Introducing lactide-based biodegradable tissue adhesives

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Lactide-based low molecular weight copolymers were synthesized and investigated as tissue adhesives. The oligomers were composed of di or trifunctional central connecting segments and lateral PLA blocks. Copolymers with glass transition temperatures in the 20–25 °C range, were found to perform better. Strong connection was found between the length of the PLA blocks, the glass transition temperature (T_g) and the Adhesive Failure Strength of the different materials. Flexible ε-caprolactone (CL) molecules were inserted into the PLA blocks, to produce longer biodegradable chains and improve the adhesive strength of the oligomers, while keeping their T_g within the appropriate temperature interval. Branched oligomers consisting of a trimethylolpropane central molecule and three LA–CL segments, displayed enhanced *in vitro* adhesive properties.

1. Introduction

Tissue adhesives are being used in a diversity of biomedical applications, to attach tissues temporarily, until healing is completed. In the surgical arena, adhering, rather than suturing or stapling planes of tissues, is highly atractive, provided it is fast acting and assures complete closure. A tissue adhesive must be biocompatible and is required to degrade within the same time interval of the healing process. In addition, it has to insure a union capable of bearing the physiological load for the time required, connect quickly to tissues, be easy to apply and affordable.

Among the existing adhesives in clinical use, only few meet these attributes, *albeit* to a limited extent. They include alkyl cyanoacrylates [1–3] and natural sealants such as gelatin [4–6] and fibrin [7–9]. Since most natural glues show substantially lower mechanical bonding capabilities, compared to their synthetic counterparts [1, 6], natural glues are rarely used as tissue adhesives, but as sealants. Furthermore, since fibrin glues are manufactured from human blood or plasma, the menace of the transmission of infective agents cannot be completely ruled out [1, 10]. On the other hand, despite their relatively high bonding strength, the clinical use of alkyl cyanoacrylates is largely limited, mainly due to their toxicity [1, 11, 12]. Most alkyl cyanoacrylates are prohibited for use on internal organs since tissue damage and impairment of function may result, and are, therefore, limited to topical skin use. Also, once the polymerization process has started, it cannot be stopped and, therefore, the surgeon has little latitude to rectify errors made during the application of the adhesive. Also, the polymerization of alkyl cyanoacrylates is exothermic and may cause thermal damage to tissues, especially if the layer spread is too thick [1, 11].

This article introduces novel biocompatible tissue adhesives that do not involve any chemical or biochemical reactions, during their application *in vivo*. The use of these new adhesives is based exclusively on their temperature-dependent rheological properties. This was achieved by engineering low molecular weight segmented copolymers that display low viscosity at the temperature of application (T_a) and attain substantially higher viscosity once at body temperature (T_b). Evidently, these materials are also required to exhibit suitable adhesive properties, once at the site of use. The initial low viscosity and enhanced flowability displayed by these materials at T_a, enable them to be easily and efficiently applied to the location, as well as to attain improved conformability and enhanced attachment to the tissues. The sharp increase in viscosity these oligomers exhibit as they cool down to T_b, is a fundamental requirement for these materials to accomplish their desired physical function as adhesives. Since biocompatibility and biodegradability are additional crucial attributes of tissue adhesives, the polymers were tailored so that they as well as their degradation products are non-toxic.

2. Experimental

The synthesis of A–B–A triblocks, comprising a polyethylene glycol (PEG), polypropylene glycol (PPG) or ethylene glycol (EG) central segment and two lateral polylactic (PLA) blocks, consisted of the ring opening polymerization of *dl*-lactide, the cyclic dimer of

lactic acid, initiated by the hydroxyl terminal groups of the diol. The same synthetic pathway was followed when trifunctional central molecules were used, as in the case of trimethylolpropane (TMP). Also, when the chains comprised not only lactoyl building blocks but also ε-caprolactone (CL) units, multistep reactions were performed, consisting of the sequential ring opening polymerization of *dl*-lactide and ε-caprolactone molecules, to generate segments of both components.

