Dopamine-functionalized polymers

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(54) **Dopamine-functionalized polymers**

(57) The invention is directed to a tissue-adhesive polymer comprising at least one polymeric chain functionalized with an ArOH group, wherein said ArOH represents a hydroxyl-substituted aromatic group which is bound to said polymeric chain with an amide, urethane, thiourethane or urea bond, optionally via a spacer. The ArOH can be positioned at one or more termini of said polymeric chain, at the backbone of the polymeric chain according to formula Ib, or both. The ArOH can be based on a compound such as dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin. In addition, the invention is directed to a caprolactam blocked hydroxyl-substituted aromatic compound, suitable for the preparation of the tissue-adhesive polymer and to the method for the preparation of the tissue-adhesive polymer.

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Title: Dopamine-functionalized polymers

BACKGROUND OF THE INVENTION

The invention is in the field of tissue-adhesive materials. In particular, the invention is directed to tissue-adhesive multi-arm polymer and applications thereof in tissue-adhesive materials such as hydrogels. The invention is also directed to the processes to prepare the polymer and the use of a reactive intermediates in this and similar processes.

Tissue adhesives are common practice in the fields of surgery, thereby providing an alternative for the traditionally perforating materials such as sutures and staples. The currently used tissue adhesives have limited adhesive strength (*e.g.* fibrin-based products) or are relatively toxic (*e.g.* cyanoacrylate). Examples of adhesive materials include Coseal™, TissuepatchDural™, Hemopatch™ (described in WO2011/079336) and Veriset™. Tissue-adhesive materials that shows improve adhesive properties over these materials are patches described in WO 2019/066657 and WO 2017/171549, the latter being commercially available under the tradename LIQOSEAL™.

The patches however, are not fluidic and limited in their application. Hydrogels at the other hand, are fluidic and can therefore be utilize in different applications. Specific examples of known hydrogels include Evicel™, Adherus™, Tisseel/Tissucol™ and DuraSeal™, that is commercially available from Integra LifeSciences Corporation and described in *e.g.* US5997895. Some of the known hydrogels (*e.g.* DuraSeal™) are NHS-esters-based hydrogels and show a limited adhesive and burst strength. Alternatively to NHS-based gels, hydrogels have been proposed that are based on dopamine-modified poly(ethylene glycol) polymers (see Liu *et al.*, ACS Applied Materials and Interfaces 6 (2014) 16982−16992). The polymers however, require laborious preparation comprising NHS functionalization of poly(ethylene glycol) polymers followed by coupling with dopamine and dialysis of the dopamine and N-hydroxysuccinimide after this coupling to prevent free dopamine. Another drawback of the dopamine-modified poly(ethylene glycol)

polymers by Liu is the presence of an ester bond, which limits the water resistance of these hydrogels.

BRIEF SUMMARY OF THE INVENTION

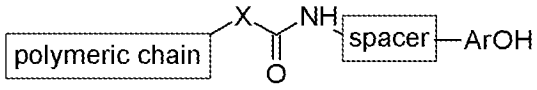
It is an object of the present invention to provide tissue-adhesive polymers that can be used in tissue-adhesive materials. A particular object of the invention is to apply this polymers for hydrogels that preferably give a higher adhesive strength than known materials. A further or alternative object is that these polymers can be prepared by less laborious methods and/or without the release of free dopamine.

Surprisingly, the present inventors found that the above objects can at least partially be met by a tissue-adhesive polymer comprising at least one polymeric chain functionalized with an ArOH group, wherein said ArOH represents a hydroxyl-substituted aromatic group which is bound to said polymeric chain with an amide, urethane, thiourethane or urea bond, optionally via a spacer. With hydroxyl-substituted aromatic group is meant an aromatic group that is substituted with a hydroxyl.

Thus, the polymer of the present invention is functionalized with the ArOH group via an amide, urethane, thiourethane or urea bond. Advantageously, such a bond is more stable to hydrolytic conditions as ester bonds. Preferably, the ArOH group is bound to the polymer via a urethane, thiourethane or urea bond, as these are even more stable than amide bonds, in particular a urea bond, which is the most stable of the group.

The ArOH group can for instance be based on dopamine, as is explained in more detail herein below. The combination of the ArOH and the aforementioned bond with which the ArOH is bound to the polymeric chain, result is very good adhesive properties and allow the preparation of hydrogels that meet the above-described objects.

The polymer of the present invention may at least partially have a structure according to formula I, wherein X is S, NH and/or O, or absent.



I

In the embodiments wherein X is absent, the polymeric chain is directly bound to the -C(O)-NH-spacer-ArOH group.

For sake of clarity it is noted that the polymeric chain may bind more than one -XC(O)-NH-spacer-ArOH groups, even though only one is illustrated in formula I. In addition, in formula I, the -XC(O)-NH-spacer-ArOH may be position at one or more termini of the polymeric chain, at the backbone of the polymeric chain, or both.

The present inventors realized that marine mussels adhere to substrates by spinning treads made from proteins which are rich in the catecholic amino acid 3,4-dihydroxyphenyl alanine (DOPA), and that introducing DOPA or other hydroxyl-substituted aromatics results in polymer materials that are water resistant and have good adhesive properties under wet conditions.

The present inventors further found that in a particularly preferred embodiment of the present invention, the tissue-adhesive polymer is a multi-arm polymer, comprising a core and two or more polymeric arms of which at least one arm comprises said one polymeric chain functionalized with an ArOH group. It was found that the multi-arm facet of the polymer further results in a particular high adhesive strength towards tissues. Accordingly, in a particularly preferred embodiment of the present invention the present invention is directed to a tissue-adhesive multi-arm polymer comprising a core from which polymeric arms extent, which polymeric arms are substituted with a hydroxyl-substituted aromatic group.

The polymer of the present invention can be tailored such that the ArOH group is positioned at one or more termini of said polymeric chain, at the backbone of the polymeric chain, or both. This tailoring is enabled by the provision of various caprolactam blocked hydroxyl-substituted aromatic compounds as building blocks. These building blocks are suitable for the preparation of the tissue-adhesive polymer by a reaction of the caprolactam blocked group with a hydroxyl, amine, and/or sulfhydryl group that is present on the other building blocks (*e.g.* a polymeric chain or chain extender).

Typically, formation of amide, urethane, thiourethane or urea bonds require reactive reagents such as isocyanates that are incompatible with the hydroxyl-substituted aromatic groups and as such require protection of the hydroxyl with a protective group (*e.g.* an ester). The present inventors however found that such protection is not required when using the caprolactam blocked hydroxyl-substituted aromatic compounds as described herein. Caprolactam blocked isocyanates can be used for isocyanate-free routes to urethane compounds (see *e.g.* Maier *et al.* Macromolecules (2003) 4727-4734).

BRIEF DESCRIPTION OF THE DRAWINGS

In Figure 1, gelation times of various multi-arm polymers in accordance with the present invention are depicted.

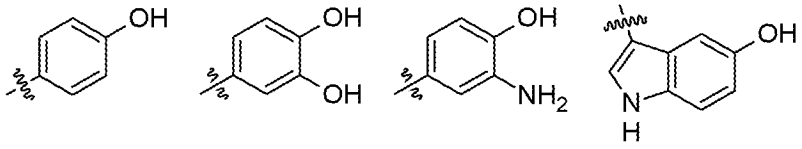
DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a tissue-adhesive polymer comprising at least one polymeric chain functionalized with an ArOH group, wherein said ArOH represents a hydroxyl-substituted aromatic group which is bound to said polymeric chain with an amide, urethane, thiourethane or urea bond, optionally via a spacer.

Similar to what is known for DOPA, hydroxyl-substituted aromatic groups can be oxidized, after which the aromatic compound can interact, in particular react with tissue, typically at the ortho-position to the hydroxyl substituent. Hydroxyl-substituted aromatic groups such as catechol groups can be oxidized to quinones. Quinones are electrophilic and capable of reaction with nucleophilic groups (*e.g.* thiols and amines) that are present in tissue. Other than a reactive interaction, a physical interaction may also be possible. As such, the polymers and materials functionalized with hydroxyl-substituted aromatic groups can used as tissue-adhesive compounds and materials.

Particularly suitable hydroxyl-substituted aromatic groups include phenol, catechol, 3-hydroxyphenol, 4-hydroxyphenol, 2-aminophenol, 3-aminophenol, 4-aminophenol, 4-hydroxyindole, 5-hydroxyindole, 6-hydroxyindole, 7-hydroxyindole. These group are oxidizable and can subsequently interact with

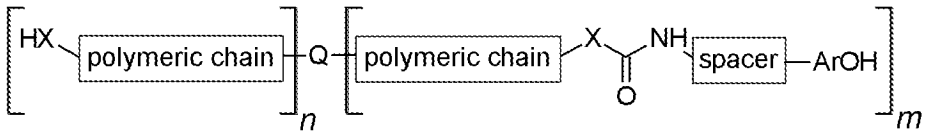
tissue. In a preferred embodiment, the hydroxyl-substituted aromatic group is one or more selected from the group of aromatic moieties having one of the following structures:



Of this group of aromatic moieties, the catechol group is particularly preferred for its presence in several naturally occurring compounds such as DL-DOPA, dopamine, L-DOPA, D-DOPA, and/or noradrenaline. The catechol-group is therefore generally biological well compatible and preferred for medical applications such as tissue-adhesive substances and devices. Accordingly, is a further preferred embodiment, the moiety "NH–spacer–ArOH" (for example in formula Ia below) is based on dopamine, DL-DOPA, L-DOPA, D-DOPA and/or noradrenaline. Other naturally occurring hydroxyl-substituted aromatic compounds such as tyramine and serotonin can also advantageously be used for the moiety "NH–spacer–ArOH".

ArOH functionalization of polymer at the termini

The ArOH group can be attached to the polymer in the backbone, to one or more termini or both. For instance, in case the ArOH is position at one or more termini of the polymer, the polymer may have a structure according to formula II.



II

wherein Q represent a core;

the polymeric chain comprises one or more polymeric groups;

X represents O, S or NH, preferably O;

the spacer represents a spacer functionality, preferably a linear or branched C_1-C_8 hydrocarbon, optionally substituted with one or more OH, SH, halide, amide and/or carboxylate;

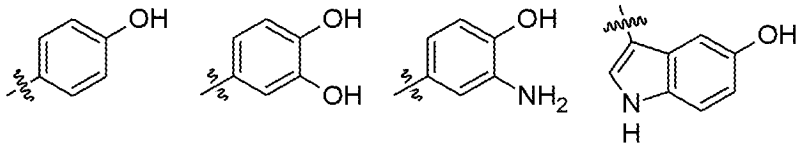
ArOH represents a hydroxyl-substituted aromatic group;

m represents the number of functionalized polymer arms and is 2 or more, preferably in the range of 3 to 12; and

n represents the number of non-functionalized polymer arms and is a number in the range of less than *m*, preferably 0.

The spacer in the polymer of the invention generally originates from an amine compound comprising the hydroxyl-substituted aromatic group. As will be described herein below, the process to functionalize the polymer with this amine compound is also an aspect of the present invention.

Particularly suitable hydroxyl-substituted aromatic groups include phenol, catechol, 3-hydroxyphenol, 4-hydroxyphenol, 2-aminophenol, 3-aminophenol, 4-aminophenol, 4-hydroxyindole, 5-hydroxyindole, 6-hydroxyindole, 7-hydroxyindole. These group are oxidizable and can subsequently interact (*e.g.* react) with tissue. In a preferred embodiment, the hydroxyl-substituted aromatic group is one or more selected from the group of aromatic moieties having one of the following structures:



Of this group of aromatic moieties, the catechol group is particularly preferred for its presence in several naturally occurring compounds such as DL-DOPA, dopamine, L-DOPA, D-DOPA, and/or noradrenaline. The catechol-group is therefore generally biologically compatible and preferred for medical applications such as tissue-adhesive substances and devices. The hydroxyl-substituted aromatic group present in tyramine and/or serotonin can also favorably be used. Accordingly, is a further preferred embodiment, the moiety "NH–spacer–ArOH" (*vide infra*) is based on dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin.

