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Development of Tissue Adhesives Based on Amphiphilic Isocyanate-Terminated Trimethylene Carbonate Block Copolymers

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Summary: In this study, novel 3-armed star-shaped isocyanate-terminated block copolymers based on trimethylolpropane ethoxylate (TMPE) and trimethylene carbonate (TMC) varying in composition were designed and synthesized. By reaction with water, networks were formed. The characteristics of these networks were compared with those prepared from analogous linear structures based on polyethylene glycol (PEG) and TMC.
Tensile testing of the networks highlighted good mechanical properties. In lap shear adhesion tests to chamois leather, very good bonding strengths of up to 0.6 MPa were obtained. These values compare favorably with those of Dermabond®, which is currently one of the strongest clinically used tissue adhesives.

Keywords: biodegradable; poly(trimethylene carbonate) isocyanate functionalization; star polymer; tissue adhesive

Introduction

Biodegradable tissue adhesives have great potential to be used in the biomedical field as replacement for sutures and staples. They allow easier and faster application and the procedure is less painful for the patient.[1,2]

In practice, only two types of adhesives are mainly applied in the clinic: fibrin glue and cyanoacrylates. Fibrin glues are natural materials that crosslink to form hydrogels via a mechanism that resembles the final stages of blood coagulation.[3] Fibrin is biocompatible and biodegradable, it rapidly degrades within 2 weeks. However, its bonding strength to tissue is too low to be used for load bearing applications and it can be a source of disease transmission.[4,5]

Secondly, cyanoacrylates are often applied. They have very good adhesive properties and cure quickly,[6] however they are hard, rigid and brittle. These compounds are degradable, but since formaldehyde is a degradation product, their toxicity is an issue.[7,8] For this reason their application is limited to topical skin wound closure. Commercial products are Dermabond® and Histoacryl®.

There is a clear need for a new tissue adhesive, with good mechanical- and adhesive properties that at the same time is biodegradable and non-toxic. It has been shown that isocyanate-functionalized polymers potentially can be used as tissue adhesives.[9] Our group reported that linear copolymers (oligomers) based on poly(-ethylene glycol) (PEG) and trimethylene carbonate (TMC), which were end-functionalized with isocyanate groups have adhesive properties to intervertebral disk tissue.[10] PEG is a biocompatible hydrophilic polymer, while TMC is hydrophobic and flexible and provides enzymatic degradability.[11] Variation of the copolymer composition allows adjusting the

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hydrophilicity of the system and optimizing the wetting of the tissue surface.[12] The isocyanate groups allow chemical reactions with the amine, thiol and hydroxyl groups present in the tissue forming covalent bonds.[13] At the same time, reaction with water leads to the formation of amine end-groups which can react with isocyanate end-groups to form biuret bonds and network.[14] As carbon dioxide is formed during this reaction as well, these amphiphilic networks can be foamed.

In this study, we designed novel 3-armed star oligomeric copolymers based on trimethylolpropane ethoxylate (TMPE), TMC and hexamethylene diisocyanate (HDI). Low molecular weight reactive copolymers with different contents of TMC were prepared, to assess the influence of the hydrophilic to hydrophobic ratio on the properties of the cured materials. The properties of the star-shaped copolymers and resultant networks were compared with those of analogous linear structures.

Experimental Part

Materials

Trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim (Germany). Polyethylene glycol (PEG) ($M_n = 400$ g/mol), trimethylolpropane ethoxylate (TMPE) ($M_n = 450$ g/mol), acetic anhydride, stannous octoate (tin 2-ethylhexanoate, $SnOct_2$), chloroform-d ($CDCl_3$), fibrinogen and thrombin (both isolated from bovine plasma) were purchased from Sigma Aldrich (Netherlands). Hexamethylene diisocyanate (HDI) was purchased from Merck Schuchardt (Germany). Dermabond® (2-octyl cyanoacrylate) was purchased from Ethicon, Johnson & Johnson (Netherlands). Phosphate buffered saline (PBS) was purchased from B Braun Melsungen AG (Germany). Diethyl ether was purchased from Biosolve (Netherlands) and dried over molecular sieves A4 prior to use. PEG and TMPE were dried at 140 °C under vacuum before use. All other products were used as received.

