors that mediate adhesion to the ECM proteins, Figure 2.6. Most cells use several integrins that recognize a range of ECM associated ligands. The integrins play important roles in differentiation and cell communication.

![Figure 2.6 Schematic picture of cell adhesion to the ECM proteins through integrins.](image)

Synthetic polymers have no natural cell binding sites, the cell adhesion occurs instead via proteins.\(^{112}\) When a material is implanted into the body, the material surface is exposed to the proteins present in blood and other body fluids. This results in a layer of proteins adsorbed to the surface. The proteins compete for the surface, since they have different affinities.\(^{113}\) The material surface smoothness, ionic and electronic charge, wettability by blood components and chemical structure determine the compositions of the proteins and bacteria that adhere to the surface.\(^{114-116}\) This in turn decides how the cells respond to the material surface. The protein layer is also the beginning of a vascular fibrous capsule, which will affect the adhesion of platelets and also influence other coagulation processes. It would be best if the implanted devices exhibited a “normal” wound healing. A normal wound healing is regulated by growth factors. These affect the protein adhesion and thereby the cell migration and proliferation. A normal wound healing after implantation would provide an integration of the implant with the body and not segregation through a vascular fibrous capsule. Therefore, ways to hinder the vascular fibrous capsule from being built are currently being sought. To improve the cell affinity, many efforts have been directed towards modifying the surface properties for example by adjusting the hydrophilicity, hydrophobicity and surface roughness.\(^{117-121}\)

2.6.1 Cell adhesion – hydrophilicity and hydrophobicity

Surface hydrophilicity plays an important role in cell adhesion, spreading and growth. This may be because the proteins that adhere prefer the hydrophilic surface. Hydrophobic surfaces have a high interfacial free energy in aqueous solutions and this
seems to be a disadvantage in terms of their cell, tissue, and blood compatibility. Surface modification to achieve a more hydrophilic polymer is the most common way of altering the surface of a particle so that it can be ingested by phagocytes and of improving the surface properties of the system. This is still a complex area which is far from being well understood. Articles describing how cells adhere less to hydrophilic surfaces have also been published.\textsuperscript{122,123}

2.6.2 Cell adhesion - morphology and topography

The key design parameter for achieving good cell responses is the sample shape and the nano-level topography of the material.\textsuperscript{124-126} The topography of natural soft tissue is dependent on the ECM, which have a nanometer length and width. Successful polymeric constructional materials should therefore have nano-dimensional surface features. It is expected that a biocompatible material that mimics the nanometer topography of the relevant tissue will enhance cellular responses, and thus lead to better tissue integration \textit{in vivo}. The interaction between the scaffold and cell also determine the cell function. It was shown that not only proliferation but also cell differentiation and cell migration depended on interactions with polymer surfaces.\textsuperscript{122} The cells recognize surface features and react to them, resulting in some kind of contact guidance. This can be used in different ways. Ligaments and tendons are well-organized fibrous connective tissues but, after an injury, cells in the healing site are found to have a non-specific orientation. The resulting collagen matrix is also less organized. The explanation of the decrease in mechanical properties of the healing tissue has been related to these unorganized structures. When the broken ligaments and tendons are treated it is important that the cells are aligned and that the collagen matrix is organized as in normal tissue. The topography of a surface can help the cells align. Orientation of the cells and also an organization of the collagenous matrix was achieved by using a structured membrane. The cells then produce aligned collagenous material similar to the uninjured state of tendons and ligaments.\textsuperscript{127} Research has also been carried out to elucidate whether a surface with nanometer feature dimensionalities has any effect on the cell adhesion. The results show a clear effect, the numbers of cells were compared to a flat surface and the number of adhered cells was 51% higher on the nano-structured surface after 5 days.\textsuperscript{120} Other reports support this claim and provide evidence that surfaces with smaller features enhance cell functions.\textsuperscript{3,121,125}

The molecular architecture of the polymer is of course a major factor during the micro-phase separation and for the topography obtained.\textsuperscript{128,129} The microphase-separated morphologies also have a pronounced effect on the mechanical properties.\textsuperscript{130,131} The modulus is dependent on the fraction of taut interfibrillar tie molecules, while the tensile strength depends on the ratio of interfibrillar area to total area.
3. EXPERIMENTAL

3.1 Materials

The L-lactide (Serva Feinbiochemica, Germany, 98%) was recrystallized from toluene several times, dried at room temperature under vacuum for 48h and stored in an inert atmosphere. \(\varepsilon\)-caprolactone (Acros) was dried over calcium hydride for 48h at room temperature and distilled under reduced pressure just before use. The synthesis of 1,5-dioxepan-2-one (DXO) has been described elsewhere\(^{132,133}\). After the synthesis DXO was purified by two distillations, recrystallization from dry diethyl ether and a final distillation under reduced pressure. m-chloroperoxybenzoic acid (mCPBA; Aldrich) was cleaned from m-chlorobenzoic acid by dissolving in dichloromethane and washing with a phosphate buffer at pH 7.4. The organic layer was dried over MgSO\(_4\), filtered and dried under a vacuum for 2 days at room temperature. Toluene (Merck, Germany) was dried over Na-wire before use. Chloroform (Labora Chemicon, Sweden) stabilized with 2-methyl-2-butene was dried over calcium hydride for at least 24 h and then distilled under reduced pressure under an inert atmosphere. Dibutyltin oxide (Aldrich, Germany), Succinyl chloride (95%, Acros Organics), Dichloromethane (VWR) and pentaerythritol ethoxylate, 3/4 EO/OH and 15/4 EO/OH, (Aldrich, Sweden) were used as received.

3.2 Synthesis of initiators

3.2.1 Germanium initiators

![Figure 3.1 Germanium initiators, 1 (n=4), 2 (n=11) and 3 (n=43).](image)

The germanium initiators, 1, 2, and 3, were a gift from Professor Kricheldorf and their synthesis was not a part of this work. Appearance: 1 had a syrupy character while 2 and 3, had a more crystalline shape, \(T_m\) (2) 72°C, \(T_m\) (3) 65°C.
3.2.2 Functionalized tin initiators

![Functionalized tin initiators, 4 and 5.](image)

The functionalized initiators 4 and 5 were synthesized from dibutylin dimethoxide and the corresponding alcohols as described in the literature.\textsuperscript{134, 135} Initiator 5 was recrystallized in dry toluene, and both initiators were distilled over a short-path apparatus under reduced pressure (10^{-3} \text{ mbar}) before use. \textit{Appearance}: 4 had a syrupy character and 5 was crystalline, \( T_m(5) \) 90°C.

3.2.3 Spirocyclic tin initiators

![Spirocyclic tin initiators, 8 (m+n+o+p=3) and 9 (m+n+o+p=15).](image)

The procedure was the same as in the synthesis of the functionalized initiators. Two different pentaerythritol ethoxylate compounds were used. The precipitated product was centrifuged before the supernatant solvent was poured off. The initiator was dried under reduced pressure for 24 h. \textit{Elemental analysis} (%): 8 \( \text{C}_{27}\text{H}_{56}\text{O}_7\text{Sn}_2 \) (731.8) calculated C 44.3 H 7.7, found C 42.4 H 8.0, 9 \( \text{C}_{51}\text{H}_{104}\text{O}_{19}\text{Sn}_2 \) (1258.8) calculated C 48.6 H 8.3, found C 46.6 H 8.2. Exposure to moisture before analysis could explain the difference between the calculated and found amounts of carbon. \textit{Appearance}: crystalline solids, white, \( T_m(8) \) 117°C \( T_m(9) \) 109°C.

3.3 Polymerization model reaction

The polymerizations were carried out in silanized round-bottomed flasks closed by a three-way valve. A magnetic stirring bar was enclosed in the reaction flask. The
equipment was flamed and stored in a glovebox (Mbraun MB 150B-G-I) purged with nitrogen. The monomer and the initiator were weighed and added to the reaction vessel in the glovebox. Distilled chloroform was transferred to the reaction vessel in the hood by a flamed syringe under strictly anhydrous conditions. During polymerization, the reaction flask was completely immersed in a thermostated oil bath preheated to the polymerization temperature. Samples for $^1$H-NMR and SEC analysis were withdrawn from the reaction vessel using a flamed syringe while flushing with inert gas. The product was precipitated in cold methanol/hexane mixture when the reaction time was over.

3.4 Epoxidation

Epoxidation was carried out in chloroform. To obtain the completely epoxidized products the amount of mCPBA used in the epoxidation reaction was set to twice the theoretical number of double bonds. mCPBA was added to a round-bottomed flask containing PLLA dissolved in chloroform. The reactions were maintained with magnetic stirring at room temperature until the conversion of double bonds was complete. A white precipitate appeared and, after filtration, the filtrate was precipitated in cold hexane to obtain the epoxidized polymer.