In the aforementioned preferred embodiment wherein the moiety "NH–spacer–ArOH" is based on dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin, the spacer may thus be based on C_2-alkylene, optionally substituted with a hydroxyl or carboxylate. Variations of this linker may however also be well possible. Esters of the carboxylate can also be applied. In

general however, it is preferred to maintain a short linker, without superfluous substituents. As such, the linker is preferably a linear or branched C_1-C_6 alkylene, preferably C_2-C_4 alkylene, more preferably C_2 alkylene, most preferably a linear C_2 alkylene. The linker may optionally be substituted with a hydroxyl and/or a carboxylic acid group.

The moiety "NH–spacer–ArOH" is however not necessarily directly based on dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin. It was surprisingly found by the present invention that certain compounds comprising two or more caprolactam blocked groups and the ArOH group, can bifunctionally be used – *i.e.* for functionalization of the polymer at one or more termini and at the backbone of the polymer. This is explained in more detail herein-below.

The polymeric chain of the polymer may be based on a variety of polymers or polymeric groups and combinations thereof. Good interaction with the tissue (*i.e.* good adhesion) can in particular be obtained if the tissue-adhesive polymer is based on a hydrophilic polymer. Examples of suitable hydrophilic polymers include hydrophilic polyether, polyester, poly(ester ethers), polycarbonates, polyurethanes, polyetherurethanes, polyurethane urea, poly(vinylpyrrolidone), poly(saccharide), poly(vinyl alcohol), polyoxazoline, or combinations thereof. The polymer preferably comprises polyether, polyester, poly(ester ether), polyamide, polycarbonate, polyurethane or a combination thereof. The presence of a hydrophobic polymeric part is not necessarily excluded, as long as this is not detrimental to the adhesive properties of the tissue-adhesive polymer. For instance, the hydrophobic part can be overruled by a hydrophilic part of the tissue-adhesive polymer such that overall the polymer remains adhesive to tissue.

Particularly preferred polymeric chains include polyether, polyester, polycarbonate such as poly(alkylene glycol) or a poly(lactic acid), poly(caprolactone), polydioxanone, poly(glycolide) or a poly(trimethylene carbonate). Although polyesters such as poly(lactic acid) and poly(caprolactone) show favorable hydrophilic properties, the presence of the ester bonds in the polymers, in particular when combined with ethers, results in a shorter adhesion than the polyether and polycarbonate, probably due to hydrolysis of the ester bonds. Accordingly, even more preferably, the polymer or polymeric group

comprises poly(ethylene glycol) (PEG), polycaprolactone (PCL), poly(lactic acid) (PLA), for instance poly(L-lactic acid) (PLLA), a co-polymer of PCL and PLA or a poly(trimethylene carbonate) (PTMC), most preferably PEG.

In the embodiments wherein the backbone of the polymer is functionalized with the ArOH group, the polymeric chain of said polymer preferably comprises a polyurethane (based on –NHC(O)O–), polythiourethane (based on –NHC(O)S–) and polyurea (based on –NHC(O)NH–), synthesized by reacting a chain extender such as respectively a polyol, polythiol or polyamine with a monomeric unit functionalized with the ArOH, in particular with a unit comprising two or more caprolactam blocked groups and the ArOH group. As such, the polymeric chain may have a structure according to formula III

$$-(E-Z-(R^{Ar})_q-/-(R^{Reg})_p-Z)_s-$$

III

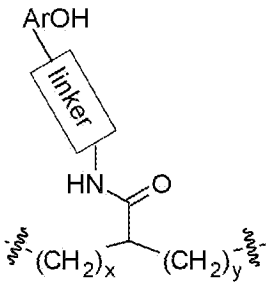
wherein E is based on a chain extender such as a polyol, polythiol or polyamine. In formula III, R^{Ar} represents a functionalized monomeric unit functionalized with the ArOH group while R^{Reg} represent a regular monomeric unit not functionalized with the ArOH group and q plus p equal 1 while q is not 0 (as such, R^{Ar} is always present in formula II). The polymeric chain may thus be based on only monomeric units R functionalized by the ArOH group ($p = 0$, $q = 1$), or on a combination or R^{Ar} and R^{Reg} ($p > 0$ and $0 < q < 1$). The ratio of R^{Ar} over R^{Reg} is preferably more than 2, more preferably more than 4 and most preferred R^{Reg} is not present. In other words, q/p is preferably at least 0.5, more preferably at least 0.75, most preferably 1.

In formula III, Z represents a urethane, thiourethane and/or urea bond while s represents the number of polymer units of the polymeric chain and proportional to its molecular weight. The s is preferably an integer from 5-500.

The chain extender on which E is based can comprise one or more of the group consisting of C_2-C_{10} alkylene, optionally substituted with C_1-C_{10} alkyl or C_1-C_{10} alkyl groups substituted with halides or protected S, N, P or O moieties and/or comprising S, N, P or O in the alkylene chain, aliphatic polyesters, polyether esters, polyethers, poly(anhydrides), polycarbonates, polyethers or combinations

thereof, optionally at least one E comprises a hydrophilic segment. A specific example of a suitable chain extenders includes polyethylene glycol (PEG).

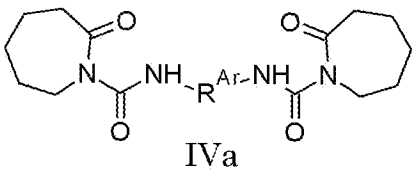
The R^{Ar} moiety in the polymeric chain typically comprises a C_2-C_{10} alkylene substituted with the ArOH group. The ArOH group and said alkylene can be linked via a spacer. For instance, the R^{Ar} group may have a structure according to formula VII



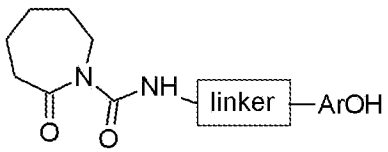
VII

wherein the spacer is as defined for formula Ia and provides a linking functionality, ArOH represents the hydroxyl-substituted aromatic group, x and y are integers each between 0 and 8 while x + y being between 3 and 9. In a particular embodiment of the present invention, wherein R^{Ar} is based on a modified lysine, x is 4 and y is 0.

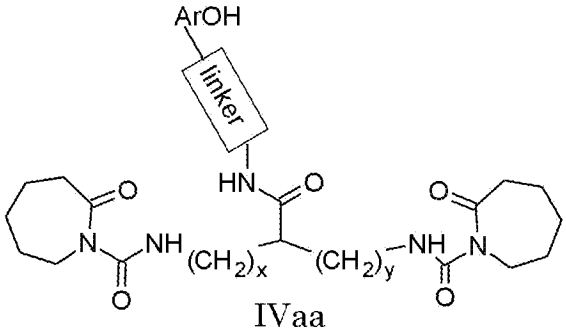
The present inventors found that the polymer can suitably be prepared with one or more caprolactam blocked hydroxyl-substituted aromatic compounds (abbreviated as CAB-ArOH or CAB_2-ArOH). Said caprolactam blocked compound can have a structure according to formula IVa (CAB_2-ArOH) or IVb (CAB-ArOH), preferably IVaa or IVba, more preferably IVab or IVba.



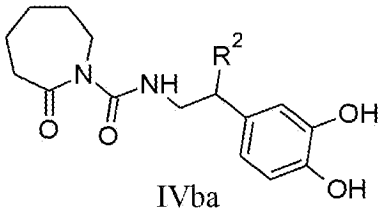
IVa



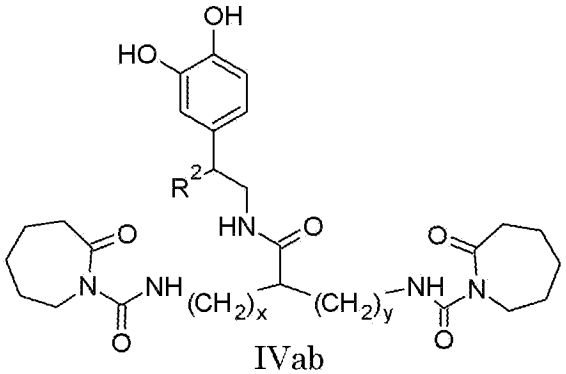
IVb



IVaa



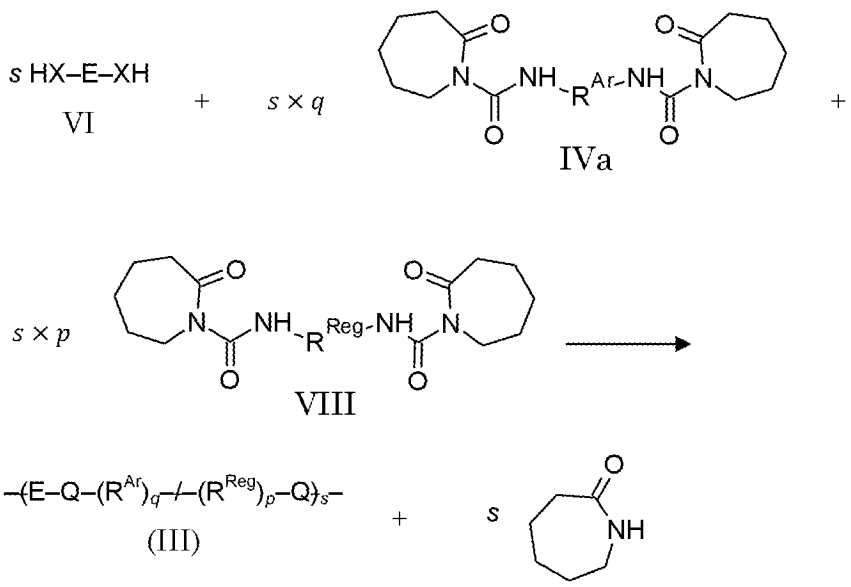
IVba



IVab

wherein R^{Ar} represents the functionalized monomeric unit functionalized with the ArOH group; the linker represents a linear or branched C_1-C_8 hydrocarbon, optionally substituted with one or more OH, SH, hale, amide and/or carboxylate; R^2 is H, OH or CO_2H or esters thereof such as CO_2Me or CO_2Et and x and y are integers each between 0 and 8 while x + y being between 3 and 9, preferably x is 4 and y is 0.

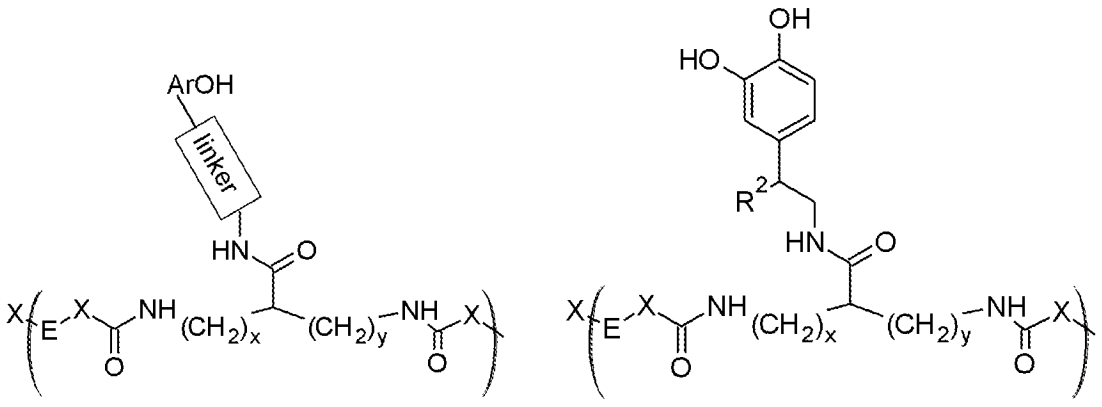
The bis-reactive CAB_2-ArOH compounds according to structures IVa, IVaa and IVab can be used for the backbone functionalization of the polymer in a polymerization reaction as illustrated in Scheme 1 (shown only for IVa).



Scheme 1

In Scheme 1, HX–E–XH represent a chain extender wherein XH is a hydroxyl, sulfhydryl and/or amine group and E is as defined for compound III.