All the reactions were carried out in the molten state, under a dry nitrogen atmosphere and with mechanical stirring. PEG, PPG, EG and TMP were dried for 1 h at 100 °C, under vacuum and stirring. PEG chains of various molecular weights (200, 400 and 1000) and ethylene glycol were supplied by Aldrich and Baker, respectively. PPG chains of various molecular weights (425, 1000, 2000 and 4000), TMP and ε-caprolactone (CL) were purchased from Aldrich. *dl*-lactide was provided by Boehringer Ingelheim and the stannous octanoate catalyst was supplied by Sigma. Since preliminary work conducted in our laboratory demonstrated that *l*-lactide generated copolymers were unsuitable for their use as tissue adhesives, this article focused on *dl*-lactide based materials.

2.1. Synthesis

Weighed amounts of *dl*-lactide or ε-caprolactone were added to dry PEG, PPG, EG or TMP, to generate the PLA or PCL segments. The amount of lactide was varied, depending on the length of the PLA blocks to be formed (from 4 to 30 lactoyl repeating units). Stannous octanoate was added at a 1/400 catalyst/lactide molar ratio. The reaction was carried out at 145 °C for 150 min, in a dry N_2 enviroment and with magnetic stirring. Lateral chains containing not only lactoyl blocks but also caprolactone segments (typically containing between 1 and 4 CL molecules), were synthesized following the same basic procedure. PLA blocks were formed initially, followed by the polymerization of CL, by reacting PLA's OH terminal groups with caprolactone rings and starting their polymerization. The same sequential synthetic scheme was followed also when complex multiblocks containing various LA and CL sequences were formed.

2.2. Characterization

The proton NMR spectra were recorded using a Bruker 300 High resolution ^{1}H- NMR spectrophotometer. Deuterated chloroform was used to dissolve the samples.

The molecular weight and polydispersity were determined by Gel Permeation Chromatography (GPC) using a Waters Alliance Model 2690 with Refractometer Detector Water 410 and Milllenium Chromatography manager.

Thermal analysis was performed using a Mettler TA 3000 DSC thermoanalyzer. The thermograms ranged from − 100 to 200 °C, at 10 °C/min rate. Sample weight was between 9 and 11 mg, and the analysis was conducted under an inert nitrogen atmosphere.

2.3. Mechanical tests

The adhesive strength tests were performed using two different types of substrates. First, 1 mm thick bovine embryo skin was used. Unfortunately, bovine skin was not homogenous and tended to deteriorate rather rapidly, even when stored at − 18 °C. In addition, the failures usually occurred within the glue itself (cohesive failure) and were not adhesive failures at the skin/glue interface. In light of the above, later tests were performed using 30 μm thick polyamide (6/6 nylon) films. The tests (T-Peel Test ASTM D 1876) were performed using an Instron machine (model 4502) and adhesive failure strength (AFS) values were determined and reported in (Newton *per* cm width) units. The tests were performed at a speed of 30 mm/min at 37 °C. Each test was repeated five times.

The **nomenclature** used in this article to designate the different materials synthesized, denotes first the central segment connecting the biodegradable blocks and its molecular weight, followed by the average number of lactoyl units of each of the PLA chains. Therefore, PPG1000$(LA_{14})_2$, for example, describes a copolymer based on a central PPG1000 segment, with two lateral PLA blocks, each containing, on average, 14 lactoyl units. The same nomenclature was used also when LA and CL sequences were formed. Therefore, TMP$(LA_8$–$CL_2)_3$, for example, denotes a polymer consisting of a TMP central segment, bound to three blocks consisting of a first PLA block containing eight lactoyl building blocks and then two CL units.