3.5 Copolymerization

The first step, polymerization of DXO, followed the procedure described in the model reaction, section 3.3. During the polymerization, the flask was immersed in a thermostated oil bath at 60°C. The initial monomer concentration was 0.5 M. The second monomer, LLA, was dissolved in chloroform following the same procedure as for DXO and transferred to the reaction vessel with a syringe at the time for full conversion of DXO.

3.6 Synthesis of networks

3.6.1 Tetra-functional acid chloride

The synthesis was performed according to an earlier report. Cis-1,2,3,4-cyclopentane tetracarboxylic acid and n-heptane were added to a round-bottomed flask containing phosphorus pentachloride. The temperature was increased gradually from 20°C to 95°C over a three-hour period. Reflux was maintained at 95°C for an additional 2 hours until no HCl evolution was detected. The yellow solution obtained was filtered through a coarse paper and then roto-evaporated to oil.

3.6.2 Crosslinking reaction

When the polymerization was complete, the crosslinker was added through a flamed syringe. In most cases a gel was formed immediately as soon as the crosslinker
was added. The reaction was held at 60°C for an additional couple of hours to ensure complete conversion of the acid chloride. The gels were extracted with CH₂Cl₂ before characterization.

3.7 Film preparation

The polymer was dissolved in chloroform to form solutions with concentrations of 0.3 wt% and 5 wt%. A freshly cleaved mica substrate (9 cm × 10 cm) was put into a glass container and 2 ml of the solution was deposited on the surface of the substrate. The samples were conditioned for 2 days at room temperature and then for at least 1h in vacuum. The samples were heated in vacuum at 170°C for 16h and then either quickly quenched to room temperature or slowly cooled to room temperature over a period of several hours.

3.8 Characterization methods

3.8.1 Nuclear Magnetic Resonance

For ¹H-NMR measurements, the samples were dissolved in deuterated chloroform in 5-mm NMR tubes at room temperature. The sample concentration was 5% by weight. ¹³C-NMR was performed with a 10% sample concentration in 5-mm tubes. Non-deuterated chloroform was used as an internal standard (δ = 7.26 ppm). NMR spectra were recorded on a Bruker AM-400 Fourier-Transform Nuclear Magnetic Resonance spectrometer (FT-NMR) operating at 400 MHz, T=25°C. When spectra of the functionalized initiators were recorded at different temperatures a Bruker DMX 500 was used. 2D ¹H- ¹³C heteronuclear multiple quantum coherence – gradient selected (invieagsssi) spectra were acquired and processed with a standard Bruker microprogram. A total of 256 experiments were accumulated using one scan with a relaxation delay of 2s. The spectrum was obtained with 9 ppm spectral width over the F₂ (proton) axis and 200 ppm for ¹³C along the F₁ (carbon) axis at -13°C.

3.8.2 Size Exclusion Characterization

Chloroform was used as an eluent and was delivered at a flow rate of 1.0 mL/min. The samples were dissolved in chloroform at a concentration of 0.06 wt%. The injection volume was 50µL. Narrow polystyrene standards in the 580-1,900,000 g/mol range were used for calibration. A Waters 717plus auto sampler and a Waters model 510 apparatus equipped with three PLgel 10 µm mixed-B columns, 300×7.5 mm (Polymer Labs., UK) connected to an IBM-compatible PC were used. Millenium³² version 3.20 software was used to process the data.
3.8.3 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were made on a Mettler-Toledo DSC instrument with a DSC 820 module. The measurements were run from -50°C to 180°C at a heating rate of 10°C/min and a cooling rate of 10°C/min. The samples were heated in a nitrogen atmosphere. T<sub>g</sub> and T<sub>m</sub> of the polymers were determined during the second heating period. T<sub>g</sub> was determined as the middle of the record step change in heat capacity, and the T<sub>m</sub> was defined as the endotherm peak of the curve.

3.8.4 Atomic Force Microscopy

The atomic force microscopy (AFM) measurements in the tapping mode were made on a Multimode Instrument from Digital Instruments equipped with a Nanoscope III software system. Commercial etched silicon nitride cantilevers of 125 µm length with a spring constant of 36 - 55 N/m and a resonance frequency of 324 - 372 kHz were used.

3.8.5. Environmental Scanning Electron Microscopy

ESEM (Environmental Scanning Electron Microscope) model 2020 produced by ElectroScan. Thermoelectric stage was used in the experiments, which alters and measures the temperature. The wetting conditions were created by maintaining the temperature at 4°C and by altering the pressure in sample chamber from 3.0 to 7.0 Torr. The water was condensed from the chamber atmosphere.

3.8.6 Swelling

The swelling of the network was studied gravimetrically. All the swelling data were obtained with extracted hydrogel specimens. In a typical case, a piece of the network film was weighed and transferred to water. At regular intervals, it was taken out, the excess water was removed from the surface with tissue paper and it was then weighed and returned to the medium. This procedure was continued until constant weight was attained. The equilibrium degree of swelling, DS, was calculated as:

\[
\text{degree of swelling (DS)} = 100 \cdot \frac{(W - W_0)}{W_0}
\]  

where W<sub>0</sub> is the initial weight of the dry sample and W is the final weight of the swollen sample. Each measurement was repeated three times and the average value was reported.
3.9 Cell response measurements

The group of Professor Biagini, Italy, made all the measurements of cell growth. Keratinocytes, NCTC 2544 cells, (ICLC Genos Italy) were grown on the heat-treated samples in a controlled atmosphere (5% CO₂; T=37°C) in Minimum Essential Medium Eagle (MEM) (Sigma, Milan, Italy) supplemented with 5% foetal calf serum (FCS), 1% non-essential amino acids, 2.0 mM L-glutamine, and antibiotics. After thawing, keratinocytes were routinely split 1:2 every 3-4 days and used between the 2nd and 4th passages. For SEM analysis the cells were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated in increasing ethanol concentrations, CPD-dried, mounted on aluminium stubs and gold-sputtered.

3.9.1 Time-lapse videomicroscopy

Cells were seeded onto the heat-treated samples in 2ml Hepes-modified E-MEM supplemented with 5% FCS, 2ml L-glutamine, 100ml U/ml penicillin, 100U/ml streptomycin and kept at 37°C. They were observed under an inverted microscope (Nikon Eclipse TS-100) equipped with a 10x objective and a colour CCD video camera (JVC TK-C1381). Phase-contrast images of living cells were recorded using a time-lapse VCR (Panasonic AG-TL700) and digitalized using a video frame grabber card and dedicated software (Image-Pro Express, Media Cybernetics).
4. **TRIBLOCK COPOLYMERS**

The polyether-polyester block copolymers are an interesting class of biomaterial. Copolymers of, for example, PLLA and PEG provide a large variety in terms of mechanical properties and degradability. PEG is hydrophilic and flexible and PLLA appears most interesting due to its degradability. In addition, both PEG and PLLA have been accepted by the U.S. Food and Drug Administration for internal use in the human body. All these properties are valuable for biomedical applications such as implanting devices, materials for tissue engineering and cell scaffolds. By using copolymerization, the hydrophilicity of PEG can be combined with the degradability of PLLA, and the high crystallinity of the PLLA will decrease due to the flexibility in PEG. Most advantageous is the ability to modulate the degradation rate and hydrophilicity of the polymer by adjusting the ratio of its hydrophilic and hydrophobic constituents.

4.1 Germanium initiators

Kricheldorf et al have earlier thoroughly investigated the cyclization of oligo- and poly(ethylene glycol) with dibutyltin dimethoxide and have also used the macrocycles in polymerizations.137-139 The same group has continued the research in this area and has recently synthesized the same kind of structures but with tin being replaced as the metal atom by germanium. The metal center plays an important role in ROP.43 The electrophilicity of the initiator metal center is of the utmost importance. The tendency toward metal-oxygen bond formation follows the accessibility of the initiator’s lowest unoccupied molecular orbital (LUMO). More electrophilic initiators polymerize cyclic esters more rapidly.140 Secondly, the molecular weight dispersity (MWD) of the polymer also depends on the metal.45 This can be explained by the energy difference between the highest occupied molecular orbital (HOMO) and LUMO. This energy difference decreases in a group of the periodic table and is directly proportional to the activation energy of the transesterification reactions. Compared to tin, which is often used as an initiator in ROP, the energy difference between HOMO and LUMO in germanium is higher, Figure 4.1, the activation energy for transesterification is therefore also higher. Since germanium is more electrophilic and also has a higher activation energy for transesterification reactions than tin it is interesting to see how these compounds serve as initiators.141, 142
Triblock copolymers

Figure 4.1 Part of the periodic table showing how the electrophilicity and the energy difference between HOMO and LUMO varies.