Synthesis and Characterization of Oligomers and Isocyanate-Functionalized Oligomers

Oligomers were synthesized by ring opening polymerization of TMC using PEG or TMPE as the initiator. In a typical procedure, TMC was reacted with PEG or TMPE using $Sn(Oct)_2$ (0.02 mmol/mol of monomer) as a catalyst. The polymerization was conducted at 130 °C for 3 days under nitrogen atmosphere. Oligomers with TMC and PEG molar ratios of 2:1 and 4:1, and oligomers with TMC and TMPE with molar ratios of 3:1 and 6:1 were prepared. The used nomenclature for the synthesized oligomers is: PEG-$(TMC_m)_2$ or TMPE-$(TMC_m)_3$, where m is the number of TMC units per oligomer arm.

The oligomers were then reacted with HDI to obtain reactive molecules. In a typical procedure, a metered volume of HDI was placed in a flask under the flow of nitrogen. The oligomer was then added drop-wise to maintain an excess of the isocyanate compound. The final molar OH/NCO ratios for the linear- and 3-armed compounds were 2.08 and 3.12, respectively. The reaction was allowed to proceed at 75 °C for 8 hours. The excess of unreacted HDI was removed by precipitation in dry diethyl ether, the product was dried under vacuum overnight. The resulting products were labeled: PEG-$(TMC_m\text{-}HDI)_2$ or TMPE-$(TMC_m\text{-}HDI)_3$. Figure 1 shows the synthesis of the oligomers and their subsequent functionalization with HDI.

The chemical structure of the oligomers and functionalized oligomers was assessed using ^1H NMR spectroscopy (Bruker 400MHz NMR spectrometer) with $CDCl_3$ as the solvent and FTIR spectroscopy using Perkin-Elmer Spectrum 1000 FTIR spectrometer. Using NMR, the TMC content, number average molecular weight (Mn) as well as the degree of functionalization (DF) after subsequent reaction with HDI could be determined with an accuracy of approximately 1%.

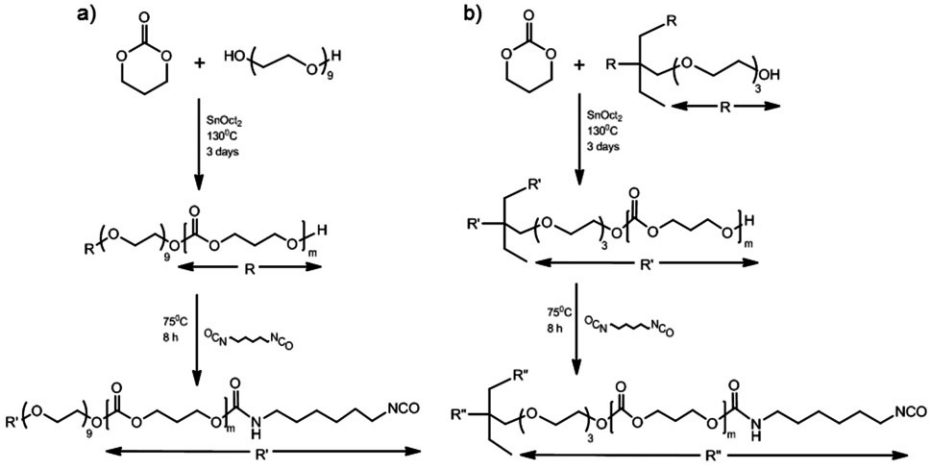

Figure 1.
Synthesis of PEG-$(TMC_m\text{-}HDI)_2$ (a) and TMPE-$(TMC_m\text{-}HDI)_3$ (b) isocyanate-functionalized oligomeric copolymers. The value of m is 1 or 2.