3. Results and discussion

The aim of this work was to develop tissue adhesives that have the ability to display large temperature-dependent viscosity differentials, within a narrow and clinically relevant temperature range. This was achieved by engineering low molecular weight segmented copolymers that exhibit low viscosity at the temperature of application (T_a) and attain substantially higher viscosity, once they cool down to body temperature (T_b). The application temperature of the adhesives was determined to be lower than 60 °C, to avoid thermal damage to the tissues. Evidently, these materials were also required to exhibit suitable adhesive properties, once at the site of use.

Since the application of these new adhesives is based on their temperature-dependent rheological properties, various basic considerations were taken into account, while tailoring the molecules. The first, focused on the relationshionship between temperature, rheological properties, and molecular weight (see Fig. 1). Due mainly to substantial chain entanglements, the viscosity of high molecular weight polymers is not only too high for tissue adhesive purposes, but also hardly changes over clinically relevant temperature intervals {region I} in Fig. 1. The viscosity of high molecular weight polymers changes sharply only in two regions, none of which is applicable: (a) around the glass transition temperature (T_g) {region II}, where the material is excessively rigid, and (b) in the melt region {region III},

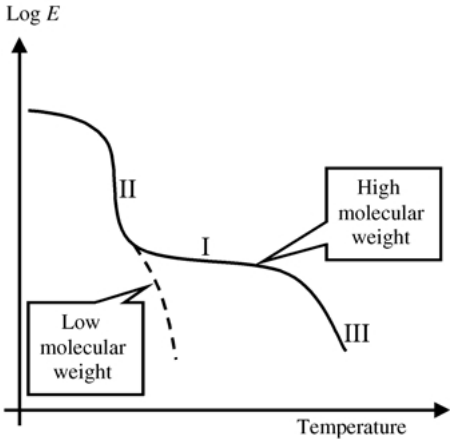

Figure 1 A schematic plot of the change in modulus as a function of temperature.

where, obviously, the temperature is utterly unacceptable from a clinical perspective.

Since low molecular weight polymers tend to display very limited crystallizability and they have substantially less chain entanglements than their high molecular weight counterparts, they exhibit a steep decrease in viscosity at a temperature slightly above their T_g. In addition to molecular weight considerations, the inherent flexibility of the chain is a key factor to take into account, when designing these molecules. In light of the above, biodegradable oligomers displaying a glass transition at a temperature somewhat lower than the body temperature were, therefore, synthesized. The viscosity differential displayed by these materials between the two relevant temperatures, T_a and T_b, as well as the adhesive properties of the oligomers, were fine tunned by incorporating lactide and caprolactone building blocks, each of them playing distinct chemical and physical roles.

Besides playing a central role in tailoring the rheological properties and degradability of the different copolymers, PLA blocks were viewed as playing a major role in rendering the oligomers with the required adhesiveness. This, based on previous work conducted in our laboratory, which revealed the adhesive properties of low molecular weight PLA chains. The main function of the caprolactone units was to enhance the segmental flexibility of the polyester chains, allowing, thus, to fine tune the glass transition temperature of the molecules being engineered. This, in the context of the efforts made to extend the length of the polyester blocks, enhancing, therefore, their cohesive strength, while keeping the T_g values in the appropriate temperature interval. PEG and PPG chains, having different molecular weights, as well as ethylene glycol were used to create linear oligomers, whereas branched molecules comprised TMP. By controlling the composition and molecular weight of the diverse components of these copolymeric systems, a variety of materials were produced, displaying different physical properties, biodegradation kinetics, and adhesive capabilities.

The different oligomers were characterized by nuclear magnetic resonance (NMR) spectroscopy, gel permeation chromotography (GPC) and differential scanning calorimetry. The ratio between the central segment and

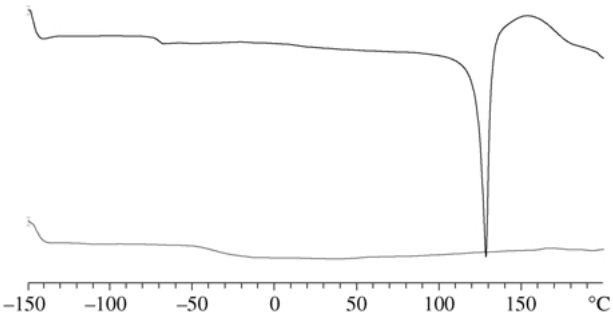

Figure 2 Thermograms of PPG2000 and dl-lactide (upper), and their copolymer PPG2000$(LA_{14})_2$ (lower).