Certain germanium compounds also have a low mammalian toxicity and they exhibit a clear activity against certain bacteria.\textsuperscript{143}

Professor Kricheldorf gave three germanium initiators to our group and their structures are shown in Figure 4.2. Both the synthesis and the mechanistic consideration of these compounds were outside the scope of this work.

![Structure of germanium initiators](image)

Figure 4.2 Structure of germanium initiators used in the synthesis of triblock copolymers, 1 \( n = 4 \), 2 \( n = 11 \), 3 \( n = 43 \).

The initiators have different lengths of ethylene oxide units in the structure. These will be incorporated into the polymer during polymerization and when low monomer-to-initiator ratios are used the polymer will end up in an ABA block structure.

The assignment of the initiators was done by \(^1\text{H}\)-NMR (Figure 4.3), \(^{13}\text{C}\)-NMR (Figure 4.4), \(^1\text{H}-^1\text{H}\) COSY NMR (homonuclear proton-proton correlation spectroscopy) (Figure 4.5) and \(^1\text{H}-^{13}\text{C}\) hmqc-gs spectra (heteronuclear multiple quantum coherence – gradient selected) (Figure 4.6). Signals typical of the \(-\text{O--CH}_2\text{-CH}_2\)- group were observed in the \(^1\text{H}\)-NMR spectrum and the assignment can be seen in Figure 4.3. The peak noted as "a" emerging at 3.61 ppm originated from the \(-\text{O--CH}_2\)- protons directly attached to the germanium atom. The peak at 3.72 ppm was assigned to the protons that are positioned next to these protons, \(-\text{O--CH}_2\text{-CH}_2\)-. In Figure 4.4 the \(^{13}\text{C}\)-NMR spectrum of initiator 1 is shown. All carbons could be seen and the coupling between the protons and carbons was recorded with a \(^1\text{H}-^{13}\text{C}\) hmqc-gs spectrum (Figure 4.6). The coupling between the protons can be seen in Figure 4.5.
**Figure 4.3** $^1$H-NMR spectra of germanium initiator 1, 2 and 3.

**Figure 4.4** $^{13}$C-NMR spectrum of germanium initiator 1.
Figure 4.5 $^1$H–$^1$H-COSY NMR spectrum of initiator 1. F1=conventional $^1$H-NMR spectrum, F2=conventional $^1$H-NMR spectrum

Figure 4.6 $^1$H–$^{13}$C hmqc-gs spectrum of initiator 1. F1=conventional $^{13}$C-NMR spectrum, F2=conventional $^1$H-NMR spectrum
4.2 Polymerization

In solution polymerization, it is extremely important that the initiator and monomer are completely soluble in the solvent. Chloroform, 1,2-dichloroethane and chlorobenzene are good solvents, in which initiators 1, 2 and 3 are soluble. Conversion tests showed that chlorobenzene as solvent, a temperature of 120°C and an initial monomer concentration of 1 M was a good option to achieve a successful ring-expansion polymerization of LLA using these germanium initiators. It should be noted that solution polymerization using chloroform and 1,2-dichloroethane respectively as solvent was first used in an attempt to carry out the reaction at a lower temperature, but with no success. 1H-NMR spectrum of initiator 1 and a spectrum of precipitated PLLA initiated by 1 are assigned in Figure 4.7. Note that the signal from the protons closest to the germanium at 3.61 ppm in the top spectrum was shifted downfield to 4.3 ppm during polymerization. The disappearance of the peak at 3.61 ppm proves that all four Ge-OCH$_2$ bonds participated in the polymerization.
Conversion tests were performed by withdrawing samples from the reaction flask during polymerization. The samples were characterized by $^1$H-NMR. Since the $^1$H-NMR signals of LLA and PLLA are different, the conversion could easily be checked by $^1$H-NMR spectroscopy. The results were used to construct a plot of monomer conversion versus time, Figure 4.8. The conversion was high in all polymerizations; the maximum conversions for the polymerizations initiated by 1, 2 and 3 were 91%, 93% and 95% respectively.
From the samples withdrawn from the flask, the semilogarithmic plot, $-\ln([M]/[M]_0)$ versus reaction time, could be obtained. Where $[M]_0$ is the initial LLA monomer concentration and $[M]$ the LLA concentration at a given reaction time. The linearity of the relationship in Figure 4.9 attests that the polymerization kinetics of LLA, $[M]/[I]$ ratio of 50 at 120°C, initiated by these germanium initiators are first order in monomer. The linearity also indicates that the amount of termination reactions is low and that the number of growing chains is constant.

Figure 4.9 Semilogarithmic plot of $-\ln([M]/[M]_0)$ as a function of time, polymerization of LLA using 1, 2 and 3 as initiators. $[M]/[I]=50$, $[M]_0=1M$, $T=120^\circ C$

Plots of number-average molecular weight, $M_n$, versus conversion also gave straight lines, Figure 4.10, which means that the frequency of transesterification reactions during the polymerizations was low.

Figure 4.10 $M_n$ as a function of conversion during polymerization of LLA using 1, 2 and 3 as initiators. $M_n$ was determined by SEC using narrow molecular weight polystyrene standards. $[M]/[I]=50$, $[M]_0=1M$, $T=120^\circ C$
Considering the long reaction times, the MWD was found to be narrow. Initiator 3 had the broadest dispersity, around 1.4 and initiators 1 and 2 had a dispersity around 1.2. All the above-mentioned results indicate that the concentration of active centers remains constant and that the transesterification reactions are low. The polymerizations were in other words controlled. For an initiator to be useful it is also necessary that the molecular weights of the polymer can be determined and can be predictable from the molecular composition of the initial reaction mixture. Two polymerizations were therefore performed with each initiator using different targets of DP, DP=25 and DP=50. The reaction times could be predicted from the conversion curves. The results are presented in Table 4.1.

<table>
<thead>
<tr>
<th>Initiator</th>
<th>[M]/[I] a</th>
<th>DP b</th>
<th>Time (h)</th>
<th>Conv. c (%)</th>
<th>Yield (%)</th>
<th>M_{n,SEC} d</th>
<th>MWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>31</td>
<td>116</td>
<td>92</td>
<td>85</td>
<td>7000</td>
<td>1.2</td>
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<tr>
<td>1</td>
<td>50</td>
<td>53</td>
<td>190</td>
<td>96</td>
<td>91</td>
<td>13400</td>
<td>1.2</td>
</tr>
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<td>2</td>
<td>25</td>
<td>29</td>
<td>103</td>
<td>96</td>
<td>94</td>
<td>7300</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>53</td>
<td>168</td>
<td>94</td>
<td>90</td>
<td>16200</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>29</td>
<td>95</td>
<td>93</td>
<td>90</td>
<td>28500</td>
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<tr>
<td>3</td>
<td>50</td>
<td>45</td>
<td>162</td>
<td>90</td>
<td>90</td>
<td>41400</td>
<td>1.4</td>
</tr>
</tbody>
</table>

a) monomer/initiator ratio added to the reaction flask  
b) degree of polymerization found after precipitation  
c) conversion, determined from crude reaction mixture  
d) number-average molecular weight determined by SEC using narrow polystyrene standards

The DP after precipitation were determined by quantification of the \textsuperscript{1}H-NMR spectrum, signals b', c' and d' in Figure 4.7 were used. Polymers with narrow MWD and with DP close to the values expected based upon monomer/initiator loading were produced.

Because of the low reaction rate, an attempt was made to increase the reaction rate. PLLA with a DP of 50 was synthesized in bulk, T=120ºC, using initiator 2. After 20h the conversion was 85%, M_{n} was 5000 and the MWD was 1.4. An even higher reaction rate was achieved when the reaction was performed at a temperature of 160ºC. The conversion was 90% after 10h, M_{n} was 4000 and MWD 2.0. The broader dispersity is a consequence of the high reaction temperature, since this increases the amount of side reactions.

### 4.3 Thermal characteristics

The thermal properties of the PLLA-PEG-PLLA copolymers were evaluated. Results of DSC measurements are shown in Table 4.2.
Table 4.2 Thermal properties of PLLA-PEG-PLLA triblock copolymers.