In preferred embodiments, the CAB_2-ArOH compounds according to structures IVaa and IVab can be used, leading to a polymer comprising at least partially a backbone according formula IIIa and IIIb respectively, wherein E, X, R_2, x, y and the linker are as defined for compounds II, IVaa and IVab.

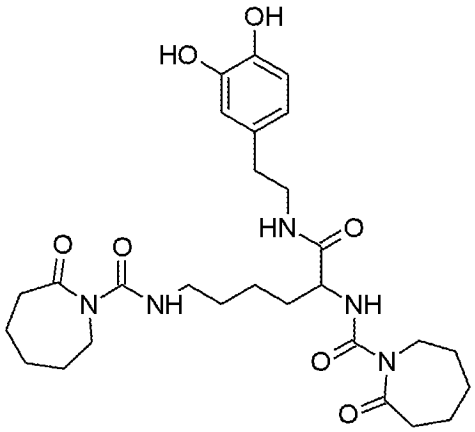


IIIa IIIb

The conditions for a polymerization reaction according to Scheme 1 typically include elevated temperatures (*e.g.* higher than 100 °C, such as about 145 °C) and reduced pressure in order to allow caprolactam to evaporate from the

reaction mixture. Suitable solvents are organic solvents such as dimethylformamide (DMF).

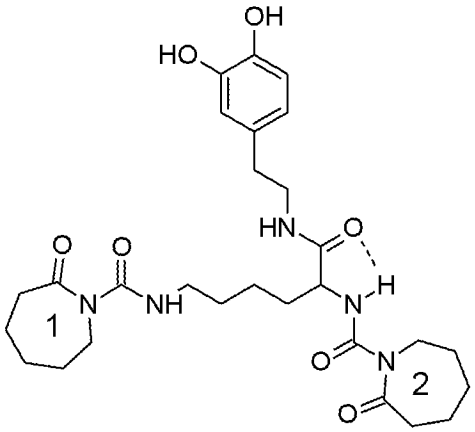
A specific example of a compound according to formula IVab is the lysine-derived caprolactam blocked lysine diisocyanate functionalized with dopamine (CBDLI-DA), which can be obtained from caprolactam blocked lysine diisocyanate methylester (CBLDI-OMe, see also Yin J. PhD thesis, *Lysine based amorphous polyurethanes decorated with pendant bio-active groups. 2012* University library of Groningen, ISBN 9789036756730).



CBLDI-DA

The inventors surprisingly found that CAB_2-ArOH compounds such as CBDLI-DA can not only suitably be used to obtain backbone ArOH-functionalized polymers through polymerization reaction, but that such compound can also suitable be used to functionalize one or more termini of the polymer. Namely, it was found that CBDLI-DA for example, reacts relatively slowly in a polymerization reaction.

Without wishing to be bound by theory, the present inventors believe that the reaction with the caprolactam blocked isocyanate number 2 (as indicated in the structure directly below) is slightly hampered (*i.e.* it reacts slower than caprolactam blocked isocyanate number 1), possibly due to intra-molecular hydrogen bonding, rendering the carbonyl to be reacted less electrophilic toward the chain extender VI (*i.e.* HX-E-XH).



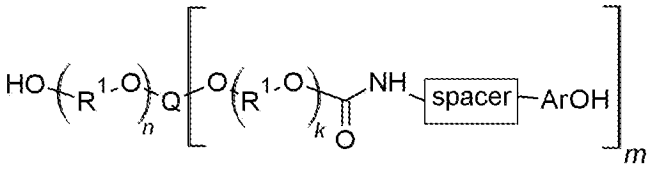
CBLDI-DA

Accordingly, CAB_2-ArOH compounds can be used to obtain backbone ArOH functionalized polymers, terminally ArOH functionalized polymers or combinations thereof. By keeping a relatively short reaction time, PEG may for instance be terminally functionalized, while avoiding dimerization or even further polymerization. On the other hand, by using a long reaction time, polymerization can be obtained.

For sake of clarity, it is noted that when CAB_2-ArOH compounds are used to obtain terminally ArOH functionalized polymers, the spacer as used in formulae II and IIa is thus typically not equal to the linker as used in compound IVaa. In such case, the moiety "NH–spacer–ArOH" is not based on dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin, even if the moiety "NH–linker–ArOH" is based on these compounds. On the other hand, when a CAB-ArOH compound such as IVb is used to obtain terminally ArOH functionalized polymers according to the invention, the moiety "NH–spacer–ArOH" is equal to the moiety "NH–linker-ArOH" and both may be based on dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and/or serotonin.

The inventors found that both CAB-ArOH and CAB_2-ArOH compounds can be used to terminally functionalized polymers with the ArOH group. More in particular, the inventors found that these groups can very suitably be used to provide a tissue-adhesive multi-arm polymer according to formula Ia, wherein m is 3 or more.

In a particular embodiment, the tissue-adhesive multi-arm polymer has a structure according to formula IIa.

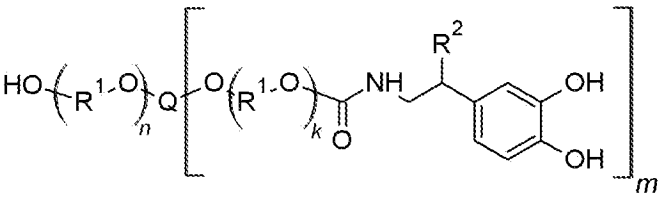


IIa

Formula IIa is a specification of formula II and the spacer, Q, *m* and *n* are accordingly the same for both formulae. In formula Ib, R^1 represents linear or branched C_1-C_4 alkylene and/or –C(O)–C_1-C_5 alkylene; and *k* represents the number of polymer units of each arm and is proportional to the molecular weight of each arm. The number of polymer units of each arm *k* is typically in the range of 5 to 1000. For example, if the polymer is based on a multi-arm PEG weighing 40 kDa, *k* is about 114. Preferably *k* is in the range of 10 to about 250, more preferably 50 to 150 such as about 114 units.

The R^1 part originates from the monomeric units on which the polymer arm is based. For instance, in embodiments wherein the arm is based on poly(ethylene glycol) or poly(propylene glycol), R^1 represents ethylene or propylene while in embodiments wherein the arm is based on a polyester, R^1 represents –C(O)–alkylene, wherein –C(O)– is the carbonyl group in the polyester. In a further preferred embodiment, R^1 represents –C(O)–$CH(CH_3)$– (*i.e.* a branched –C(O)–C_2 alkylene originating from *e.g.* lactide) -or –C(O)–$(CH_2)_5$ (*i.e.* a linear –C(O)–C_5 alkylene originating from *e.g.* ε-caprolactone).

An yet another particularly preferred embodiment, the tissue-adhesive multi-arm polymer has a structure according to formula IIb.



IIb

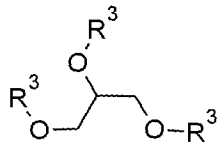
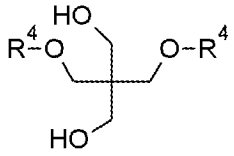
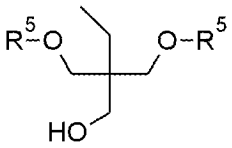
Formula IIb is a specification of formulae II and IIa and Q, *m* and *n* are accordingly the same for all formulae. The R^1 and *k* for formulae Ib and Ic are also the same. In formula IIb, R^2 represents H, OH, CH_3 or CO_2H or ester thereof, preferably H, and typically originates from the amine on which the hydroxyl-substituted aromatic group and spacer is based. Suitable esters of the CO_2H group include C_1-C_6 alkyl and alkyl esters such as CO_2Me and CO_2Et.

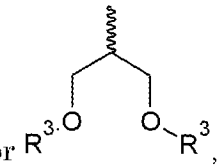
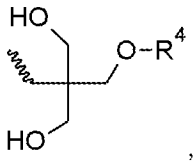
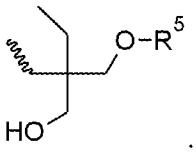
The length of the arms can be expressed with their molecular weight. Accordingly, on average, the number-average molecular weight (M_n) of each arm is preferably in the range of 500 Da to 50 kDa, more preferably 1-25 kDa, most preferably 2 to 10 kDa. In addition, number-average molecular total weight of the multi-arm tissue-adhesive polymer is preferably in the range of 5 to 100 kDa, more preferably in the range of 10-80 kDa, most preferably in the range of 20-60 kDa. For instance, very good results were obtained with an 8-armed (PEG) having a number-average molecular weight of 40.000 g/mol (*i.e.* 40 kDa), of which each arm is thus about 5 kDa.

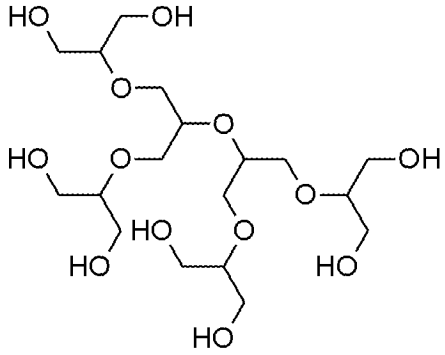
The number-average molecular weight can be determined by known analytical techniques such as size exclusion chromatography (SEC) and/or matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS).

The multi-arm nature of the polymer can be attributed to a core. The number of functionalized arms in the polymer *m* depends on the selected core and the substitution degree of the arms (*i.e.* the ratio of functionalized arms to non-functionalized arms). In general, the core may be based on any poly-functional compound to which the polymeric chains (*i.e.* the arms) can suitably be connected. In a preferred embodiment, the core is based on a poly-functional compound which is an initiator in polymerization reactions. This enables that the polymeric chain may be grafted from the core. Therefore, the core is preferably based on a polyol that is suitable for initiation polymerization to form polyethers or polyesters. Examples of such polyols include ethylene glycol, glycerol (GL), pentraerythritol (P), hexaglycerol (HG), tripentaerytritol (TP), trimethylolpropane (TMP), dipentaerythritol (DP) and combinations thereof.

As such, in a preferred embodiment, Q of formula Ia, Ib and Ic is based on a polyol comprising $m+n$ hydroxyl groups, preferably Q is based on a polyol of any of structures depicted below,

wherein each R^3 is individually H or  , each R^4 is individually H or  , each R^5 is H or  . The R^3, R^4 and R^5 can be selected based on the amount of desired hydroxyl groups. For instance, hexaglycerol (HG) can be represented as follows.



In a particular embodiment, the core is the initiator for the preparation of a multi-arm PEG, which is then used as a subsequent initiator in a polymerization reaction with lactide, trimethylenecarbonate, glycolide of caprolacton to form a multiarm block copolymeer comprising a multi-arm PEG segment extended with one or more of these monomers.

In a further preferred embodiment, the number of functionalized arms m is 4 to 10, preferably 6 to 8. It was surprisingly found that gelation time decreases with an increasing m. Thus, the gelation time for the polymer wherein m is 6 is less than when m is 4, while for the polymer wherein m is 8, the gelation time is even less than when m is 6. In addition, a higher number of functionalized arms were surprisingly found to result in better adhesive properties as well as in

better mechanical properties. As such, pentraerythritol (P), hexaglycerol (HG), tripentaerytritol (TP), trimethylolpropane (TMP) and dipentaerythritol (DP), in particular hexaglycerol (HG), dipentaerythritol (DP), tripentaerytritol (TP) are preferred core structures.

Generally, it is preferred that all arms are functionalized, but it may be that not all arms are substituted with the hydroxyl-substituted aromatic group, *e.g.* due to limitation in the method for preparation (*vide infra*) or by design. The substitution degree of the arms as defined by m divided by $(m+n)$ is preferably more than 60%, more preferably more than 80%, even more preferably more than 90%, most preferably about 100%. In general, a higher substitution degree leads to better adhesion and better mechanical properties.

The substitution degree can be determined by ^{1}H-NMR in combination with the following mathematical formula

$$substitution\ degree(\%) = \frac{A}{Q \times R} \times \frac{Z \times M_n}{B \times M_w} \times 100\%$$

wherein:

A is the area of the peak or peaks corresponding to all the protons of the moiety "NH–spacer–ArOH";

Q is the number of protons in the moiety "NH–spacer–ArOH";

R is the total number of arms of the polymer;

B is the area of the peak or peaks corresponding to all the protons of polymer arms;

Z is the number of protons in the monomer on which the polymeric chain is based;

M_w is the molecular weight of monomer on which the polymeric chain is based;

M_n is the number-average molecular weight of the polymer without the moiety "NH–spacer–ArOH".