Network Formation and Network Characterization

Network films were prepared by casting the isocyanate-functionalized oligomers on glass with a 0.3 mm thick casting knife and immersing in excess of water. The cast films were left overnight at room temperature (RT) to allow reaction with water and curing.

To evaluate the network characteristics, their gel content, degree of swelling in chloroform and water uptake were determined. The prepared networks were dried in a vacuum oven at 40 °C until constant weight, then their initial mass was determined (m_i). The specimens were then placed in chloroform or water for 24 hours and their wet mass (m_{wc} and m_{ww}) was determined. Finally, the samples were re-dried in a vacuum oven at 40 °C until constant weight and their dry mass was determined (m_d). The experiments were performed in triplicate.

The gel content (GC) in chloroform was calculated using:

$$GC = \left(\frac{m_d}{m_i}\right) \cdot 100\%$$

The degree of swelling (DS) in chloroform was calculated using:

$$DS = \left(\frac{m_{wc}}{m_d}\right) \cdot 100\%$$

The water uptake (WU) was calculated using:

$$WU = \left(\frac{m_{ww} - m_d}{m_d}\right) \cdot 100\%$$

The thermal properties of the water-cured networks were determined by differential scanning calorimetry (DSC) using Perkin Elmer Pyris 1 before and after drying. Samples of 5–10 mg were heated from −100 °C to 100 °C at 10 °C/min. Glass transition temperatures and maximum melting temperatures could be determined with an accuracy of ±1 °C.

Dumbbell-shaped test specimens of the water-cured network films were prepared according to ISO 37:1994 (E), Type 3 and subjected to tensile testing using a Zwick Z020 universal tensile tester. The grip separation was 35 mm and the crosshead speed was 50 mm/min. The mechanical properties of the films were evaluated both in the wet and in the dry state after drying in a vacuum oven at 40 °C for 24 hours. All measurements were performed in triplicate.

Application as Tissue Adhesive

Chamois leather (dermal sheep collagen) was chosen as a model for tissue. Strips of approximately $0.5 \times 10 \times 30$ mm were cut, immersed in water and blotted with tissue

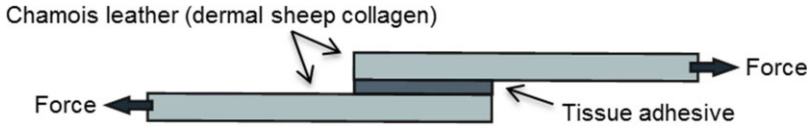

Figure 2.
Testing of the lap shear adhesion strength after curing of the different tissue adhesives.

to remove excess water. The different isocyanate-functionalized compounds were applied onto the chamois leather to evaluate their adhesive properties. On each strip, the adhesive covered an area of approximately 100 mm^2. Two of these strips were pressed together, immersed in water and left overnight at RT to allow curing and network formation.

To determine the adhesion strength, the wet specimens were subjected to a lap shear tensile test analogous to ASTM F2255-05 using a Zwick Z020 universal tensile tester. This is shown schematically in Figure 2. The grip to grip separation was 25 mm and the crosshead speed 50 mm/min. The shear adhesive bond strength expressed in MPa is the maximum shear force divided by the glued area. Each measurement was performed at least in triplicate.

Two currently applied tissue adhesives Dermabond® and fibrin glue were used as controls. Dermabond® was used as received, fibrin glue was prepared by dissolving fibrinogen and thrombin in PBS at concentrations of 100 mg/mL and 100 U/mL, respectively. Subsequently, these two solutions were mixed in double syringe system with a static mixer and directly applied onto the wet chamois leather.