<table>
<thead>
<tr>
<th>Initiator</th>
<th>DP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>T&lt;sub&gt;g&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (°C)</th>
<th>T&lt;sub&gt;cPLLAc&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;m PEGd&lt;/sub&gt; (°C)</th>
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<td>1</td>
<td>31</td>
<td>39.5</td>
<td>88.6</td>
<td>54.0</td>
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<tr>
<td>1</td>
<td>53</td>
<td>46.2</td>
<td>92.8</td>
<td>51.3</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>54.4</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>32.4</td>
<td>95.1</td>
<td>51.2</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>55.7</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>54.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> degree of polymerization found after precipitation, <sup>b</sup> the glass transition temperature of PLLA, <sup>c</sup> the crystallization temperature of PLLA, <sup>d</sup> the melting temperature of PEG

Each chromatogram had two T<sub>m</sub> that corresponded to the PEG and PLLA melting transitions respectively. It is of interest to note that the melting temperature of the PLLA-PEG-PLLA was shifted to a lower temperature region (120-150°C) than the typical T<sub>m</sub> of normal PLLAs with equivalent molecular weights (150-160°C). The decrease in T<sub>m</sub> is due to the presence of the PEG unit. The incorporation of PEG into the backbone of the polymer hinders the chain ordering of the polymer, and leads to a decrease in crystallinity. The melting point depression of the PLLA block with an increasing molar content of PEG can clearly be seen in Figure 4.11.

Figure 4.11 DSC chromatogram of PLLA-PEG-PLLA triblock copolymers initiated by germanium initiators. [M]/[I]=25

Another noticeable phenomenon is the multiple melting peaks in each case, which indicates the dispersion of crystal thickness due to the greater MWD of the polymer chains. Polymers initiated by initiator 1 and 2 showed only a very small melting peak associated with PEG. The melting peak of the PEG segments has a tendency to disappear. The explanation of this observation is that the PLLA hard segments are
the first to solidify upon cooling, which makes it hard for the PEG segments to move so that the crystallization of PEG is thereby blocked. This is most apparent in a block copolymer with a small content of PEG. The observed exothermic peak at 90-95°C is related to the cold crystallization of the PLLA segments. No crystallization was observed during the second heating when initiator 3 was used. This is considered to be due to the differences in PEG chain length and the low crystallizability of low molar mass PLLA.
5. FUNCTIONALIZED POLYESTERS

In the introduction, the importance of reaction sites in the polymer chain was emphasized. In order to modify polymers by chemical reactions, they should contain reactive groups either in the backbone or in side groups. A versatile functional group is the C=C double bond. In this work, functionality in the form of a double bond has been introduced into the initiators and subsequently incorporated into the macromonomers during the polymerization. Two different tin initiators have been used, and in both cases a double bond has been introduced into the main chain. Tin initiators were used since their reaction rates are high and the knowledge concerning their reactions have been thoroughly investigated and are well understood.

5.1 Functionalized cyclic tin(IV)alkoxides

The functionalized initiators were synthesized by the condensation reaction between dibutyltin dimethoxide and the appropriate alcohol. The driving force in the reaction is the gain in entropy during cyclization, where many small molecules are formed instead of one large. The macrocycles must of course be strain-free. An additional stabilizing effect results from the intramolecular donor-acceptor interactions between oxygen and tin. Such interactions have been shown to occur.\(^{147}\) The cyclic tin alkoxides in Figure 5.1 have previously been mentioned briefly in the literature.\(^{148}\) Published \(^{119}\)Sn-NMR analysis data for these initiators showed that the unimer form of initiator 4, noted as 4/1 in Figure 5.1, predominates at room temperature, whereas initiator 5 is present mainly as a dimer, 5/2. In the continuation of this work, the initiators will be referred to only as 4 and 5.

![Figure 5.1](image-url)
$^1$H-NMR spectra of initiator 4 at different temperatures, T= +25, +7, -13°C, are shown in Figure 5.2. The peak appearing at 4.49 ppm originated from the two $-\text{O-CH}_2$ protons next to the double bond in the initiator. This peak was broad at room temperature, but when the temperature was lowered this broad peak split into two peaks. To understand this behavior, a $^1\text{H}^\text{-}^{13}\text{C}$ hmqc-gs spectrum was recorded (not shown), from which it became clear that the splitting of the signal originated from a fast equilibrium between the unimeric and dimeric forms, 4/1 and 4/2 respectively. At room temperature, the rate of exchange between the monomeric and dimeric forms was too fast for the signals to be resolved.

![Figure 5.2 $^1$H-NMR spectra and assignment of initiator 4 at different temperatures. T= +25°C, +7°C, -13°C](image)

Other oligomers than the dimers are probably present to some extent, causing the broadening of the peaks. The dimerization is probably due to the favorable Sn–O donor-acceptor interactions.
The $^{1}$H-NMR spectrum (T=+25°C) of initiator 5 with assignments is shown in Figure 5.3. Also with this initiator, spectra were recorded at lower temperatures, +7°C and –13°C, but this lowering of the temperature did not have any effect. The reason is that the rate of exchange between the two forms is slow enough for the peaks to be resolved at 25°C. Kricheldorf has reported the same observation for similar cyclic tin alkoxides.149

**Figure 5.3** $^{1}$H-NMR spectra and assignment of initiator 5.

### 5.2 Synthesis of functionalized polyesters

The polymerizations were carried out in chloroform at 60°C with an initial monomer concentration of 0.5 M. The reaction rate was monitored by $^{1}$H-NMR spectroscopy. Samples were withdrawn from the reaction mixture at different times and analyzed until no further changes in the conversion could be observed. As can be seen in Figure 5.4, the reaction rates of the two functionalized tin initiators when polymerizing LLA were similar. No induction period could be seen and the conversions were almost complete. With ε-CL, the reaction rate was faster. Conversion of ε-CL was complete within 140 minutes, in contrast to LLA, which was only converted to 45% during the same period of time, indicating that ε-CL was more reactive than LLA.
Figure 5.4 Conversion curves for polymerizations of LLA and ε-CL using the two functionalized tin initiators, 4 and 5. \([M]/[I]=50, [M]_0=0.5\text{M}, T=60^\circ\text{C}\)

The spectra of crude PLLA initiated by 4 at two different times during polymerization together with the spectrum of pure initiator 4 are shown in Figure 5.5.

Figure 5.5 \(^1\text{H-NMR spectrum of initiator 4 (at the bottom) and spectra of two samples taken during polymerization of LLA.}\)

In all the studied cases, the signal at 4.49 ppm associated with the unreacted initiators disappeared. The resulting polymers should contain the initiator fragment as one type of block. The peak from the incorporated \(-\text{CH}_2\text{-CH=CHCH}_2\)– group of initiator 4
appeared at 4.72 ppm and the peak at 4.35 ppm originates from the $\text{-O-CH}_2$ protons directly attached to the tin atom. Polymerization using 5 revealed the same results, peaks from the initiator were seen in NMR spectra of the polymers. The results confirmed that the alkoxide groups in all the initiators were completely reacted under these reaction conditions, which is consistent with earlier investigations with a similar initiator.61, 150

The progress of these reactions was also followed by SEC. In every polymerization, the MWD of PLLA and PCL was narrow, indicating fast initiation with respect to propagation. It also indicates fast propagation compared to chain transfer or other adverse termination reactions. The molecular weight increased linearly with the conversion, which supports these statements. A typical polymerization course using 4 or 5 as initiator is presented in Figure 5.6.

![Figure 5.6](image_url)

*Figure 5.6 Results from LLA polymerization using 4 as initiator. $M_n$ was determined by SEC using narrow polystyrene standards. $[M]/[I]=50$, $[M]_0=0.5M$, $T=60^\circ C$*

Figure 5.7 shows a semilogarithmic diagram of the data obtained during the polymerizations. The straight lines confirm the previous results, that the reactions are proceeding in a controlled way.
A number of polymerizations were performed using 4 and 5 as initiators and LLA or ε-CL as monomer. The target DP was selected to be between 20 and 500, which was controlled by adjusting the molar ratio of monomer to initiator. Table 5.1 lists the results.

**Table 5.1 Results from the polymerizations of LLA and ε-CL using the functionalized initiators. A) PLLA, initiated by 4 B) PCL, initiated by 4 C) PLLA, initiated by 5**

<table>
<thead>
<tr>
<th>[M]/[I]</th>
<th>Time (h)</th>
<th>DP</th>
<th>MWD</th>
<th>Conv. (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6</td>
<td>21</td>
<td>1.06</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>50</td>
<td>16</td>
<td>53</td>
<td>1.07</td>
<td>98</td>
<td>86</td>
</tr>
<tr>
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<td>28</td>
<td>130</td>
<td>1.05</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td>250</td>
<td>71</td>
<td>270</td>
<td>1.10</td>
<td>78</td>
<td>68</td>
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<tr>
<td>500</td>
<td>140</td>
<td>550</td>
<td>1.09</td>
<td>83</td>
<td>76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[M]/[I]</th>
<th>Time (h)</th>
<th>DP</th>
<th>MWD</th>
<th>Conv. (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.5</td>
<td>55</td>
<td>1.22</td>
<td>92</td>
<td>83</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>108</td>
<td>1.23</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>250</td>
<td>15</td>
<td>260</td>
<td>1.24</td>
<td>98</td>
<td>93</td>
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<tr>
<td>500</td>
<td>30</td>
<td>530</td>
<td>1.23</td>
<td>99</td>
<td>90</td>
</tr>
</tbody>
</table>
The find DP agreed well with the monomer-to-initiator ratio; and the MWD was narrow. To determine DP by quantification of the $^1$H-NMR spectrum, the signals in the polymer and the signal from the incorporated double bond were used. This excellent control of molar mass indicates that the initiator efficiency is high with little loss of active sites.