A further aspect of the present invention is a kit of parts comprising a first container comprising the tissue-adhesive polymer and a second container comprising an oxidizing agent. As described herein-above, the tissue-adhesive properties are obtained by oxidizing the hydroxyl-substituted aromatic group. Suitable oxidizing agents are generally known agents capable of oxidizing such

groups and include periodates, peroxides, permanganates and the like, such as sodium periodate, potassium permanganate.

By mixing the contents of both containers, a hydrogel is formed that can be injected. Such a hydrogel is another aspect of the present invention.

Advantageously, the hydrogel according to the present invention demonstrates improved adhesion and/or mechanical properties vis-à-vis conventional hydrogels. The hydrogel of the present invention may have a lap shear adhesion strength of more than 0.50 N, preferably more than 0.7, more preferably more than 1, even more preferably more than 1.5, most preferably more than 2 N as determined by ASTM F2255-05.38. The burst pressure may be more than 15 mbar, preferably more than 20 mbar, more preferably more than 25 mbar as determined by ASTM F2392-04.

The tissue-adhesive polymer and the injectable hydrogel of the present invention can be used in a medical treatment of a human or animal, in particular for sealing or closing of tissue, preferably of tissue that is otherwise difficult to treat using conventional methods such applying suture, patches or staples. Due to their typical injectable nature, the polymer and hydrogel of the invention can very suitably be used in laparoscopic treatments. The polymer and hydrogel are thus well suitable for sealing the ventral cavity (including the abdominal, thoracic and pelvic cavities) like liver, lung, pancreas, spleen, bladder, kidney and/or intestine tissues.

Oxidation of the polymer may occur on-site (*i.e.* after appliance), before appliance, or a combination thereof (*e.g.* using a syringe comprising two containers corrected to a mixing section wherein the oxidation can at least partially occur).

A particular application for tissue-adhesive polymer and hydrogel is the sealing of dura mater or spinal tissue. Dura mater is the outermost membrane layer that surrounds the brain and spinal cord of the central nervous system. After *e.g.* trauma or cranial surgery, opened dura mater needs to be sealed to prevent leakage of cerebrospinal fluid. Even when in an operation dura mater is closed by suture, staples and such, cerebrospinal fluid may still leak, in particular through remaining small openings. It is therefore typically required that the dura mater is sealed by a surgical sealant, which preferably, is based on a tissue-adhesive material such that no glue or other type of adhesive is required to apply the sealant

and seal the dura mater. *Inter alia* for these reasons, tissue-adhesive polymer and hydrogel in accordance with the invention is very suitable for use in methods of surgery.

Yet a further aspect of the invention is directed to the preparation of the tissue-adhesive polymer. The present inventors found that the polymer can efficiently be prepared by reacting the non-functionalized polymer, for example a non-functionalized multi-arm polymer with with one or more of the caprolactam blocked hydroxyl-substituted aromatic compounds CAB-ArOH and CAB_2-ArOH.

Backbone functionalization can be carried out with CAB_2-ArOH as described for and illustrated by Scheme 1.

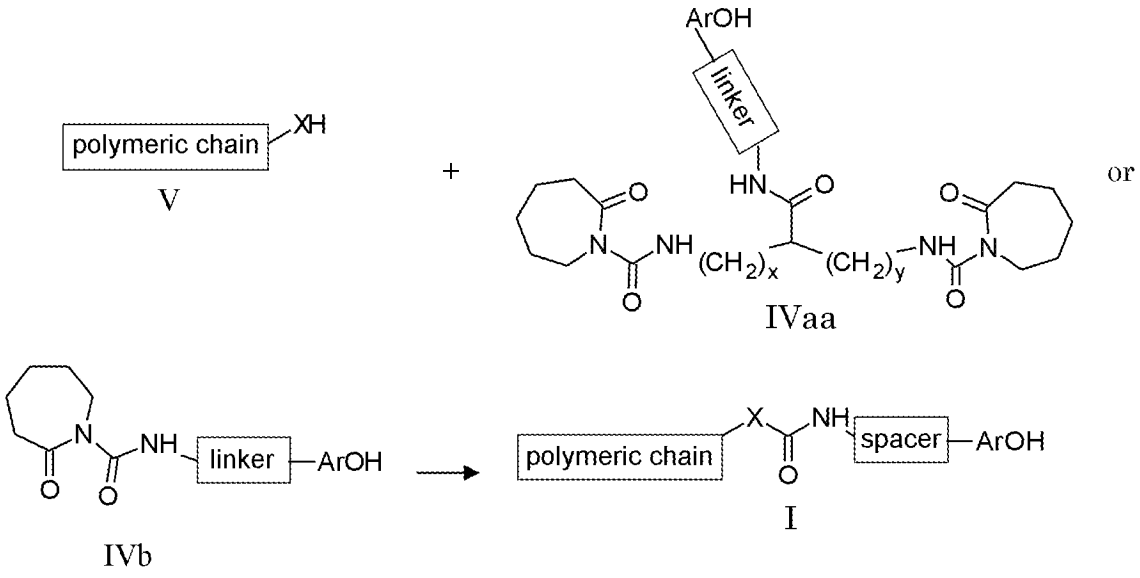
Functionalization of one or more termini of the non-functionalized polymer can be carried out with CAB-ArOH and CAB_2-ArOH for reasons described herein above. It may be appreciated that a combination of backbone and terminal functionalization is also according to the present invention and that this for instance can be obtained by first carrying out a polymerization as illustrated in Scheme 1, followed by a functionalization at one or more termini. Non-functionalized polymer thus refers to the polymer as existing before (further) functionalization and may already be functionalized with the ArOH in a preceding process.

Advantageously, in contrast to Liu *et al.*, ACS Applied Materials and Interfaces 6 (2014) 16982−16992, the present tissue-adhesive polymer can be prepared without the requirement of dialysis or other elaborate and/or cumbersome purification method.

The present inventors found that CAB-ArOH and CAB_2-ArOH can be a suitable substitute for isocyanates and offers several advantages. Isocyanates are generally toxic and there is a possibility for incomplete reactions. Furthermore, undesired crosslinking may take place in the presence of moisture and/or elevated temperatures. In contrast, the CAB-ArOH and CAB_2-ArOH is non-toxic and stable up to a temperature of 100°C or more.

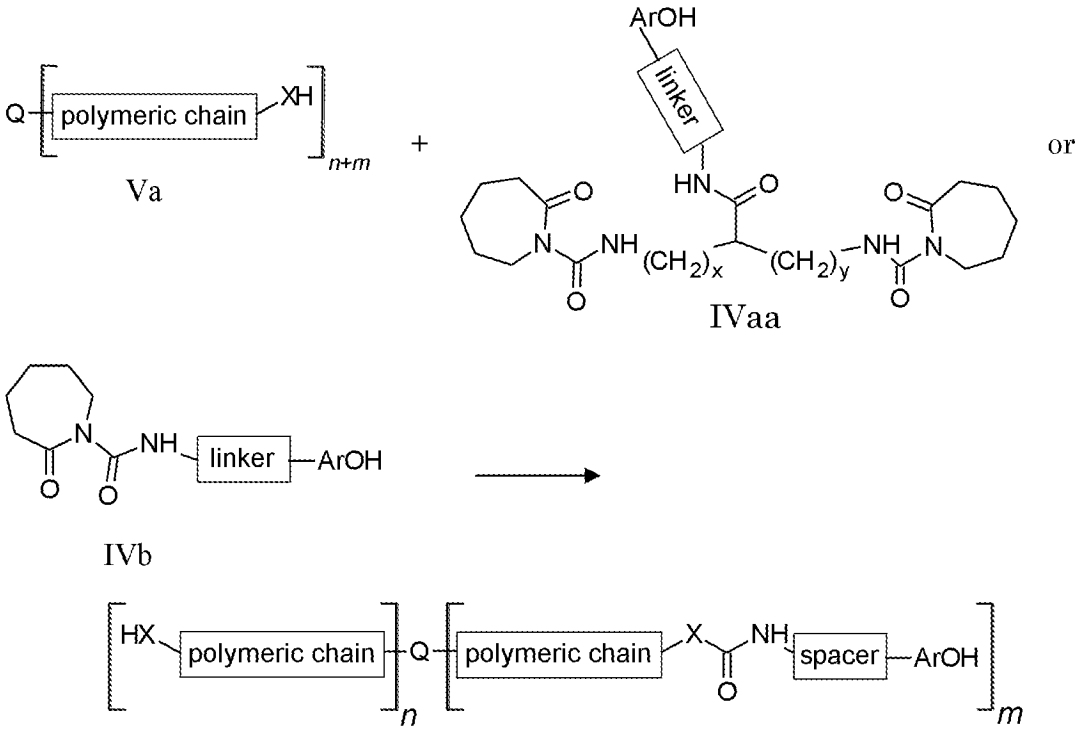
Accordingly, a particular aspect of the invention is the preparation of the tissue-adhesive multi-arm polymer of formula I, said method comprising reacting the polymer having a structure according to formula V with the

caprolactam blocked hydroxyl-substituted aromatic compounds IVaa or IVb, as illustrated in Scheme 2, wherein XH is a hydroxyl, sulfhydryl and/or amine group.



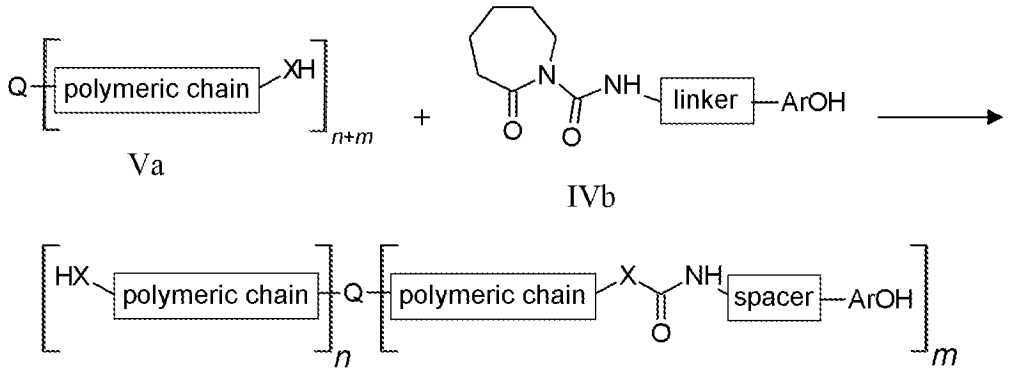
Scheme 2

Naturally, in the particular embodiment wherein the polymer is a multi-arm polymer according to formula II, the process can be carried out similarly, as illustrated in Scheme 3, wherein XH is a hydroxyl, sulfhydryl and/or amine group.



Scheme 3

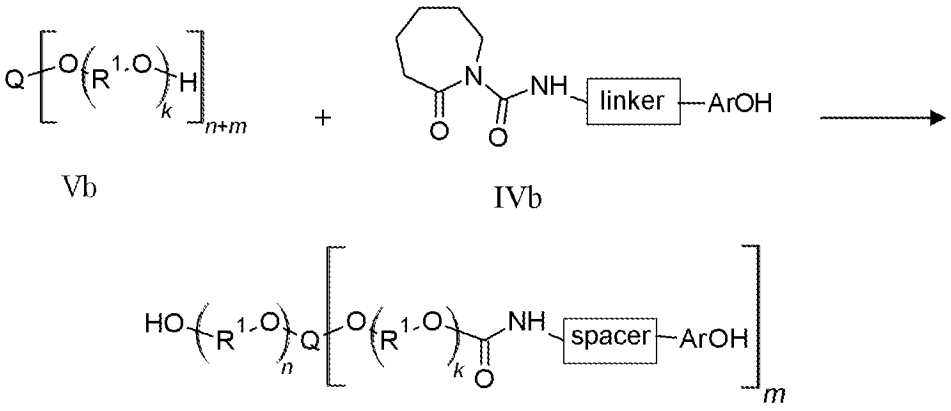
Preferably, for terminal functionalization of the polymer, the CAB-ArOH according to any of formulae IVb and IVba is used, as is illustrated in Scheme 4 for IVb. In this embodiment, the linker is equal to the spacer.