Preliminary Cytotoxicity Evaluation

The cytotoxicity of PEG-$(TMC_1$-$HDI)_2$ was evaluated using primary human annulus fibrosus cells (ScienCell, USA) and corresponding media (ScienCell). The cells were incubated at 37 °C and 5% CO_2 for 3 d to reach 90% confluence. The PEG-$(TMC_1$-$HDI)_2$ was added to the cell culture medium (0.3 g/ml) and incubated overnight at 37 °C. The extract was then filter-sterilized and added to the annulus fibrosus cells in culture at different concentrations varying from 1 to 20% (ml extract per ml culture medium), 5 wells not containing the extract were used as control. The cells were then incubated for a further 24 h at 37 °C and 5% CO_2. At this time point, cell viability was assessed by basic light microscopical morphological investigation.

Results and Discussion

Synthesis and Characterization of Oligomers and Functionalized Oligomers

Several oligomers varying in composition were synthesized by ring opening polymerization of TMC using PEG or TMPE as initiator and subsequently reacted with HDI giving isocyanate-terminated reactive oligomers. NMR analysis showed that TMC conversion was higher than 98% in all cases.

The molecular weight of PEG was determined by NMR after functionalization with acetic anhydride, the molecular weight of TPME was confirmed by comparing the integral values of the methylene group at 0.9 ppm with the repeating ethylene oxide units (-O-CH_2-CH_2-) at 3.5–3.75 ppm. The TMC content and the number average molecular weights (M_n) of the non-functionalized oligomers were then determined by comparing the integral values of the TMC peak (-COO-CH_2-CH_2-CH_2-O-) at 4.1–4.3 ppm or at 2.1 ppm and the integral values of the PEG peak (-O-CH_2-CH_2-) at 3.5–3.7 ppm in the ^{1}H NMR spectra. In case of TMPE, the integral values of TMC were compared with those of the TMPE methylene group at 0.9 ppm.

A typical spectrum of a PEG-$(TMC_1)_2$ oligomer is shown in Figure 3a.

Table 1 shows that the TMC contents and the determined number average molecular weights of PEG, TMPE and the

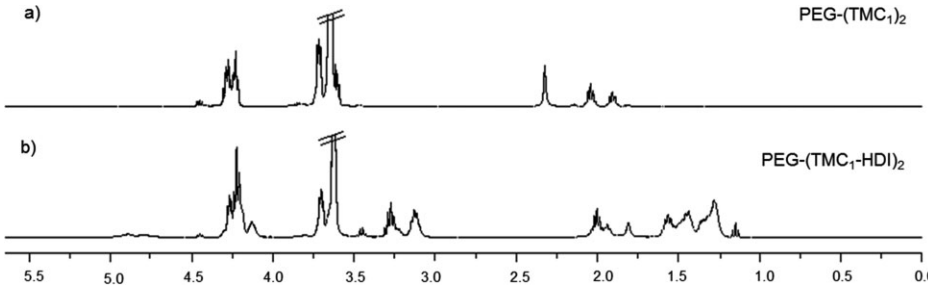

Figure 3.
Characteristic ^{1}H NMR spectra of a PEG-$(TMC_1)_2$ oligomer (a) and a corresponding isocyanate-functionalized PEG-$(TMC_1$-HDI$)_2$ oligomer (b).

synthesized block copolymeric oligomers are close to the expected values.

The extent of functionalization of the oligomers was also assessed by ^{1}H NMR spectroscopy, see Figure 3b for a characteristic spectrum. The degree of functionalization was calculated by comparing the integral values of the TMC peaks at 2.1 ppm with those of the methylene peaks of HDI (-OOCHN-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-NCO) at 3.3 ppm or at 1.44–1.6 ppm. As shown in Table 1, the degree of functionalization was high with values above 91% in all cases. In addition, FTIR spectroscopy showed the presence of both the unreacted free isocyanate end-groups at 2235–2300 cm^{-1} and the urethane bonds at 3250–3500 cm^{-1}.

Formation and Characterization of Networks

To evaluate the physical properties of the polyurethane networks, networks in the form of films were formed by reaction of cast isocyanate-functionalized oligomers with water. In a series of reactions, the formation of biuret linkages leads to covalent crosslinking of the block copolymeric chains. Also carbon dioxide, which can lead to foaming, is formed. In our experiments, foaming was limited and films with thicknesses of 0.3–0.4 mm were obtained.