the biodegradable chains (% w/w) was determined using ^{1}H-NMR spectroscopy, by comparing peaks representative of each of the constituents of the molecule. This can be illustrated for PPG4000$(LA_6)_2$, for example, where the peaks ratioed were that assigned to PPO's protons (α to the oxygen), at 3.5 ppm (duplet) and the peak at 5.2 ppm (quartet), due to the proton of PLA's methine groups. When PEG-containing oligomers were synthesized, the peak used was that assigned to PEG's methylene protons, at 3.65 ppm (singlet). GPC provided strong supporting evidence of the occurrence of the ring opening polymerization, as revealed by chromatograms of the final oligomers, that differed markedly from those of the starting materials.

Fig. 2 presents the thermograms of PPG2000$(dl-LA_{14})_2$ at time 0 (the mixture of the reagents before the reaction) and at the end of the synthesis. As expected, the mixture of the reagents exhibits two peaks, a T_g at $-73\,^{\circ}C$, attributed to the PPG2000 chains and the melting endotherm of the *dl*-lactide, at 125 °C. The total disappearance of the latter, as well as the shift of PPG's T_g to $-39\,^{\circ}C$, due to the stiffenning effect of the PLA blocks, are clearly indicative of the occurrence of the reaction. GPC data showing the absence of the lactide peak in the chromatogram, provided additional evidence of the incorporation of the lactide into the oligomer.

The type of the failure – adhesive as opposed to cohesive – exhibited by the different materials, was determined by assessing the appearance of the substrates surfaces, after failure, using colored glue (as described in Fig. 3). Clearly, the photograph on the left, where two surfaces equally colored are apparent, demonstrates that

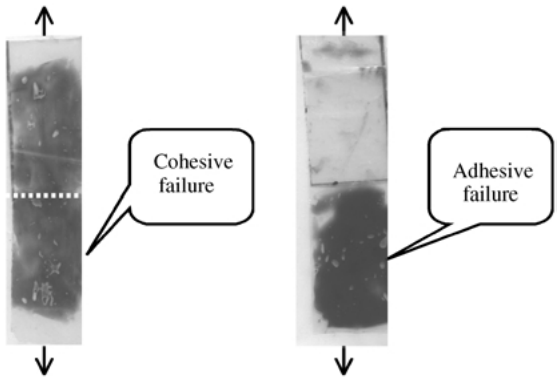

Figure 3 Types of failure occurring when T-Peel tests were performed on nylon 6/6 films.

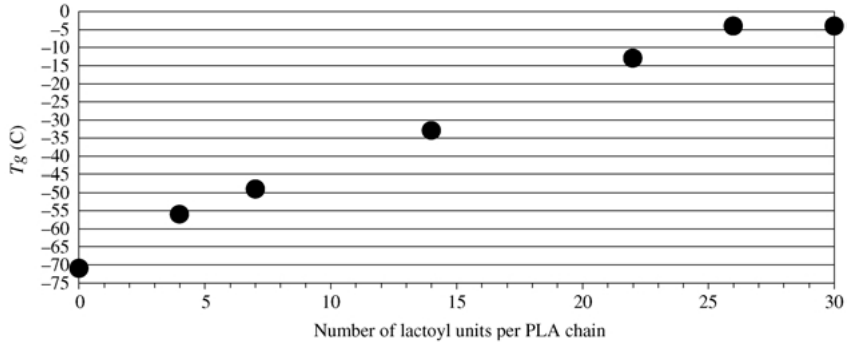

Figure 4 Effect of the length of the PLA blocks on the T_g of PPG2000-containing copolymers.

a cohesive failure mechanism was in action. This, as opposed to the system on the right, were an adhesive failure is evident.