Scheme 4

For all of the reactions illustrated in Schemes 2-4, the reaction of compound V or Va with a compound IV (*i.e.* one of IVa, IVb, IVaa, IVab and IVba) can be carried out at relatively high temperature because to the good stability of compounds IV. In general, the reaction temperature may be about 100 to 200 °C, preferably between 130 to 180° such as about 145 °C. Full conversion of compound V and Va is then typically obtained in 8 to 24 hours, for instance in about 16 hours. Advantageously, the ArOH moiety remains intact and does not degrade nor interfere with the reaction.

In a preferred embodiment of the method according to the present invention, a compound IV is reacted with the preferred embodiment of the multi-arm polymer according to formula Vb. This results in the formation of the tissue-adhesive multi-arm polymer of formula IIa, as illustrated in Scheme 5 for IVb.



Vb IVb

IIa

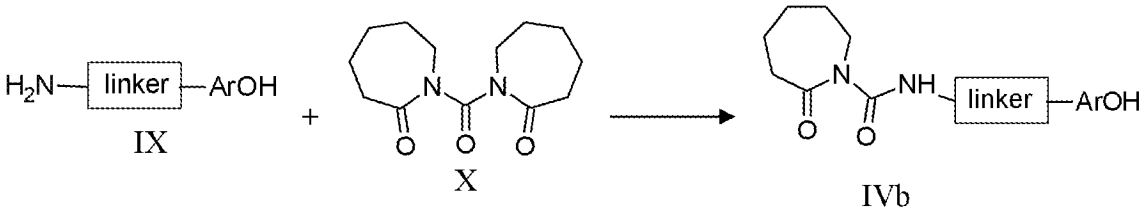
Scheme 5

Caprolactam blocked isocyanates are known in the art and are typically used in an isocyanate-free route to urethane compounds (see *e.g.* Maier *et al.* Macromolecules (2003) 4727-4734).

In a preferred embodiment of the preparation of the tissue-adhesive polymer, the method further comprises preparing the CAB-ArOH and/or CAB_2-ArOH in methods as described herein-below.

The CAB-ArOH compound IVb can be readily prepared by a reaction of the compound NH_2–spacer–ArOH (compound IX) with carbonylbiscaprolactam

(CBC, or compound X), as illustrated in Scheme 6. This reaction is preferably carried out in the presence of a base such as an organic amine (*e.g.* triethyl amine).

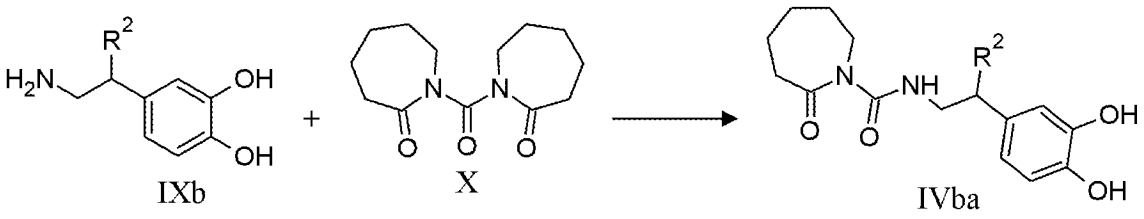


Scheme 6

Surprisingly, conditions for the preparation of caprolactam blocked isocyanates of primary amines reported in Maier *et al.* Macromolecules (2003) 4727-4734 ($CHCl_3$, 75°C for 4 hour) did not result in a good yield of the product. It was found that preferably, compound IX is used in excess vis-à-vis CBC (*e.g.* more than 1.3 molar equivalents such as 1.5 molar equivalents or more). The base is preferably present in an amount of more than 2 molar equivalents with respect to CBC, more preferably 3 molar equivalents or more, especially when compound IX is introduced in the reaction as a salt (*e.g.* a HCl salt). In addition, it is preferred that compound IX and CBC are reacted at a temperature of more than 80 °C, more preferably 90 °C or more. Typical reaction times range from 8 hours to 14 days, for instance 1 to 12 days and is generally about 7 days.

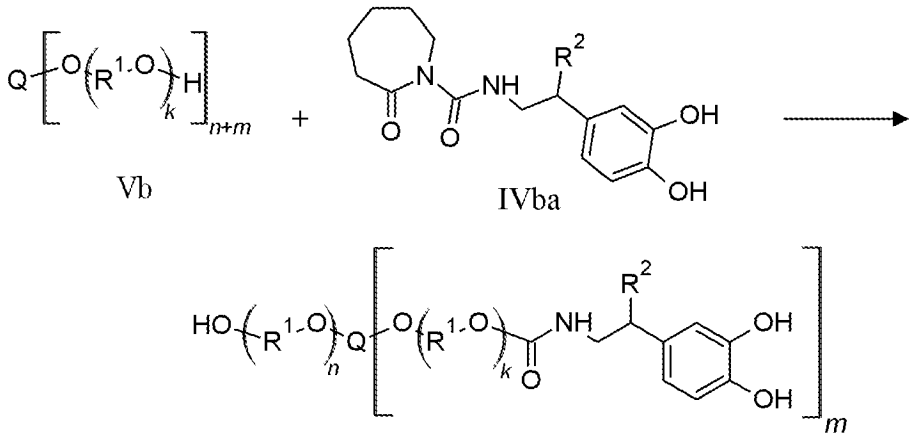
Advantageously, the hydroxyl substituent at the ArOH moiety does not require a protective group as a chemoselective reaction between the amine and CBC can be utilized.

Specific examples of NH_2–spacer–ArOH are dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline and serotonin. In preferred embodiments of the present invention as described, CBC is reacted with dopamine, DL-DOPA, D-DOPA, L-DOPA or noradrenaline (compound IXb, wherein R^2 is respectively H, CO_2H or OH), as illustrated in Scheme 7.



Scheme 7

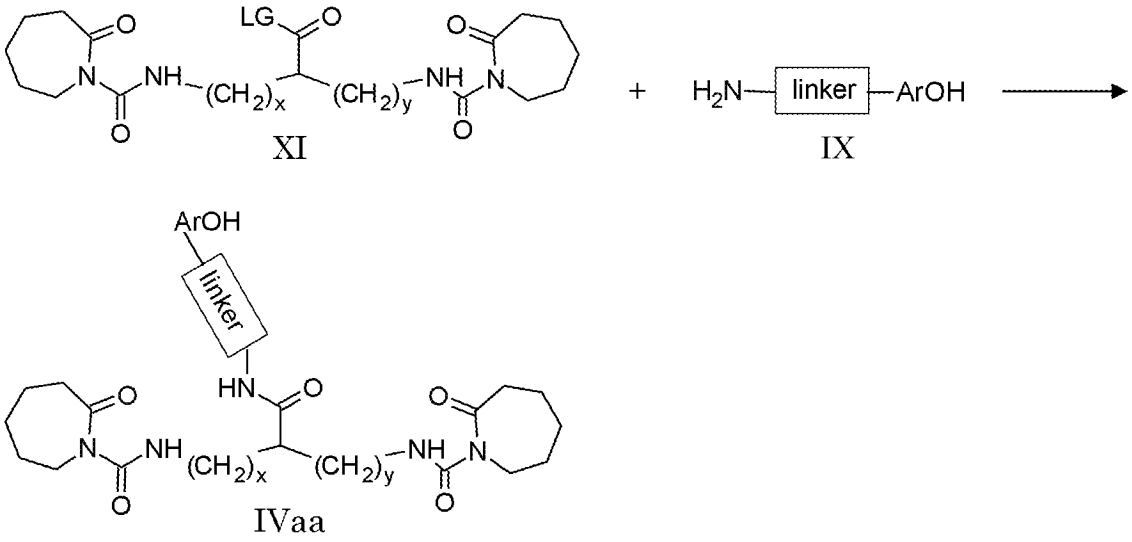
It may be appreciated that in a preferred embodiment, compound IVba is reacted with the multi-arm polymer of formula Vb, to provide the tissue-adhesive multi-arm polymer of formula IIb, as illustrated in Scheme 8.



Scheme 8

The compound CAB_2-ArOH according formula IVaa to can be prepared by reacting an actives ester according to formula XI as illustrated in Scheme 9, wherein x and y are as defined for formula IVaa and LG is a leaving group such as an alcohol radical comprising an electron withdrawing group or an halide such as a iodide bromide or chloride. Suitable examples of alcohol radicals comprising an electron-withdrawing group as the leaving group include alcohol radicals wherein the alcohol is selected from the group consisting of perfluoroalkyl alcohol, *p*-nitrophenol, 3,4,5-trichlorophenol, pentafluorophenol, 1-benzotriazolyl alcohol, 1-hydroxy-7-azabenzotriazole, 1-hydroxybenzotriazole, and *N*-hydroxysuccinimide alcohol and derivatives thereof such as *N*-hydroxymaleimide, *N*-hydroxyphthalimide, endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide and a *N*-

hydroxysulfosuccinimide salt, more preferably wherein the alcohol is a *N*-hydroxysuccinimide alcohol.



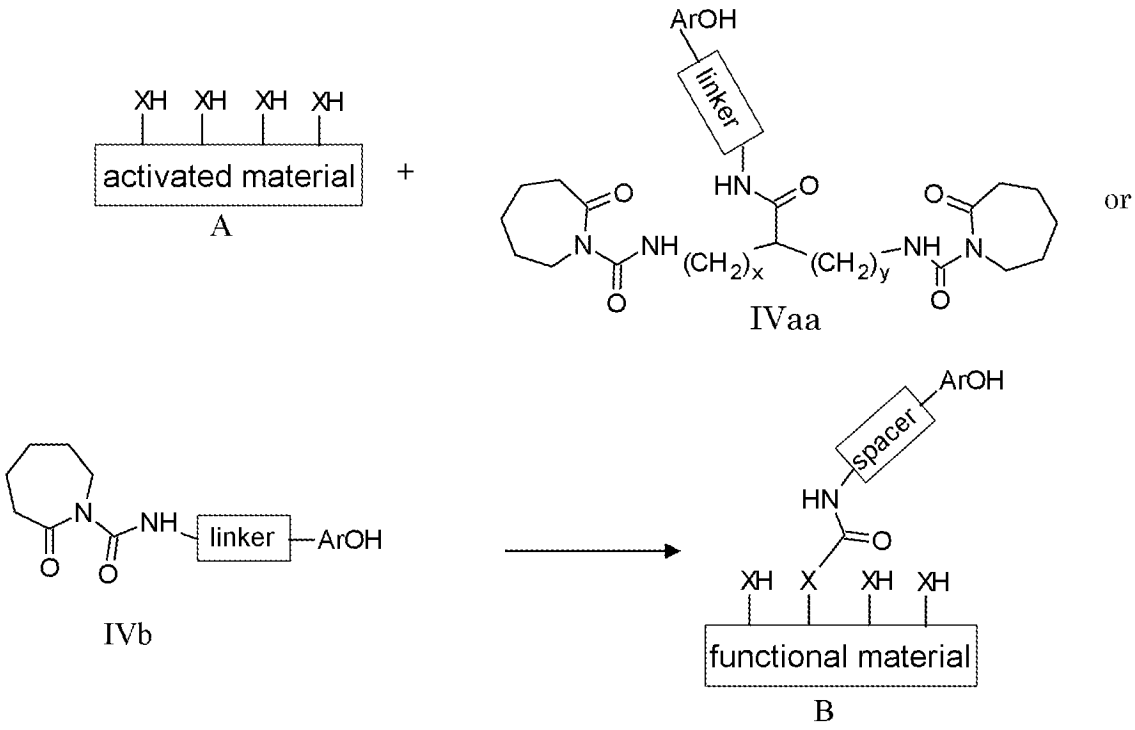
Scheme 9

The compound according to XI can be prepared according to a procedure as described in Yin J. PhD thesis, *Lysine based amorphous polyurethanes decorated with pendant bio-active groups. 2012* University library of Groningen, ISBN 9789036756730.