The network properties of these crosslinked amphiphilic films are presented in the Table 2. It can be noted that the gel content of the networks is high for all the compositions, confirming successful functionalization of the oligomers with HDI and crosslinking with water at room temperature. The degree of swelling of the networks in chloroform varied with architecture of the functionalized oligomers. Networks prepared from the linear functionalized oligomers based on PEG could be swollen 800 to 1000%, while networks prepared from TMPE based functionalized oligomers could only be swollen up to approximately

Table 1.
Characteristics of the prepared oligomers. The TMC content, number average molecular weight (M_n) as well as the degree of functionalization (DF) after subsequent reaction with HDI were determined by ^{1}H NMR.

Oligomer	TMC content in oligomer [mol%]	M_n [a)] [g/mol]	M_n [g/mol]	DF after reaction with HDI [%]
PEG	–	400	398	–
TMPE	–	450	439	–
PEG-$(TMC_1)_2$	66.5	604	601	94
TMPE-$(TMC_1)_3$	74.5	756	739	100
PEG-$(TMC_2)_3$	80.0	809	806	99
TMPE-$(TMC_2)_3$	85.7	1062	1037	91

[a)] expected value

Table 2.
Characteristics of the prepared networks. The gel content (GC), degree of swelling in chloroform (DS) and water uptake (WU) of the networks were determined in triplicate.

Network	GC [%]	DS [%]	WU [%]	TMC content [wt%]	PEG content [wt%]
PEG-$(TMC_1\text{-HDI})_2$	100 ± 0	813 ± 73	65 ± 2	21.8	43.6
TMPE-$(TMC_1\text{-HDI})_3$	99 ± 1	340 ± 38	11 ± 1	24.1	35.3
PEG-$(TMC_2\text{-HDI})_2$	86 ± 5	1019 ± 59	29 ± 4	35.4	35.2
TMPE-$(TMC_2\text{-HDI})_3$	99 ± 1	341 ± 18	9 ± 1	40.0	29.3

300%. This indicates that the latter networks are more densely crosslinked. The effect of composition seems to be minimal. (Note that the PEG-$(TMC_2\text{-HDI})_2$ network with the highest degree of swelling had the lowest gel content).

Table 2 also shows that the water uptake of the networks depends on composition as well as on architecture of the functionalized oligomers used. For more or less comparable compositions, water uptake is significantly higher for networks based on PEG than for those based on TMPE as was the case for swelling in chloroform. The effect of TMC content is clear as well, especially for the networks prepared using linear structures. The highest water uptake is observed for PEG-$(TMC_1\text{-HDI})_2$, in which the weight content of the hydrophilic PEG component is 43.6%. It was found that after uptake of water, the PEG-containing networks were opaque, while those based on TMPE remained transparent.

The thermal properties of the networks were evaluated, results are shown in Table 3. In the wet state, only single glass transition temperatures (T_g) can be observed. The networks prepared using PEG-containing functionalized oligomers had a significantly lower glass transition temperatures than those prepared using TMPE. For both architectures an increase in TMC content leads to an increase in T_g.

After drying the water-cured networks, melting points corresponding to the PEG or TMPE component in the soft segment ranging from approximately 35 to 48 °C were measured. Melting points at higher temperatures of approximately 80 °C that correspond to the urethane bonds in the hard segments could also be observed.

In the dry state, T_g of the networks is higher than in the hydrated state and varies from −40 to −34 °C for networks prepared from linear PEG-TMC structures and from −16 to −14 °C for three-armed TMPE-TMC structures. In dry PEG-TMC networks, the glass transition temperature increases with increasing TMC contents, this effect is not evident in TMPE-TMC networks.