5.3 Epoxidation of the incorporated double bond

Non-linear polymers have many structural variables (composition, backbone length, branch length, branch spacing, etc.) that give a great potential for new properties. To make a graft polymer with specific properties, the structure must be controlled. The macromonomer method is one of the most useful ways to design and obtain well-defined graft copolymers in a controlled way. The previous sections described how a double bond was incorporated into the polyester chain backbone. The double bond is a perfect grafting point where the distance between interconnecting points can be precisely regulated by the DP. In order to test the reactivity of the unsaturated group, epoxidation has been carried out at room temperature with m-chloroperoxybenzoic acid (mCPBA) in chloroform, Scheme 5.1. Aliphatic polyesters containing epoxides are interesting since they can be used as precursors of degradable networks. mCPBA was used because it is commercially available, stable in solution at a moderate temperature for prolonged periods and reacts under mild conditions.

Scheme 5.1 Epoxidation of the double bond using mCPBA.

\[
\text{[M]/[I]} \quad \text{Time (h)} \quad \text{DP} \quad \text{MWD} \quad \text{Conv.} \quad \text{Yield}
\]

\[
\begin{array}{cccccc}
\text{20} & 8 & 21 & 1.07 & 95 & 40 \\
\text{50} & 16 & 51 & 1.11 & 96 & 83 \\
\text{100} & 32 & 100 & 1.11 & 95 & 78 \\
\text{500} & 160 & 503 & 1.08 & 65 & 63 \\
\end{array}
\]

\[\text{a) molar feed ratio calculated from the unimeric species} \quad \text{b) calculated from } ^1\text{H-NMR on precipitated polymer} \quad \text{c) determined by SEC analysis calibrated with narrow polystyrene standards} \quad \text{d) conversion obtained from crude samples before precipitation} \quad \text{e) amount of polymer formed after precipitation}\]
The epoxidation was monitored by the use of NMR spectroscopy. Figure 5.8 shows $^1$H-NMR spectra at different times during the epoxidation reaction of the LLA macromonomer with $[M]/[I] = 8$.

![NMR spectra](image)

Figure 5.8 $^1$H-NMR spectra of the functionalized LLA macromonomer (lower) and the epoxidized LLA macromonomer (upper). $[M]/[I]=8$

The conversion of the double bonds were complete, as proven by the disappearance of the peaks at 5.73 and 4.72 ppm and the appearance of two multiplets signals at 3.27 and 4.15 ppm from the epoxide protons. No signals at 3.50 ppm (–OH) and 3.43/3.35 ppm (>CH–OH) were observed, which shows that no epoxide hydrolysis occurred during the acidic conditions for epoxide formation and polymer isolation. Assignments of the signals shown in Figure 5.8 for the epoxidized macromonomer were primarily based on comparisons with DEPT analysis (Figure 5.9), $^{13}$C-NMR analysis (Figure 5.10) and 2D $^1$H-$^{13}$C hmqc-gs (not shown) of the same compound. DEPT is a good help when carbons in a molecule are to be assigned. The carbons attached to an odd number of hydrogens (methine (–CH–) and methyl carbons (–CH$_3$–)) produce signals with positive amplitudes while carbons coupled to an even number (–CH$_2$–) give signals with negative amplitudes. Figure 5.9 shows the DEPT spectrum of the epoxidized polymer.

40
The $^{13}$C-NMR spectrum before and during the epoxidation is shown in Figure 5.10. The spectrum at the bottom is from the precipitated unsaturated LLA macromonomer with a DP of 8 and the spectrum at the top is from a crude sample during the epoxidation. It is clearly seen that the two peaks corresponding to unsaturated carbons in the spectrum are disappearing and that two new signals at 53.4 and 63.4 ppm, noted as "i" and "j" are appearing. The peaks at 53.4 and 63.4 ppm were shown to be associated with -CH- and -CH$_2$- groups respectively due to the amplitudes of the peaks in the DEPT spectrum and they were assigned to the carbons near the epoxide unit. From the 2D spectrum (not shown), these peaks were found to be associated with peaks in the $^1$H-NMR spectrum at 3.27 ppm and 4.15 ppm. The peaks at around 130 ppm in the top spectra belong to the aromatic carbons of mCPBA. To obtain a more legible spectrum, the chloroform peaks at 77.0 ppm were manually deleted when processing of the data.
The double bond in three low molecular weight PLLAs, [M]/[I] ratios 8/1, 20/1 and 50/1, were exposed to mCPBA. The conversion of the double bond was complete in all cases and the yields obtained were good (>95%). Interestingly, the epoxidation reaction did not lead to chain degradation but provided versatile intermediates for further functionalization of the polyester backbone. The unimodal SEC chromatogram, Figure 5.11, the narrow MWD and the agreement between the DP before and after epoxidation are consistent with a non-degradative derivatization reaction.

Figure 5.10 $^{13}$C-NMR spectra of LLA macromonomer containing a double bond (lower) and of a crude sample during epoxidation (upper).

Figure 5.11 SEC chromatogram of functionalized PLLA and epoxidized PLLA. [M]/[I]=8
As a result of the controllable nature of the graft placement by this method, the molecular weights of both the main backbone and the grafts are predictable. The polymers obtained have many structural variables such as composition, backbone length and branch length, which gives rise to the possibility of producing "perfect" polyesters with different kinds of structures, Figure 5.12.

*Figure 5.12 Presentation of one possibility of using the functionalized macromonomers.*
6. **STAR-SHAPED POLYESTERS**

There are several routes to obtain star-shaped polymers. The pathway that will be described here serves as a useful method to overcome several problems discussed in the introduction.

### 6.1 Spirocyclic tin alkoxides

The condensation reaction described in Scheme 6.1 between dibutyltin oxide and pentaerythritol ethoxylate compounds were used to synthesize spirocyclic tin initiators. Two different commercial pentaerythritol ethoxylate compounds were used, one with approximately 3 ethylene oxide units, 6, and the other with 15 ethylene oxide units, 7.

**Scheme 6.1 Synthesis of spirocyclic tin initiators.**

Similar spirocyclic tin initiators have been synthesized using ethylene glycol. These exhibited a low solubility in organic solvents and they were difficult to use in polymerizations. The low solubility was a result of undesirable oxygen interactions. The complexations were caused by the free O-electrons, which interacted with the free d-orbitals of the Sn-atoms. The initiator therefore oligomerized as soon as the reaction mixture temperature was lowered. These drawbacks are eliminated when
Star-shaped polyesters

Pentaerythritol ethoxylate compounds are used instead. The reason is that the added ether oxygen donates electrons to the tin atom and this has a stabilization effect on the molecule. Such O–Sn donor-acceptor interaction is typical for all tin alkoxides and stannoxanes.\textsuperscript{137}

Initiators 8 and 9 were characterized by $^1$H-NMR. The methylene protons in the ether chains could easily be seen in the spectra as a chemical shift of 3.4-3.8 ppm. Figure 6.1 shows the spectrum of initiator 8.

![Figure 6.1 $^1$H-NMR spectrum of initiator 8.](image)

6.2 Synthesis of star-shaped poly(L-lactide)

When new advanced architectures are developed, the polymerization system needs to be fully evaluated and optimized. As in the previous sections, both the polymerization process and the polymer obtained have been evaluated. LLA was used as monomer. To monitor the polymerization, samples were withdrawn from the reaction mixture at different times. The conversion tests showed that the initiation was instantaneous and that the conversions were high, 95\% for initiator 9 and 97\% for initiator 8. In Figure 6.2 $M_n$ and MWD are shown as a function of conversion for both initiators. $M_n$ increases linearly and the MWD is narrow, indicating a low amount of transesterifications.
Figure 6.2 \( M_n \) and MWD as a function of conversion for LLA polymerization using the spirocyclic tin initiators. \([M]/[I] \) (initiator 8) = 120, \([M]/[I] \) (initiator 9) = 100, \([M]_0 = 0.5M\), \( T = 60^\circ C \)

The semilogarithmic plot of \(-\ln([M]/[M]_0)\) versus the reaction time also gave straight lines, Figure 6.3. This linearity shows that the occurrence of undesirable termination reactions is low. It can be concluded that the initiation of LLA using spirocyclic tin initiators in ROP was controlled.