The CAB-ArOH and CAB_2-ArOH may further be used to functionalize materials, which is yet another aspect of the present invention. Typically, such materials require reactive groups to be able to react with the CAB-ArOH. Examples of reactive groups are hydroxyl, sulfhydryl and amine reactive groups. Certain materials (*e.g.* cellulose) may intrinsically comprise one of more reactive groups, while other typically of materials (*e.g.* polyesters, polyamide, polyethers, polyurethanes, polyolefins and the like) may require activation (*e.g.* hydrolysis, aminolysis or electron-beam treatment).

Accordingly, the invention is further directed to a method of functionalizing an activated material A, which surface comprises at least one hydroxyl, sulfhydryl and/or amine reactive group, with a catechol derivative, said method comprising contacting said activated material A with the caprolactam

blocked hydroxyl-substituted aromatic compound to provide functional material B, as illustrated in Scheme 10 wherein XH is the hydroxyl, sulfhydryl and/or amine reactive group.



Scheme 10

Examples of materials that can be functionalized in accordance with this method are the polyurethane foams, sheets and materials as for instance described in WO 99/64491 (Biomedical PUs), WO 2004/062704 (Nasal Sponge) WO 2017/171549 (Duraseal) and PCT/NL2018/050649 (Liver sealant), which are all incorporated herein in their entirety.

Accordingly, in a preferred embodiment, the functional material of the present invention comprises a foam structure, a sheet structure, a gel-like structure or combinations thereof.

As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. The term "and/or" includes any and all combinations of one or more of the associated listed items. It will be understood that the terms "comprises" and/or "comprising"

specify the presence of stated features but do not preclude the presence or addition of one or more other features.

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having

EXAMPLES

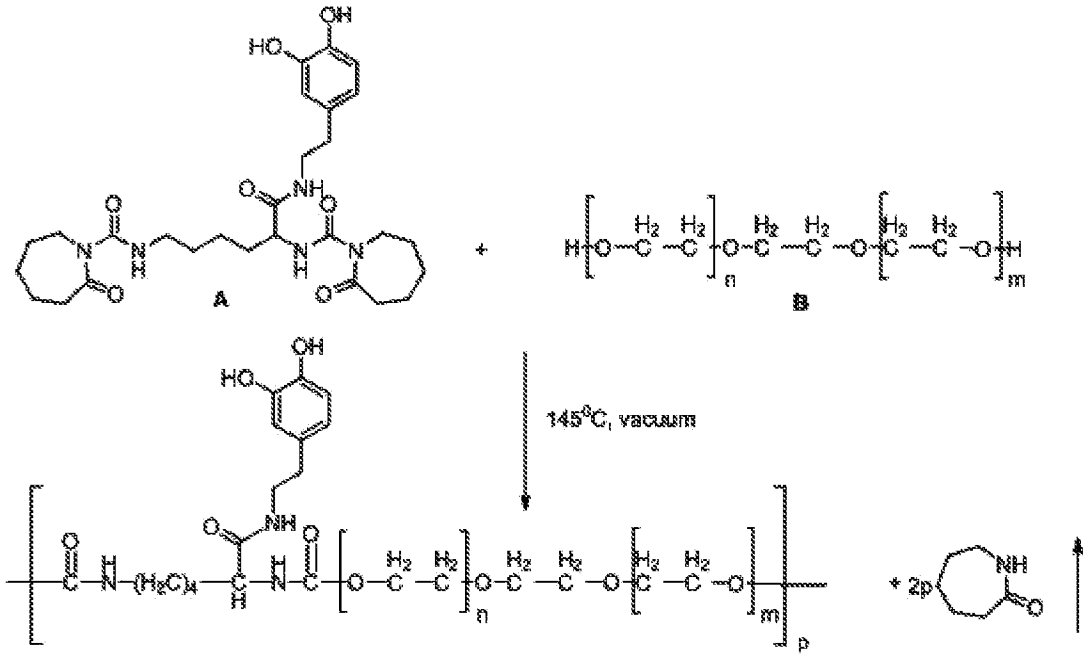
The invention can be further illustrated by the following non-limiting examples.

Example 1 – synthesis of CBDLI-DA

CBLDI-OMe prepared and hydrolyses according to Yin J. (PhD thesis, *Lysine based amorphous polyurethanes decorated with pendant bio-active groups. 2012* University library of Groningen, ISBN 9789036756730) was hydrolysed to the acid (CBDLI-COOH) in 72% yield using KOH (pH 13) in a 3:1 mixture of THF and water at room temperature using the procedure described.

CBLDI-COOH was activated with dicyclocarbodiimide (DCC) and converted to an *N*-hydroxysuccinimide (NHS) ester, after which acetonide protected dopamine (prepared according to Liu, Z., Hu, B. H., & Messersmith, P. B. (2010). Acetonide protection of dopamine for the synthesis of highly pure N-docosahexaenoyldopamine. *Tetrahedron letters*, *51*(18), 2403-2405) was added which gave acetonide protected caprolactam blocked lysine diisocyanate. Hydrolysis in trifluoroacetic acid (TFA) in a mixture of chloroform and water provided the desired monomer (CBDLI-DA).

Example 2 – preparation of backbone dopamine functionalized polymer

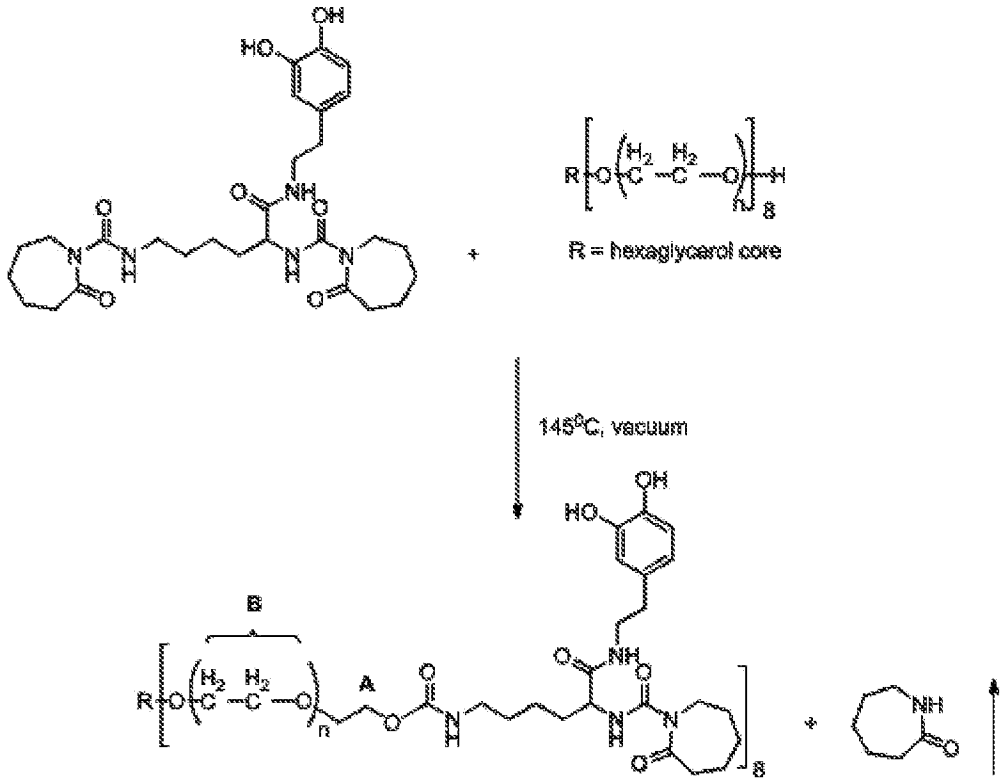


A mixture of about 1:1 CBDLI-DA (A) and PEG2000 having a M_n of 2000 (B) was heated at 145 °C for 72 h under vacuum. The results are shown in Table 1.

Table 1: Size exclusion chromatography (SEC) results after 72h polymerization at 145°C under vacuum.

Entry	A	B	Mol. eq. CBDLI-DA	Mol. eq. PEG2000	Mn (g/mol)	Mw (g/mol)	PDI	% Unreacted A
1	CBLDI-DA	PEG2000	1.025	1	3405	3511	1.03	5
2	CBLDI-DA	PEG2000	1.1	1	3411	3518	1.03	6

Example 3 – preparation of 8-armed lysine-dopamine functionalized PEG (8-ArmPEG40k-LD)



8-armed lysine-dopamine functionalized PEG (8-ArmPEG40k-LD) was synthesized by end-capping 8-arm PEG (Mn = 42320 g/mol, hexaglycerol core) with CBLDI-DA. The monomer CBLDI-DA was allowed to react with 8-armPEG-OH (Mn = 42320 g/mol, f=8) in a 1:1 molar ratio under vacuum at 145°C for a period of 16h). The reaction time was kept rather short to prevent dimerization:

An NMR analysis showed a coupling efficiency of 72% for the obtained hydrogel.

Example 4 – Gelation time (min) at various $NaIO_4$/Dopamine molar ratios

A fast curing time is an important characteristic for biomedical tissue adhesives. Therefore, 8-ArmPEG40k-LD was mixed with $NaIO_4$ in order to oxidize the catechol hydroxyl groups, as described in Liu, Z., Hu, B. H., & Messersmith, P. B. (2010). Acetonide protection of dopamine for the synthesis of highly pure N-docosahexaenoyldopamine. *Tetrahedron letters*, *51*(18), 2403-2405. Oxidation results in the formation of reactive quinone moieties which gives intermolecular crosslinking. The curing time depends on the $NaIO_4$/Dopamine molar ratio, as can

be derived from the results shown in Table 2. The fastest curing time was observed at a $NaIO_4$: dopamine ratio of 0.5:

Table 2 : Gelation time (min) at various $NaIO_4$/Dopamine molar ratios

$NaIO_4$: Dopamine molar ratio	**Gelation time (min)**
0.25	11
0.50	0.5
0.75	26
1.00	35

Example 5 – Determination of tissue adhesive properties of the 8-ArmPEG40k-LD

The tissue adhesive properties of the 8-ArmPEG40k-LD was determined on porcine dura mater. Lap shear adhesion (ASTM F2255-05.38) and burst pressure testing (ASTM F2392-04) were performed. The results (Table 3) were compared with commercially available DuraSeal™ (Medtronic Inc.). Results show that the lap shear strength of the 8-ArmPEG40k-LD is much higher (4 times) than with DuraSeal™ (Medtronic Inc.).

Table 3

Tissue adhesive material	**Lap shear strength (N) (n=5)**
DuraSeal™	0.15±0.06
8-ArmPEG40k-LD	0.66±0.25

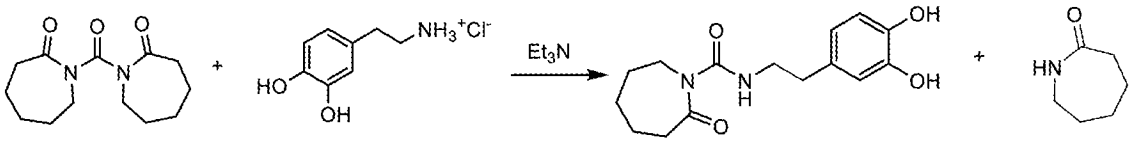
Example 6 – Determination of tissue adhesive properties of the 8-ArmPEG40k-LD

The in vitro burst pressure test was performed according to ASTM F2392-04 (Standard Test Method for Burst Strength of Surgical Sealants). The results (Table 4) were compared with commercially available DuraSeal™ (Medtronic Inc.). Results show that the burst pressure of the 8-ArmPEG40k-LD is higher than commercially available DuraSeal™ (Medtronic Inc.).