The tensile properties of the prepared networks were assessed in the wet and in the dry state, the data is presented in Table 4. Especially for the networks based on linear isocyanate-functionalized PEG-TMC oligomers, composition and water content (see Table 2) has a significant influence on the mechanical properties. The tensile strength (σ), elasticity modulus (E)

Table 3.
Thermal properties of the prepared networks in the water swollen state and in the dry state.

Network	Wet	Dry		
	T_g [a)] [°C]	T_g [a)] [°C]	$T_{m(soft)}$ [b)] [°C]	$T_{m(hard)}$ [c)] [°C]
PEG-$(TMC_1\text{-HDI})_2$	−53	−40	38	80
TMPE-$(TMC_1\text{-HDI})_3$	−36	−14	49	80
PEG-$(TMC_2\text{-HDI})_2$	−36	−34	35	81
TMPE-$(TMC_2\text{-HDI})_3$	−24	−16	40	80

[a)] glass transition temperature
[b)] melting temperature of PEG or TMPE in the soft segment
[c)] melting temperature of the urethane bond in the hard segment

Table 4.
Tensile properties of the prepared networks in the water-swollen state and in the dry state.

Network	Wet			Dry		
	σ [MPa]	E [MPa]	ε [%]	σ [MPa]	E [MPa]	ε [%]
PEG-$(TMC_1$-HDI$)_2$	0.1 ± 0.01	1.2 ± 0.2	14.0 ± 1.1	2.6 ± 0.3	14.4 ± 0.2	52.6 ± 14.3
TMPE-$(TMC_1$-HDI$)_3$	3.3 ± 1.4	34.5 ± 4.8	21.6 ± 10.5	3.8 ± 0.2	33.9 ± 4.9	26.4 ± 4.7
PEG-$(TMC_2$-HDI$)_2$	3.9 ± 0.5	21.5 ± 5.0	108.6 ± 27.4	3.5 ± 0.1	35.3 ± 4.1	48.0 ± 0.7
TMPE-$(TMC_2$-HDI$)_3$	5.0 ± 2.8	45.1 ± 11.6	22.7 ± 7.6	4.4 ± 0.5	48.9 ± 10.2	21.7 ± 1.3

and elongation at break (ε) of PEG-$(TMC_1$-HDI$)_2$ networks that contain the lowest amounts of TMC are very much increased upon drying. For networks prepared from PEG-$(TMC_2$-HDI$)_2$ this effect is not as pronounced. As networks based on TMPE-TMC do not take up as much water (Table 2), the difference in tensile properties in the dry state of these networks do not differ very much from those in the wet state. In all cases the tensile strength and the elasticity modulus of the networks increase with the TMC content.

Application as Tissue Adhesives

PEG-TMC and TMPE-TMC isocyanate-functionalized oligomers were applied as tissue adhesives using chamois leather (dermal sheep collagen) as a model for tissue. The reactive oligomers were spread onto the leather and allowed to cure overnight in a wet environment. Under these conditions the terminal isocyanate groups can react with amine- thiol- and hydroxyl groups present on the surface of the leather as well as with water to form the network.

The adhesive strength of the cured networks (the strength of the adhesive bond) was assessed in a lap shear test. Their strength in gluing chamois leather was compared with that of fibrin glue and Dermabond®. The results are shown in Figure 4.

The figure shows that not only the composition (and concomitant water-uptake) has an important effect on the adhesive bond strength, but that also the architecture of the used isocyanate-functionalized oligomers is of great importance. With increasing TMC content of networks prepared from linear isocyanate-functionalized PEG-TMC oligomers the bond strength increased from 0.03 to 0.35 MPa. The bond strength of networks based on 3-armed isocyanate-functionalized TMPE-TMC was even higher and increased from 0.56 to 0.68 MPa. In all cases, we observed cohesive failure of the glue, the interface between the cured adhesive and the leather remained intact. It is noteworthy that the TMPE-(TMC1-HDI)3 and TMPE-(TMC2-HDI)3 networks with the lowest water uptake had the highest adhesive bonding strengths to chamois leather. Apparently, in this system, it seems that further increasing hydrophilicity in order to enhance wetting of the leather surface is not necessary.