Figure 6.3 Semilogarithmic plot of LLA monomer conversion expressed as \(-\ln([M]/[M]_0)\) versus reaction time. \([M]/[I] \) (initiator 8) = 120, \([M]/[I] \) (initiator 9) = 100, \([M]_0 = 0.5M\), \( T = 60^\circ C \)
The $^1$H-NMR spectrum of the resulting precipitated PLLA initiated by 8, Figure 6.4, indicates that an acylation of the Sn-OCH$_2$ groups has taken place. The signal from the protons in the ether chains closest to the first lactyl unit was shifted to 4.3 ppm.

The multiplet around 4.4 ppm ("b" in Figure 6.4), results from the CH-\text{OH} end protons. The multiplet character is a consequence of the simultaneous coupling with the neighbouring -CH$_3$ and -OH protons. Signals from the methine protons in the PLLA chains and from the protons in the incorporated initiator unit can also be seen. The assignment has been made by using $^1$H–$^{13}$C hmqc-gs spectrum and DEPT. Results from the polymerizations using various [M]/[I] ratios with initiators 8 and 9, with LLA as monomer, are compiled in Table 6.1.

**Table 6.1** Results from the polymerization of PLLA initiated by A) initiator 8 and B) initiator 9.

<table>
<thead>
<tr>
<th>[M]/[I]$^a$</th>
<th>Time (h)</th>
<th>Conv.$^b$ (%)</th>
<th>Yield (%)</th>
<th>MWD</th>
<th>$M_{n,NMR}$$^c$</th>
<th>$M_{n,th}$$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>27</td>
<td>97</td>
<td>85</td>
<td>1.09</td>
<td>6200</td>
<td>7000</td>
</tr>
<tr>
<td>140</td>
<td>66</td>
<td>97</td>
<td>97</td>
<td>1.06</td>
<td>21100</td>
<td>19600</td>
</tr>
<tr>
<td>270</td>
<td>132</td>
<td>97</td>
<td>97</td>
<td>1.07</td>
<td>38500</td>
<td>37700</td>
</tr>
<tr>
<td>500</td>
<td>290</td>
<td>97</td>
<td>93</td>
<td>1.07</td>
<td>79500</td>
<td>69800</td>
</tr>
</tbody>
</table>
The molecular weights of the star-shaped polymers were estimated by $^1$H-NMR from the integration values of the resonance at 5.2 ppm originated from -CH ("a") of the lactyl block and -CH$_2$ ("h") of the initiator residues at 3.4 ppm, as shown in the equation:

$$M_n = \frac{I_a}{I_h} \cdot 4 \cdot M_n,\text{LLA} + M_n,\text{initiator}$$

[6.1]

where $I_a$ and $I_h$ are the integration values for peaks "a" and "h" respectively in the $^1$H-NMR spectrum of the precipitated polymer. It was assumed in the calculations that one molecule of initiator starts the growth of exactly four PLLA chains. The molecular weights obtained when 8 was used as initiator were calculated on the assumption that only two arms were involved in the $\beta$-methylene group signal. It is worth mentioning the low dispersities obtained during the polymerizations (1.04-1.13). All these results indicate that the molecular weight of the PLLA can be accurately predicted by controlling the molar ratio of monomer to initiator, that the PLLA obtained has four arms and that we successfully developed a controlled system for synthesizing star-shaped polymers.

The hydrodynamic volumes of linear and star-shaped polymers are different. Therefore, linear polymers were synthesized according to an earlier published method using a cyclic tin initiator and SEC measurements of the linear and star-shaped PLLA were compared. As expected, the $M_n$ for the star-shaped polymers were lower compared to linear ones with the same DP, Figure 6.5. The elution times are longer because of the smaller hydrodynamic volume.
Different architectures also result in different thermal behaviors. The melting point of a branched structure is lower than that of the corresponding linear structure. The reduction in melting point with branching is attributed to the decrease in chain length and, of course, to the increase in the number of free chain ends. This affects the orderly crystalline pattern. It is not therefore surprising that the polymers synthesized in this work have a lower $T_m$ than their linear counterparts. The $T_m$ were examined by DSC and were compared with those for the corresponding linear polymers, Table 6.2.

Table 6.2 Comparison of $T_m$ of linear and star-shaped PLLA.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DP</th>
<th>$T_m$ $^a$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear PLLA</td>
<td>20</td>
<td>130.4</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 8</td>
<td>20</td>
<td>129.2</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 9</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Linear PLLA</td>
<td>250</td>
<td>165.0</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 8</td>
<td>250</td>
<td>160.3</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 9</td>
<td>250</td>
<td>155.6</td>
</tr>
<tr>
<td>Linear PLLA</td>
<td>500</td>
<td>172.8</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 8</td>
<td>500</td>
<td>166.2</td>
</tr>
<tr>
<td>Star-shaped PLLA using initiator 9</td>
<td>500</td>
<td>166.3</td>
</tr>
</tbody>
</table>

$a^a$ melting temperature, determined by DSC
7. NETWORKS

7.1 Synthesis of networks

Degradable networks were synthesized in an effort to apply the presently investigated star-shaped polymers in an advanced technology area. This was carried out in a one-pot reaction through polycondensation of the resulting spirocyclic polymers using acid chlorides according to Scheme 7.1.

Scheme 7.1 The reaction pathway used for network synthesis with a controlled crosslink density.
Star-shaped and linear polymers have been crosslinked through the polycondensation technique using di-functional- or tetra-functional acid chlorides, structures 10 and 11 respectively.

\[
\begin{align*}
\text{10} & \quad \text{Cl} - \text{O} - \text{Cl} \\
\text{11} & \quad \text{Cl} - \text{O} - \text{Cl} - \text{O} - \text{Cl} - \text{O} - \text{Cl}
\end{align*}
\]

An advantage of this approach is that it is possible to control the segment length between the crosslinks via the [M]/[I] ratio in the feed in the ROP reaction.

Homopolymers of PLLA are hydrophobic and do not swell to any great extent in water. Using copolymerization, more hydrophilic networks could be obtained. PDXO or copolymers of PLLA and PDXO were used. Scheme 7.2 shows the different synthesized networks.

*Scheme 7.2 Summary of networks synthesized using spirocyclic polymers and acid chlorides.*
Networks C and F were synthesized using linear polymers. The linear triblock copolymers were synthesized according to earlier reported methods.\textsuperscript{61, 150}

### 7.2 Characterization of networks

The resulting networks were characterized after soxhlet extraction with CH\textsubscript{2}Cl\textsubscript{2} and drying. The networks were swollen in chloroform and could be analyzed by \textsuperscript{1}H-NMR. The crosslinking density were calculated and compared with the theoretical value given by the feed ratio. The results are summarized in Table 7.1.

**Table 7.1 Summary of the results of the crosslinking reactions.**

<table>
<thead>
<tr>
<th>Network code</th>
<th>[M]/[I]\textsuperscript{a}</th>
<th>Conv. DXO \textsuperscript{a}</th>
<th>Conv. LLA \textsuperscript{a}</th>
<th>[M]/[Cr]\textsuperscript{b}</th>
<th>[M]/[Cr]\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>-</td>
<td>99</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>-</td>
<td>97</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>-</td>
<td>97</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>240</td>
<td>120</td>
<td>99</td>
<td>92</td>
<td>180</td>
</tr>
<tr>
<td>E</td>
<td>240</td>
<td>120</td>
<td>99</td>
<td>96</td>
<td>360</td>
</tr>
<tr>
<td>F</td>
<td>120</td>
<td>60</td>
<td>98</td>
<td>95</td>
<td>180</td>
</tr>
</tbody>
</table>

\textsuperscript{a} monomer/initiator ratio \textsuperscript{b} theoretical crosslink density \textsuperscript{c} crosslink density of the network, obtained from \textsuperscript{1}H-NMR characterization
The tetrafunctional acid chloride exhibited an analytical disadvantage. When DXO was used in the networks the crosslinking density could not be calculated because the NMR signals overlapped. The first preliminary tests were carried out using LLA as monomer and the crosslinking densities obtained were close to the theoretical values.