Table 4

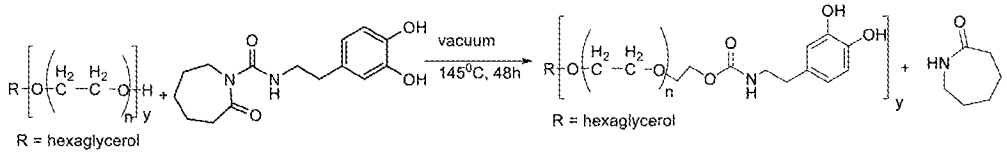
Tissue adhesive material	Burst pressure (mbar) (n=5)
DuraSeal™	10.4 ±2
8-ArmPEG40k-LD	23.8 ±3

Example 7 – preparation of a caprolactam blocked dopamine (CABDA)



At a round-bottom flask was added carbonyl bis caprolactam (1,0 eq; 19,82 mmol; 5,0 g) and dissolved in $CHCl_3$ (50 mL). The mixture was stirred at room temperature until dissolved, and of all times kept under inert conditions. Dopamine hydrochloride (1,5 eq; 29,73 mmol; 5,64 g) was added to the mixture and heated to 40°C and followed by the addition of triethylamine (3,0 eq; 59,46 mmol; 6,02 g; 8,29 mL). The reaction mixture was heated to 90°C and stirred for 48h in a closed system under nitrogen gas and reflux conditions. The mixture was slowly cooled to room temperature. The white precipitation in the mixture was removed by Büchner filtration. The solvent was removed *in vacuo*, and the residue was dissolved in ethyl acetate/hexane (2/1; 90 mL). The mixture was treated with a solution of 0,5M HCl/5% $CaCl_2$ and 5% NaCl (90mL), 5% $CaCl_2$ (90 mL), 1M Na_2CO_3 (90 mL) and brine (90 mL). The organic layer was dried with $MgSO_4$ and filtrated. The solvents were removed *in vacuo*. To give a yellow solid (yield 92%).

Example 8 – Functionalization of multi-arm PEG polymers



An 8-arm poly(ethylene glycol) polymer (PEG), 6-arm PEG and 4-arm PEG, having a molecular weight of 40 kDa, 30 kDa and 20 kDa respectively (8-arm-PEG40k, 6-arm-PEG30k and 4-arm-PEG20k respectively) were separately reacted with CABDA (1 equiv.) at 145 °C for 48h under vacuum. Tissue-adhesive multi-arm polymers based on multi-arm-PEG40k and dopamine 8-ArmPEG40k-DA, 6-armPEG30k-DA and 4-armPEG20k-DA were individually obtained.

Example 9 – Gelation time of 8-ArmPEG40k-DA, 6-armPEG30k-DA and 4-armPEG20k-DA

The polymers prepared according to Example 8 were mixed with $NaIO_4$ in order to oxidize the catechol hydroxyl groups. This resulted in the formation of reactive quinone moieties which gives intermolecular crosslinking or gelation. The results are depicted in Figure 1.

It was found that faster gelation occurs for 8-ArmPEG40k-DA > 6-armPEG30k-DA > 4-armPEG20k-DA. In addition, faster gelation occurs with increasing relative amounts of oxidation agent $NaIO_4$.

Example 10 – Lap shear adhesion of 8-ArmPEG40k-DA, 6-armPEG30k-DA and 4-armPEG20k-DA

The tissue adhesive properties of the multi-arm PEG-DA polymers prepared according to Example 8 were determined on porcine dura mater. Lap shear adhesion test were carried out according to ASTM F2255-05.38. The results are depicted in Table 5 and are compared with commercially available DuraSeal™. Results show that the lap shear strength of the 8-ArmPEG40k-DA is much higher than with DuraSeal™.

Table 5

Tissue adhesive material	Lap shear strength (N) (n=5)
4-armPEG20k-DA	0.00
6-armPEG30k-DA	0.55
8-ArmPEG40k-DA	2.28
DuraSeal™	0.15

Example 11 - *In vitro* burst pressure test of 8-ArmPEG40k-DA, 6-armPEG30k-DA and 4-armPEG20k-DA

The tissue adhesive properties of the multi-arm PEG-DA polymers prepared according to Example 8 were determined on porcine dura mater. The in vitro burst pressure test was performed according to ASTM F2392-04 (Standard Test Method for Burst Strength of Surgical Sealants). The results are depicted in Table 6 and are compared with commercially available DuraSeal™. Results show that the burst pressure of the 8-ArmPEG40k-DA is higher than commercially available DuraSeal™.

Table 6

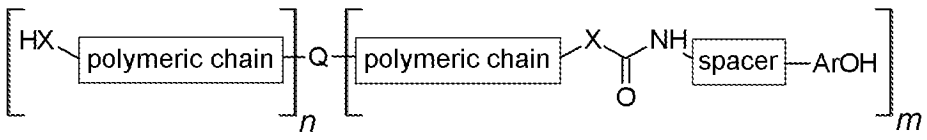
Tissue adhesive material	Burst pressure (mbar) (n=5)
4-armPEG20k-DA	0.00
6-armPEG30k-DA	25.4
8-ArmPEG40k-DA	29.3
DuraSeal™	10.4

Conclusies

1. Weefsel-hechtend polymeer, ten minste één polymeerketen omvattende die gefunctionaliseerd is met een ArOH-groep, waarbij voornoemde ArOH-groep een hydroxyl-gesubstitueerde aromatische groep vertegenwoordigt die is gebonden op voornoemde polymeerketen met een amide-, urethaan-, thio-urethaan-, of ureumbinding, optioneel via een aftstandhouder.

2. Weefsel-hechtend polymeer volgens de voorgaande conclusie, waarbij voornoemd polymeer een meertakkig polymeer is dat een kern en twee of meerdere polymeertakken omvat waarvan ten minste één tak voornoemde ene polymeerketen omvat die gefunctionaliseerd is met een ArOH-groep.

3. Weefsel-hechtend polymeer volgens een der voorgaande conclusies, waarbij voornoemd polymeer een structuur heeft volgens formule II



II

Polymeric chain = polymeerketen

Spacer = afstandhouder

waarin Q staat voor een kern;
de polymeerketen één of meerdere polymeergroepen omvat;
X staat voor O, S, of NH, bij voorkeur voor O;
de aftstandhouder staat voor een aftstandhouderfunctionaliteit, bij voorkeur een lineair of vertakt C_1-C_8 koolwaterstof, optioneel gesubstitueerd met één of meerdere OH, SH, halide, amide, en/of carboxylaat;
ArOH staat voor de hydroxyl-gesubstitueerde aromatische groep;

m staat voor het aantal gefunctionaliseerde polymeertakken, en ten minste gelijk is aan 2, bij voorkeur gelegen in het bereik van 3 tot en met 12, bij meer voorkeur waarbij *m* 4 tot en met 10 is, en bij de meeste voorkeur 6 tot en met 8; en

n staat voor het aantal niet-gefunctionaliseerde polymeertakken, en een getal is dat in het bereik ligt dat kleiner is dan *m*, bij voorkeur 0.

4. Weefsel-hechtend polymeer volgens een der voorgaande conclusies, waarbij voornoemde polymeerketen die gefunctionaliseerd is met een ArOH-groep een structuur omvat volgens formule III

$$-(E-Z-(R^{Ar})_q-I-(R^{Reg})_p-Z)_s-$$

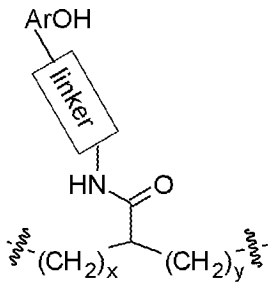
III

waarin E is gebaseerd op een ketenverlenger zoals een polyol, polythiol, of polyamine, R^{Ar} staat voor een gefunctionaliseerde monomere eenheid die gefunctionaliseerd is met de ArOH-groep, R^{Reg} staat voor een gewone monomere eenheid die niet gefunctionaliseerd is met de ArOH-groep, Z staat voor een urethaan-, thio-urethaan-, en/of ureumbinding, *s* staat voor het aantal polymeereenheden van de polymeerketen en proportioneel is met het molecuulgewicht ervan, waarbij *s* bij voorkeur een geheel getal is van 5 tot en met 500, *q* plus p gelijk is aan 1; en *q* niet gelijk is aan 0, waarbij bij voorkeur *q*/*p* ten minste gelijk is aan 0,5, bij meer voorkeur ten minste gelijk is aan 0,75, en bij de meeste voorkeur gelijk is aan 1;

waarbij bij voorkeur E één of meerdere omvat uit de groep bestaande uit C_2-C_{10} alkyleen, optioneel gesubstitueerd met C_1-C_{10} alkyl of C_1-C_{10} alkylgroepen die gesubstitueerd zijn met haliden of beschermende S-, N-, P-, of O-groepen en/of S, N, P, or O omvatten in de alkyleenketen, alifatische polyesters, polyetheresters, polyethers, poly(anhydriden), polycarbonaten, polyethers, of combinaties daarvan, waarin optioneel ten minste één E een hydrofiel segment

omvat;

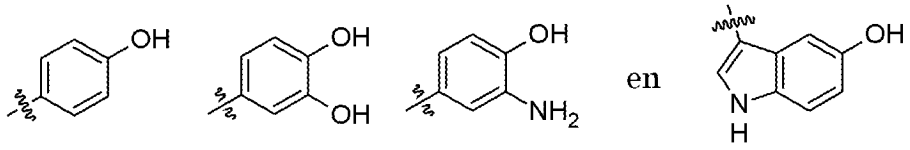
en/of waarbij bij voorkeur voornoemd R^{Ar} een C_2-C_{10} alkyleen omvat dat gesubstitueerd is met de ArOH-groep, optioneel via een aftstandhouder, bij voorkeur waarbij voornoemd R^{Ar} een structuur heeft volgens formule VII



VII

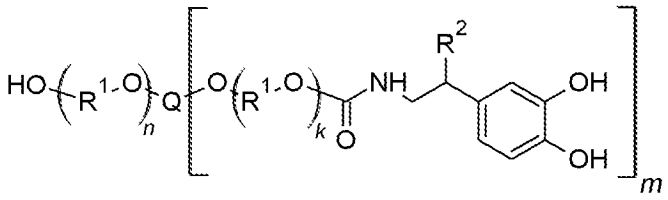
waarbij de aftstandhouder voorziet in een linker-functionaliteit, ArOH staat voor een hydroxyl-gesubstitueerde aromatische groep, x en y gehele getallen zijn die elk liggen tussen 0 en 8, terwijl x+y ligt tussen 3 en 9, bij voorkeur waarbij x gelijk is aan 4, en y gelijk is aan 0.

5. Weefsel-hechtend meertakkig polymeer volgens een der voorgaande conclusies, waarbij de ArOH is gekozen uit de groep bestaande uit fenol, catechol, 3-hydroxyfenol, 4-hydroxyfenol, 2-aminofenol, 3-aminofenol, 4-aminofenol, 4-hydroxyindol, 5-hydroxyindol, 6-hydroxyindol, 7-hydroxyindol, en combinaties daarvan, bij voorkeur één of meerdere uit de groep bestaande uit aromatische groepen die één van de volgende structuren hebben:



6. Weefsel-hechtend meertakkig polymeer volgens een der voorgaande conclusies, waarbij de groep NH-aftstandhouder-ArOH is gebaseerd op dopamine, DL-DOPA, L-DOPA, D-DOPA, tyramine, noradrenaline, en/of serotonine.

7. Weefsel-hechtend meertakkig polymeer volgens een der voorgaande conclusies, met een structuur volgens formule IIb,



IIb

waarin Q, *m*, en *n* zijn gedefinieerd zoals voor formule II;
R^1 staat voor een lineair of vertakt C_1-C_4 alkyleen en/of –C(O)–C_1-C_5 alkyleen, bij voorkeur ethyleen;
R^2 staat voor H, OH, CH_3, of CO_2H, of esters daarvan, zoals CO_2Me of CO_2Et, bij voorkeur H;
k staat voor het aantal polymeereenheden van elke tak, en is proportioneel met het molecuulgewicht van elke tak.

8. Weefsel-hechtend polymeer volgens een der voorgaande conclusies, met een op het aantal gebaseerd gemiddeld molecuulgewicht dat ligt in het bereik van 500 Da tot en met 100 kDa, dat bij voorkeur ligt in het bereik van 10 kDa tot en met 80 kDa, en dat nog beter ligt in het bereik van 20 kDa tot en met 50 kDa, zoals ongeveer 40 kDa.