The adhesive bonding strength of fibrin glue was found to be very low, as is known from literature.[15] Dermabond® in contrast, is a much stronger adhesive,[16] and in glueing chamois leather we found high adhesive strength values of approximately 0.4 MPa. The adhesive bond strength of the TMPE-TMC based networks was found to be even better than that of Dermabond®.

Cytotoxicity of Isocyanate-Functionalized Oligomers

As isocyanates can be toxic to cells, we conducted a first experiment to assess the cytotoxicity of PEG-$(TMC_1$-HDI$)_2$ using human annulus fibrosus cells. The adhesive was placed in cell culture medium (0.3 g/ml), the extract of this large quantity was then added to proliferating annulus fibrosus cells. It was found that the extract was not toxic to the cells at concentrations lower than 2% (ml extract per ml cell culture medium) as cell viabilities remained equal

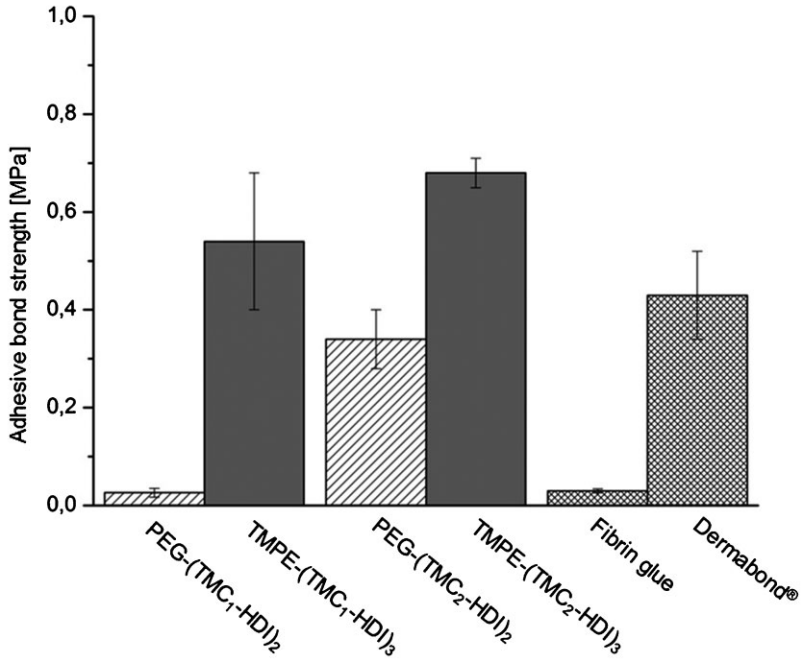

Figure 4.
The adhesive bond strength in the wet state of glued chamois leather upon curing isocyanate-functionalized PEG-TMC and TMPE-TMC oligomers with water. Fibrin glue and Dermabond® were used as controls. Values are expressed as mean ± standard deviation.

to cell viabilities in cell culture medium only. With increasing concentration of extract in the culture medium, cell viabilities decreased: at 3, 5, 8, 10 and 20%, the values of the cell viabilities were respectively 80, 40, 0, 0, 0% compared to the control. This indicates that cytotoxicity is strongly dependent on the amount of extract administered to the cells. At the lowest concentrations, cell viability is not much affected. Nevertheless, it will be important to conduct more extensive investigations of possible cytotoxicity.

Conclusion

We have designed and synthesized PEG-TMC and novel 3-armed star-shaped copolymers based on TMPE and TMC, which were both end-functionalized with isocyanate groups for use as water-curing tissue adhesives. The mechanical properties and the bond strength to chamois leather of the 3-armed star-shaped adhesives were significantly better than those of the linear structures and those of fibrin glue and Dermabond®, which were used as controls. Although more extensive experiments are needed to confirm the compatibility of these adhesives with cells and tissues, the developed materials are promising for use as degradable and resorbable tissue adhesives.

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