The swelling characteristics of networks provide information indicating possible applications. Properties like permeability, biocompatibility, degradation rate and mechanical properties can be directly related to the water content. The swelling properties of the networks are therefore a critical factor. The degree of swelling of a network is dependent on the pore size of the polymer network and on the interaction between the polymer and the solvent. In this work, the equilibrium degree of swelling, DS, was calculated according to the equation:

$$\text{degree of swelling (DS)} = 100 \cdot \frac{(W - W_0)}{W_0}$$  \hspace{1cm} [7.1]

where $W_0$ is the initial weight of the dry sample and $W$ is the final weight of the swollen sample. Average values from three measurements are reported and water has been used as solvent in all cases. In Figure 7.1, the DS is presented as a function of time for hydrogels A and B.

![Figure 7.1 DS as a function of time for network A and network B.](image)

The water absorption increased with increasing immersion time and then reached a maximum value. Network B, with longer arms, had a higher swelling ratio. The DS decreases with increasing crosslinking density, since there is less space for swelling of the network. All the swelling results are summarized in Figure 7.2.
Network D and network F have the same block composition, which exclude any swelling effects obtained from hydrophilicity differences, and the same crosslinking density. Regardless of the different synthetic pathways, they show almost the same degree of swelling. Network B have a higher DS compared to network C despite the higher crosslink density, revealing the influence from the ethylene oxide units. They make the network more hydrophilic and add flexibility into the chains. Network E has the lowest DS of all the networks built up of both PDXO and PLLA, as would be expected due to the highest crosslinking density. In comparison, Peppas and coworkers prepared PEG star polymer hydrogels by \(\gamma\)-irradiation. The hydrogels obtained from star-PEG with \(M_n=450,000\), 75 arms, swelled to 29 times their dry weight and star-PEG with \(M_n=624,000\), 31 arms, swelled 38 times their dry weight in deionised water.\(^9\) Figure 7.3 is a typical scanning electron microscopy (SEM) picture of the networks placed in a moist environment (environmental SEM), showing the soft and smooth surface.

**Figure 7.2** Summary of the swelling experiments of network B-F.

**Figure 7.3** ESEM picture of network D.
As a complement to the swelling measurements, thermal characterization was carried out using DSC. Varying the structure readily alters the physical properties of the polymer networks, as can be seen in Table 7.2.

**Table 7.2 Thermal characteristics of the networks.**

<table>
<thead>
<tr>
<th>Network code</th>
<th>DP&lt;sup&gt;a&lt;/sup&gt; DXO</th>
<th>DP&lt;sup&gt;b&lt;/sup&gt; LLA</th>
<th>DS (%&lt;sup&gt;c&lt;/sup&gt;)</th>
<th>T&lt;sub&gt;g&lt;/sub&gt; (&lt;sup&gt;d&lt;/sup&gt;°C)</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (&lt;sup&gt;d&lt;/sup&gt;°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>-</td>
<td>180±19</td>
<td>-32</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>-</td>
<td>103±10</td>
<td>-31</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>-</td>
<td>103±10</td>
<td>-31</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>240</td>
<td>120</td>
<td>528±59</td>
<td>-22</td>
<td>128</td>
</tr>
<tr>
<td>E</td>
<td>240</td>
<td>120</td>
<td>351±26</td>
<td>-25</td>
<td>118</td>
</tr>
<tr>
<td>F</td>
<td>120</td>
<td>60</td>
<td>498±15</td>
<td>-33</td>
<td>133</td>
</tr>
</tbody>
</table>

<sup>a</sup> degree of polymerization of DXO in the copolymer.<br>
<sup>b</sup> degree of polymerization of LLA in the copolymer.<br>
<sup>c</sup> glass transition temperature, determined by DSC.<br>
<sup>d</sup> melting temperature, determined by DSC.
8. Surface Characterization

In order to engineer material surfaces with optimal properties for cell adhesion and proliferation, the surface properties need to be considered. The triblock copolymers of hydrophobic PLLA and hydrophilic PDXO are an interesting option as scaffolds in tissue engineering. Surface characterization was therefore included in this work. However, a more complete investigation is needed and is also in preparation, and this will form a basis for further studies. The triblock copolymers were synthesized according to the earlier reported method. The ratio between the block lengths was altered, the ratio of LLA in the triblock copolymers being varied between 30 and 65 mol%. Table 8.1 summarizes the different synthesized triblock copolymers and their characteristics.

The hydrophilicity of the surface is interesting with respect to cell growth. Results of the contact angle measurements confirm earlier results that these surfaces are hydrophilic and that the hydrophilicity is dependent on the triblock composition. For example, the advancing contact angles of polymer 2 and polymer 4 were 83 and 63 respectively. A contact angle of zero indicate full wetting, whereas an angle between 0 and 90 indicates a spreading of the drop caused by molecular attraction.

The morphology and topography may be taken as an advantage and be used for example to optimize the cell adhesion. This is because the cells adhere differently to surfaces depending on the topography. Here are topography results relating to the linear triblock copolymers of PLLA and PDXO presented. Films were prepared on

<table>
<thead>
<tr>
<th>Polymer notion</th>
<th>Polymer composition PLLA:DXO:PLLA</th>
<th>mol% LLA</th>
<th>Mₐᵃ·10³</th>
<th>Mₐᵇ·10³</th>
<th>MWD</th>
<th>Tₘᶜ(°C)</th>
<th>T₉ᵈ(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer 1</td>
<td>50:230:50</td>
<td>30</td>
<td>41.1</td>
<td>28.3</td>
<td>1.29</td>
<td>162.3</td>
<td>-35.7</td>
</tr>
<tr>
<td>Polymer 2</td>
<td>150:160:150</td>
<td>65</td>
<td>61.8</td>
<td>52.3</td>
<td>1.25</td>
<td>164.5</td>
<td>-33.5</td>
</tr>
<tr>
<td>Polymer 3</td>
<td>120:300:120</td>
<td>44</td>
<td>69.5</td>
<td>54.3</td>
<td>1.35</td>
<td>158.8</td>
<td>-32.5</td>
</tr>
<tr>
<td>Polymer 4</td>
<td>180:290:180</td>
<td>55</td>
<td>85.5</td>
<td>69.3</td>
<td>1.12</td>
<td>161.1</td>
<td>-30.6</td>
</tr>
<tr>
<td>Polymer 5</td>
<td>100:400:100</td>
<td>33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ᵃ) number-average molecular weight of the PDXO block, calculated from ¹H-NMR spectrum of the precipitated polymer b) number-average molecular weight of the PLLA block, calculated from ¹H-NMR spectrum of the precipitated polymer c) melting temperature, determined by DSC d) glass transition temperature, determined by DSC

The hydrophilicity of the surface is interesting with respect to cell growth. Results of the contact angle measurements confirm earlier results that these surfaces are hydrophilic and that the hydrophilicity is dependent on the triblock composition. For example, the advancing contact angles of polymer 2 and polymer 4 were 83 and 63 respectively. A contact angle of zero indicate full wetting, whereas an angle between 0 and 90 indicates a spreading of the drop caused by molecular attraction.

The morphology and topography may be taken as an advantage and be used for example to optimize the cell adhesion. This is because the cells adhere differently to surfaces depending on the topography. Here are topography results relating to the linear triblock copolymers of PLLA and PDXO presented. Films were prepared on
f freshly cleaved mica sheets and during the melt-induced phase-separated crystallization in an inert atmosphere the materials developed a distinct surface roughening, cf. Figures 8.1 A and B.

As-cast specimens were observed to be featureless because they were in a disordered state, the heating causes the block copolymers to develop an ordered structure.

8.1 Influence of process parameters

The morphologies of triblock copolymers 1, 2, 3 and 4 in Table 8.1 were studied after heat treatment. Both the polymer composition and the heating process have earlier been shown to affect the morphological structures and the physical properties. AFM has been used in the tapping mode to observe the melt-induced phase-separated crystallization of these triblock copolymers. The AFM technique has advantages over other microscopic techniques since it allows uncoated crystalline regions to be studied.

8.1.1 Solution concentration and cooling procedure

The thin films were made at two different solution concentrations, 0.3 wt% and 5 wt%. The samples were either quenched or slowly cooled to room temperature after the heating procedure. This resulted in different surface characteristics. Figure 8.2 shows a typical example of polymer 3 obtained from a 0.3 wt% solution. The fiber structures developed when the cooling rate was low, the picture to the right in Figure 8.2. When the sample was quenched to room temperature, a sheet-like structure was instead formed, the left-hand picture. It must be noted that the fiber structure was not
observed on all occasions when the low concentration was used together with a slow cooling, but the fiber structure never appeared with the fast cooling.

![Figure 8.2 AFM phase images of solution-cast films of polymer 3, 0.3 wt\% solution, the sample was quenched to room temperature (left picture) and slowly cooled to room temperature (right picture) after heat treatment.](image)

At the higher concentration, the frequency of the fiber structures obtained increased significantly. In this case, no difference between the cooling procedures could be seen. As shown in Figure 8.3, the fiber structure appeared using both methods.