The effect of the length of the biodegradable chains on the glass transition of these oligomers is exemplified in Fig. 4, which presents the T_g values of PPG2000-containing copolymers, with increasingly long *dl*-lactide blocks (up to 30 lactoyl units *per* lateral segment). While PPG2000 shows a very low glass transition (around $-71\,^{\circ}C$), expectedly, T_g shifts to higher temperatures as the number of lactoyl units increases, leveling off for segments containing 25 and 30 repeating units.

The same effect was observed when other central segments were used, even though the range of T_g changes, varied accordingly. For example, as the molecular weight of the PLA chains in PEO1000-containing oligomers increased from $(LA)_4$ to $(LA)_{14}$, the T_g values measured were -42 and $-17\,^{\circ}C$, respectively. Fig. 5 presents the increase in T_g as a function of the length of the *dl*-PLA blocks, with ethylene glycol (EG) central segments. Even though the general pattern was the same, it is apparent that the temperature interval covered by the glass transition temperatures of these materials is much higher, starting from $10\,^{\circ}C$, for $EG(LA_8)_2$, increasing steadely up to $22\,^{\circ}C$ for $EG(LA_{22})_2$.

Expectedly, also the length of the central segment largely affected the glass transition of these materials. This can be exemplified for two copolymers, both containing the same LA blocks, $(LA)_{14}$, but differing in the length of the central segment. While the T_g of the PPG2000-containing oligomer was $-33\,^{\circ}C$, the material containing a short PPG400 chain had a glass transition $45\,^{\circ}C$ higher, at around $12\,^{\circ}C$.

The data graphically presented in Fig. 6 clearly demonstrate the large effect the length of the PLA blocks has on the adhesive strength of the material. The AFS values measured at $37\,^{\circ}C$, increased by a factor of more then 20, as the length of the PLA blocks attached to the central EG molecule increased from 8 to 16 units.

T_g and AFS values followed the same pattern described above, also when branched central segments, were used. Figs. 7 and 8 present the change in T_g and AFS, respectively, as the length of the PLA blocks bound to TMP, increased.

Ample experimental evidence revealed that copolymers with glass transition temperatures in the 20–25 °C range, performed better at both temperatures, T_a and T_b, namely, these materials displayed satisfactory flowability at $60\,^{\circ}C$, and achieved higher AFS values at $37\,^{\circ}C$.

Having said that, it should be stressed that the AFS values ($37\,^{\circ}C$) displayed by the LA-containing oligomers described above, were relatively low and that these materials failed cohesively. It is also worth underscoring the fact that, even though the increase in the molecular weight of the PLA blocks resulted in an increase in cohesive strength, it resulted also in high T_g values and unacceptably high viscosity levels at clinically tolerable temperatures ($T_a < 60\,^{\circ}C$). Only molecules consisting of rather short PLA segments could be applied at T_a, but the low molecular weight of these blocks resulted in weak materials that failed cohesively, at T_b.

The viscosity differential attained by these polymers between the temperature of application and body temperature, was fine tuned by incorporating flexible caprolactone building blocks along the PLA chain. The main function of the caprolactone units was to enhance the segmental mobility of the polyester chains, and

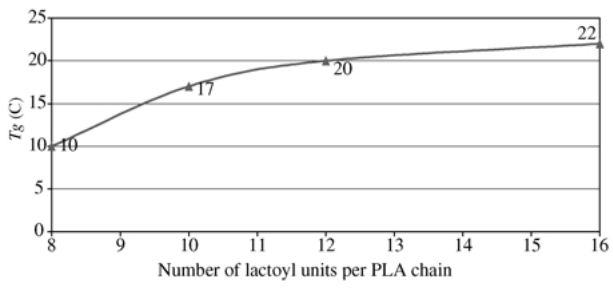

Figure 5 Effect of the length of the PLA blocks on the T_g of EG-containing copolymers.