![Figure 8.3 AFM phase images of solution-cast films of polymer 3, 5 wt\% solution, the sample was quenched to room temperature (left picture) and slowly cooled to room temperature (right picture) after heat treatment.](image)
An explanation of these results may be that, owing to the difference in film thickness, the morphology was not the same in the two cases. The average film thickness using the low concentration was 40 nm whereas the film thickness obtained at the higher concentration was 190 nm. It has been reported by other researchers that the growth of crystals is changed by thinning of the films. Phenomena such as reduced mobility, which resulted in a diffusion-controlled growth, and that the morphological transition takes place from a layered to an in-plane phase separation with decreasing film thickness have been discussed.\textsuperscript{157-159}

8.1.2 Variation of the segment lengths

The segment length in the triblock copolymers influenced the fiber structure. Figure 8.4 shows one example. In the left-hand picture, the PLLA blocks are much shorter than the PLLA blocks in the right-hand picture, which resulted in thinner fibers. This is an interesting result because it shows that changing the thickness of the fibers also alters the topography, and this creates opportunities to optimize the cell adhesion.

The mechanism of the structural organization cannot be explained at this point. To answer this question, additional experiments are planned, but searching the literature reveals a probable model for the chain folding.\textsuperscript{129, 160} The ordered line in Figure 8.5 represents the folding of the PLLA. The turn is perpendicular to the plane of the paper and, when the DP of the PLLA blocks is changed the lamella width decreases.
Nevertheless, the final morphology exhibits interesting highly ordered micro-domain structures, which makes it an interesting research topic.

### 8.2 Topography

To better understand the height variations on the surfaces of the triblock copolymer, a three-dimensional image of polymer 3 is shown in Figure 8.6. The height variations can clearly be seen, the light and dark parts correspond to height differences. If it were possible to control these height variations, it would be possible to vary the cell adhesion.
8.3 Cell adhesion

One method of evaluating the biocompatibility of a material is to measure the cell adhesion \textit{in vitro}. The adhesion is critical for the success of a biomaterial because the cells need to adhere appropriately to the substrate in order for subsequent cellular functions to occur in the appropriate way at the implant site. This is a cost-effective procedure in the early stages of the biocompatibility testing of potential biomaterials. In this chapter, the first cell adhesion results using the triblock copolymers are presented, obtained in cooperation with the group of Professor Biagini.\textsuperscript{161} Keratinocytes were grown onto the heat-treated samples of polymer 2, polymer 4 and polymer 5. Keratinocytes are the cells that make the protein keratin and are the predominant type of cells in outer layer of the skin. Analysis by SEM showed that the keratinocytes adhered most to polymer 5 and less to polymers 2 and 4. The spreading of cells onto polymers 2 and 4 was poor; the cells maintained a round shape or became only poorly spread, Figure 8.7. The cells grown on polymer 5 developed a more elongated shape, Figure 8.8.
More specifically, the cells that adhered to polymer 5 had an intermediate morphology between round and elongated. On polymer 2, mainly rounded cells were observed and the cells grown on polymer 4 showed a spreading morphology, Figure 8.9. As mentioned in the introduction, the adhesion is most often higher on hydrophilic surfaces. This was the case here as well. Cells adhered best to polymer 5, the most hydrophilic sample, and polymer 4 is more hydrophilic than polymer 2.
The existence of cells with an elongated shape is an indication of movement. It suggests that the nano-structured substrate obtained during the heat treatment was able to provide some degree of "direction" to the grown cells. To investigate this further cells were grown on a reference material without any structure and the number of cells with an elongated morphology became less. Time-lapse studies showed that cells seeded on polymers 2 and 4 remained motionless on the substrate, showing no tendency of movement, Figure 8.10. Cells seeded on polymer 5 showed a migration tendency, Figure 8.11.

Figure 8.9 The morphology of the keratinocytes grown onto polymers 2, 4 and 5.

Figure 8.10 Time-lapse studies of keratinocytes on polymer 2.
These data are in agreement with the hypothesis that these materials have a good compatibility. The polymers seem to be compatible with \textit{in vitro} cell lines. However, the material biocompatibility needs to be more thoroughly evaluated, as these results show only the major trends. Work using fibroblasts (which synthesize for example collagen and elastin) and chondrocytes (cartilage cells) is in progress and a SEM picture of a fibroblastic murine cell line grown on polymer 5 is shown in Figure 8.12.
9. **CONCLUSIONS**

The over-all conclusion of this work is that all the evaluated initiators can be used to synthesize well-defined structures. New controlled synthesis pathways have been established to produce usable aliphatic polyesters with a crucial functionalization and advanced architectures. The architectures obtained could be utilized in subsequent reactions to further vary the structure and obtain new, even more advanced and controlled architectures. The polymer surface showed an interesting pattern after the heating process, which could be varied and cells were able to adhere and grow on these surfaces.

- Triblock copolymers of poly(ethylene glycol) and poly(L-lactide) have been synthesized using different germanium initiators. These copolymers are very attractive as biomaterials and the results revealed that the macromonomers can be synthesized in a controlled way.

- Poly(L-lactide) have been functionalized with a double bond in the backbone. New tin initiators were used and the initiator fragment including the double bond was inserted into the lactide chain during polymerization. The double bond was subsequently epoxidized without any degradation. These polymers can be used to build up advanced architectures with unique possibilities of obtaining well-controlled structures. The structure will be unique, where the branches are built up essentially of a monodisperse macromonomer while the backbone is built up of another monomer.

- Two new spirocyclic tin alkoxide initiators have been synthesized and used in the reaction of star-shaped poly(L-lactide). The results showed that the spirocyclic tin initiators are a very attractive class of new tin alkoxides. For example, hydrogels with a predetermined length between the crosslinks were synthesized *in situ*. These structures were synthesized as homopolymers and copolymers. Different crosslinking agents were also used. Swelling tests in water showed very high swelling ratios and a dependence on the internal structure within the network.

- Surface characterization of the poly(L-lactide)-poly(1,5-dioxepan-2-one)-poly(L-lactide) triblock copolymers showed that phase separation during heat treatment created a well-defined fiber structure, whose dimensions could be altered by changing the copolymer structure. Preliminary results showed that the surfaces promote the adhesion and growth of cells.
10. Future Perspectives

One of the goals of tissue engineering is to find polymer structures useful for exchanging the ECM. The polymer will need a biospecific surface that provides interactive bonds and the polymer should also, after fulfilling its role, be replaced by a native ECM. This work evaluated a route to obtain functionality in the polymer, and it is possible in future work to use this technique and create more advanced structures. Using this method, the grafting points will be known and the material properties can then be related to a predetermined structure. There is a continuing interest in the preparation of multifunctional polymeric materials and the evaluation of their potential in the biomedical field.

Since the synthesized networks have a known and predetermined structure, the degradation profile can be varied. This is of importance in, for example, controlled drug delivery applications where the erosion rate of the drug is dependent on the degradation and also on the network mesh size. The ability to influence the hydrophilicity of the network makes it possible to use different drugs by tuning the network properties through copolymerization. The effect of the presence of chemical crosslinks on the microphase separation, in relation to the morphologies in the linear block copolymers, is another interesting area that needs to be studied. The ability to control the phase separation will make it possible to produce surfaces with a known morphology and topography.

Process polymers and achieve desired physical and other characteristics without any chemical modification is also fascinating. This would reduce the cost of taking a new material through the time-consuming regulatory pathway. Research on composites provides opportunities to achieve desired goals without chemically altering a biomaterial. Shape-memory polymers, whose permanent shape differs from the initial shape, are also interesting. With this technique it is possible to achieve smart materials.162
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12. References

4) Chardack, W. M.; Gage, A. A.; Greatbatch, W., Surgery 1960, 48, 643-664
6) Reed, A. M.; Gilding, D. K., Polymer 1981, 22, 494-498
20) Claridge, D. V., Cyclic ether lactones, US Patent 1,272,733, 1972
26) Stridsberg, K.; Albertsson, A. C., Polymer 2000, 41, 7321-7330
References

References

58) Sbarbati-Del Guerra, R.; Cascone, M. G.; Tricoli, M.; Cerrai, P., *ATLA* 1993, 21, 97-101
References

120) Thapa, A.; Miller, D. C.; Webster, T. J.; Haberstroh, K. M., *Biomaterials* 2003, 24, 2915-2926
References

125) Dalby, M. J.; Riehle, M. O.; Johnstone, H.; Affrossman, S.; Curtis, A. S. G., *Biomaterials* 2002, 23, 2945-2954
136) Ikeda, K., *Desalination* 1994, 96, 113-118


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