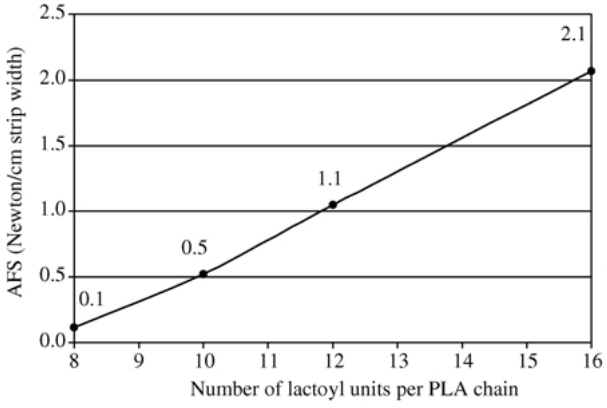

Figure 6 Effect of the length of the PLA blocks on the adhesive failure strength (AFS) of EG-containing copolymers, at $37\,^{\circ}C$.

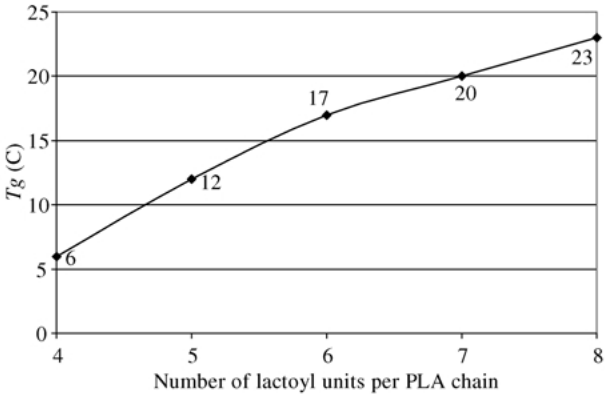

Figure 7 Effect of the length of the PLA blocks on the T_g of TMP-containing copolymers.

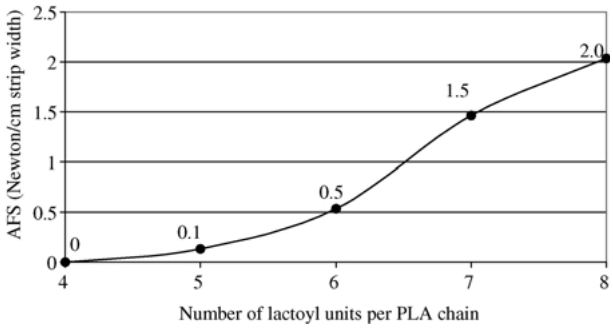

Figure 8 Effect of the length of the PLA blocks on the AFS of TMP-containing copolymers, at 37 °C.

compensate for PLA's stiffening effect, allowing, therefore, to adjust the glass transition temperature of the molecules being engineered. That, aiming at extending the length of the polyester blocks and enhancing their cohesive strength, while keeping the T_g values in the clinically appropriate temperature interval. Since caprolactone had a detrimental effect on the adhesive properties of the oligomers, CL's content was fine tuned so to combine in an optimal way, enhanced adhesiveness and suitable rheological behavior.

Whereas LA-CL linear as well as branched oligomers were synthesized, the reminder of this article will focus on TMP-based materials. The number of LA and CL segments along the chains, as well as their respective segmental degree of polymerization were varied in a controled manner. The different oligomers were characterized by NMR Spectroscopy and GPC. The ratio between the central segment and the two biodegradable components (% w/w) was determined using ^{1}H-NMR spectroscopy, by comparing peaks representative of each of the constituents of the molecule. This can be illustrated for TMP(LA_4–CL_1–LA_4)$_3$, for example, where the following peaks were ratioed: (a) the protons of TMP's methyl group, at 0.9 ppm (triplet), (b) the peak at 5.2 ppm (quartet), due to the proton of PLA's methine group, and (c) the protons of CL's methylene groups, α to the carbonyl bond, at 2.4 ppm (triplet). GPC provided strong evidence of the occurrence of the sequential polymerization of both types of units and the concomitant increase in the molecular weight of the oligomer. This is exemplified for TMP(LA_4–CL_1–LA_4–CL_1–LA_4)$_3$ (see Fig. 9), which presents the chromatograms obtained at each of the steps along the synthesis of the final copolymer.

As already stated, the purpose of incorporating the caprolactone units, was to compensate for the stiffening effect of increasingly long PLA blocks, by enhancing the segmental mobility of the polyester chains, while the T_g values remained essentially constant. Fig. 10 presents T_g values for four different oligomers, two of which comprised LA blocks only, whereas the other two incorporated also CL units. While TMP(LA_8)$_3$ had a glass transition around 23 °C, doubling the length of the PLA chains TMP(LA_{16})$_3$ resulted in a large, 14 °C increase in T_g. That, in clear contrast to TMP(LA_8–CL_1–LA_8)$_3$, which, even though had approximately twice the molecular weight of TMP(LA_8)$_3$, exhibited the same T_g value. The strong flexibilizing effect of CL, can be further underscored by comparing the glass transitions exhibited by TMP(LA_{16})$_3$ and TMP(LA_8–CL_1–LA_8)$_3$. Even though they have a very similar molecular weight, the CL-containing oligomer exhibited a transition temperature around 22 °C, strinkingly lower than the $T_g = 37$ °C, displayed by TMP(LA_{16})$_3$. Moreover, when the polyester chain

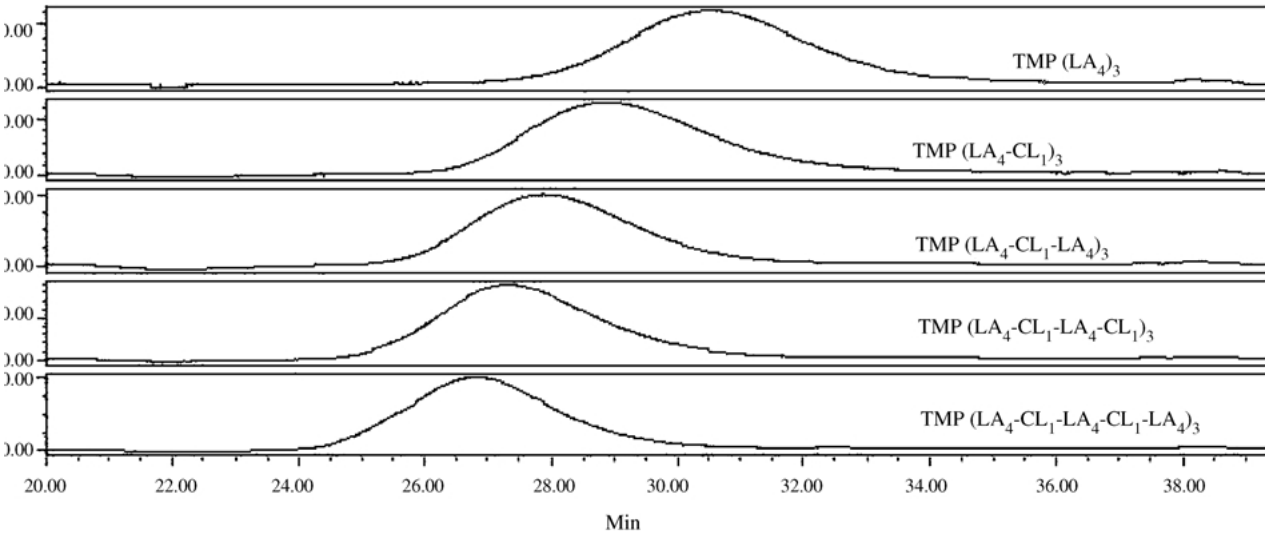

Figure 9 Gel permeation chromatograms obtained at each of the steps along the synthesis of TMP(LA_4–CL_1–LA_4–CL_1–LA_4)$_3$.

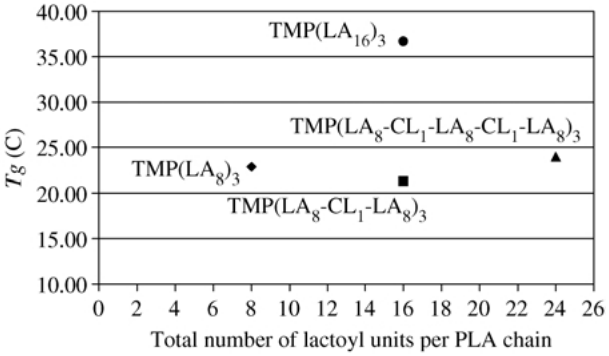

Figure 10 Effect of the incorporation of CL units into the PLA blocks on the T_g of TMP-containing copolymers.

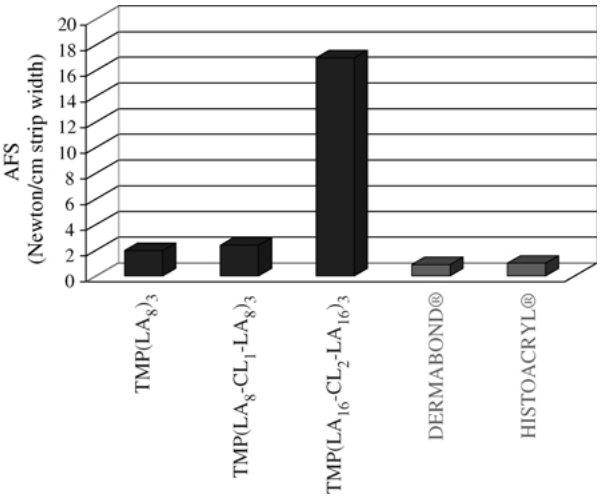

Figure 11 AFS values for TMP-containing copolymers and two cyanoacrylate adhesives in clinical use: Dermabond® and Histoacryl®.

was lengthened even further, as in TMP(LA_8–CL_1–LA_8–CL_1–LA_8)$_3$, the introduction of the caprolactone units along the chain, allowed the glass transition to remain unchanged, at around 24 °C.

The purpose of the incorporation of caprolactone units was to allow the lengthening of the biodegradable blocks (enhancing, therefore, their cohesive strength), while keeping the T_g values within the appropriate temperature range. The AFS data presented in Fig. 11, are in full agreement with the theoretical considerations. It is worth stressing that the three copolymers displayed in Fig. 11, have very similar T_g values, all of them falling within the 21–24 °C interval, described previously. It is apparent also, that oligomers comprising longer LA–CL chains, performed better as adhesives at 37 °C. This behavior was especially impressive for TMP(LA_{16}–CL_2–LA_{16})$_3$. This copolymer, which comprised rather long LA-CL segments, achieved adhesive failure strength levels, 16 times higher than those showed by the tissue adhesives presently in clinical use.

4. Conclusions

Linear and branched oligomers, consisting of a central binding segment and two or three biodegradable lateral blocks, were synthesized and characterized.

A strong correlation was found between the molecular weight of the biodegradable chains, T_g and the adhesive failure strength of the different materials, with copolymers with glass transition temperatures in the 20–25 °C range, performing better. The incorporation of flexible ε-caprolactone (CL) molecules along the PLA blocks, allowed to form longer biodegradable chains and improved the adhesive strength of the oligomers, while keeping their glass transition within the appropriate temperature range.

The degradation kinetics and the *in vivo* adhesive properties of these copolymers are currently under investigation and will be published separately.

Acknowledgment

G.L. dedicates this article to Shmuel and Ester Lando.

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Received 4 March
and accepted 25 July 2002