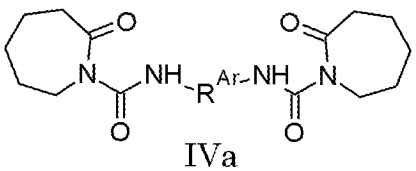
9. Kit met onderdelen, een eerste container omvattende die een weefsel-hechtend polymeer volgens een der voorgaande conclusies omvat, alsook een tweede container die een oxiderend middel omvat.

10. Injecteerbare hydrogel, bij voorkeur gebaseerd op een geoxideerd weefsel-hechtend polymeer volgens een der conclusies 1 tot en met 8, waarbij voornoemde hydrogel een overlapafschuivingshechtsterkte heeft van meer dan 0,50 N, bij voorkeur van meer dan 0,7 N, nog beter van meer dan 1 N, nog beter van meer dan 1,5 N, en nog beter van meer dan 2 N, zoals bepaald in overeenstemming met ASTM F2255-05.38, en/of waarbij voornoemde hydrogel

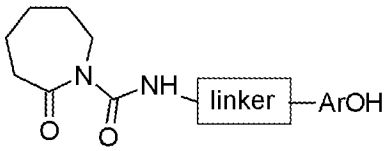
een barstdruk vertoont van meer dan 15 mbar, bij voorkeur van meer dan 20 mbar, en nog beter van meer dan 25 mbar, bepaald in overeenstemming met ASTM F 2392-04.

11. Weefsel-hechtend polymeer volgens een der conclusies 1 tot en met 8, of injecteerbare hydrogel volgens conclusie 10, voor toepassing bij een medische behandeling, bij voorkeur waarbij de behandeling het afdichten of het sluiten van weefsel omvat.

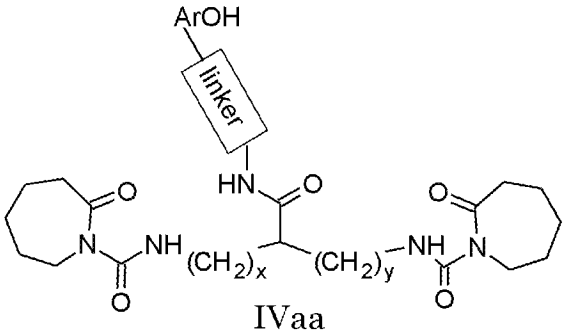
12. Caprolactaam-geblokkeerde hydroxyl-gesubstitueerde aromatische verbinding (afgekort als CAB-ArOH of CAB_2-ArOH), geschikt voor de bereiding van een weefsel-hechtend polymeer volgens een der conclusies 1 tot en met 8, waarbij voornoemde caprolactaam-geblokkeerde verbinding een structuur heeft volgens formule IVa (CAB_2-ArOH) of IVb (CAB-ArOH), bij voorkeur IVaa of IVba, liever IVab of IVba.



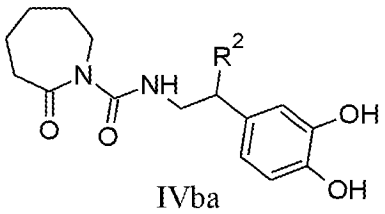
IVa



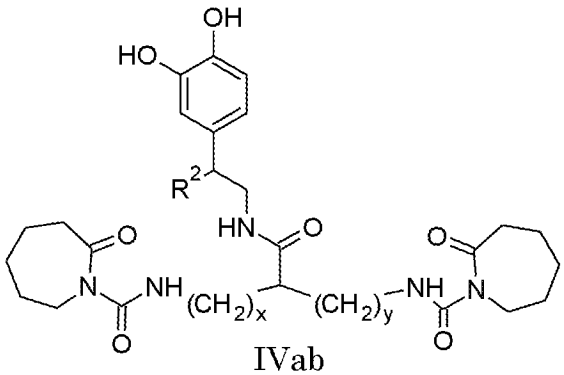
IVb



IVaa



IVba

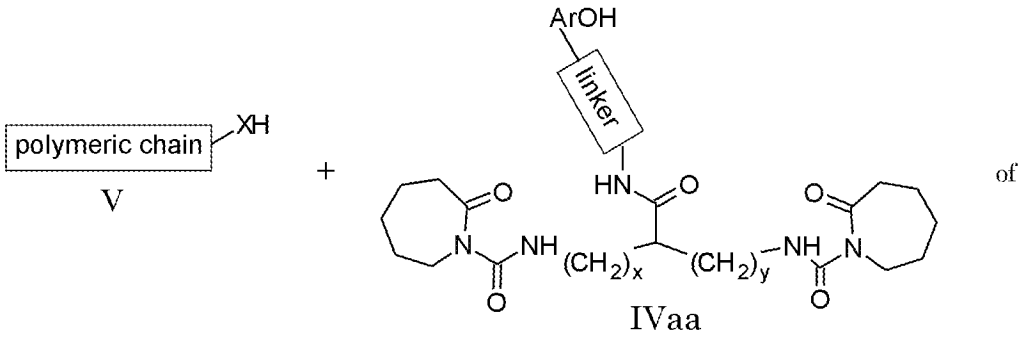


IVab

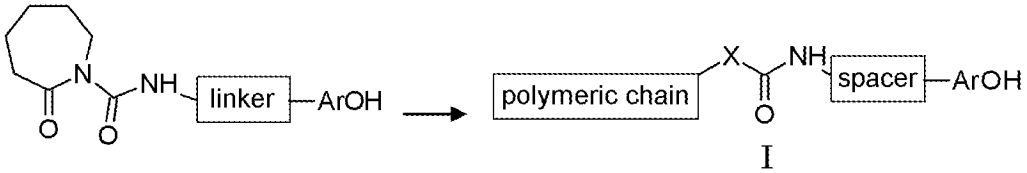
waarin R^{Ar} staat voor een gefunctionaliseerde monomere eenheid die gefunctionaliseerd is met de ArOH-groep; de linker staat voor een lineair of vertakt C_1-C_8 koolwaterstof, optioneel gesubstitueerd met één of meerdere OH, SH, halide, amide, en/of carboxylaat;

R^2 staat voor H, OH, CH_3, of CO_2H, of esters daarvan, zoals CO_2Me of CO_2Et, en x en y gehele getallen zijn die elk liggen tussen 1 en 8, terwijl x+y ligt tussen 3 en 9, waarbij x bij voorkeur gelijk is aan 4, en y gelijk is aan 0.

13. Werkwijze voor het bereiden van een weefsel-hechtend polymeer volgens een der conclusies 1 tot en met 8, waarbij voornoemde werkwijze het laten reageren omvat van een polymeer die een polymeerketen omvat die gefunctionaliseerd is met XH, zoals geïllustreerd met formule V met CAB-ArOH of CAB_2-ArOH volgens conclusie 12,



V + IVaa of



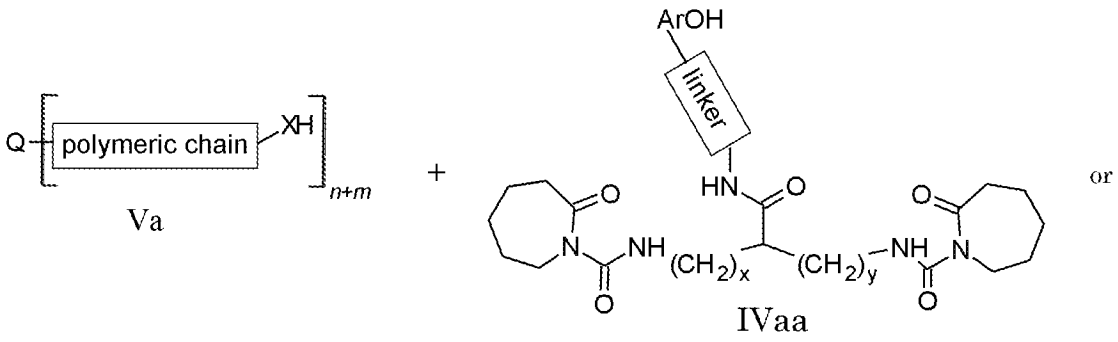
IVb I

Polymeric chain = polymeerketen

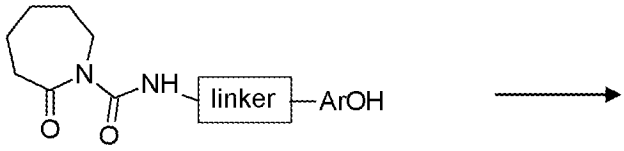
Spacer = afstandhouder

waarin XH een hydroxyl-, een sulfhydryl-, en/of een aminegroep is, en waarbij de aftstandhouder staat voor een aftstandhouderfunctionaliteit,

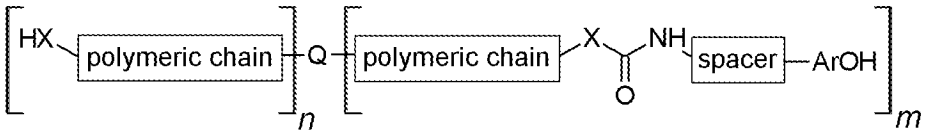
waarbij bij voorkeur voornoemd weefsel-hechtend polymeer een meertakkig weefsel-hechtend polymeer volgens een der conclusies 3 tot en met 8, in zoverre afhankelijk van conclusie 3, is, en waarbij voornoemde werkwijze het laten reageren omvat van een meertakkig polymeer met een structuur volgens formule Va met CAB-ArOH of CAB_2-ArOH volgens conclusie 12,



Va IVaa



IVb



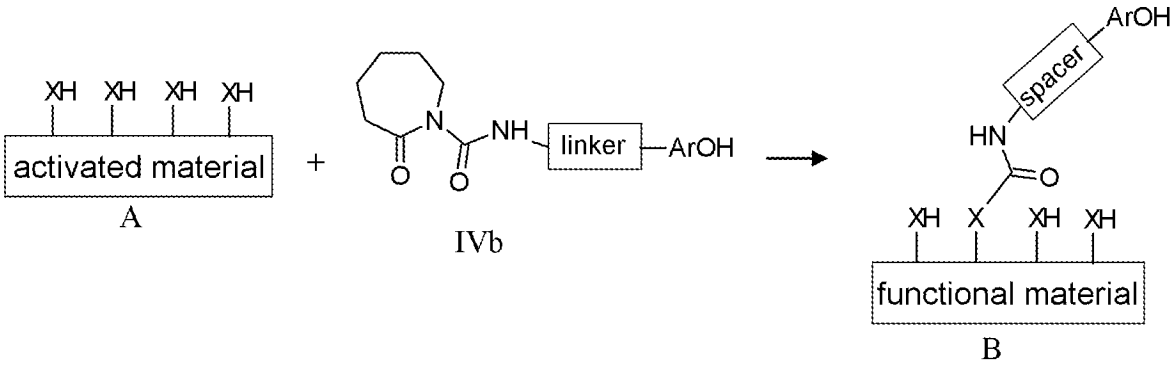
II

Polymeric chain = polymeerketen

Spacer = afstandhouder

waarin XH een hydroxyl-, een sulfhydryl-, en/of een aminegroep is, en waarbij de aftstandhouder staat voor een aftstandhouderfunctionaliteit,

14. Werkwijze voor het functionaliseren van een geactiveerd materiaal A waarvan het oppervlak ten minste één reactieve hydroxyl-, sulfhydryl-, en/of aminegroep omvat, met een catecholderivaat, waarbij voornoemde werkwijze het in contact brengen omvat van voornoemd geactiveerd materiaal A met de CAB-ArOH volgens conclusie 12, teneinde functioneel materiaal B te verkrijgen,



Activated material = geactiveerd materiaal
Linker = linker
Spacer = aftstandhouder
Functional material = functioneel materiaal

waarin XH de reactieve hydroxyl-, sulfhydryl-, en/of aminegroep is.

15. Gefunctionaliseerd materiaal, verkrijgbaar aan de hand van een werkwijze volgens de voorgaande conclusie.

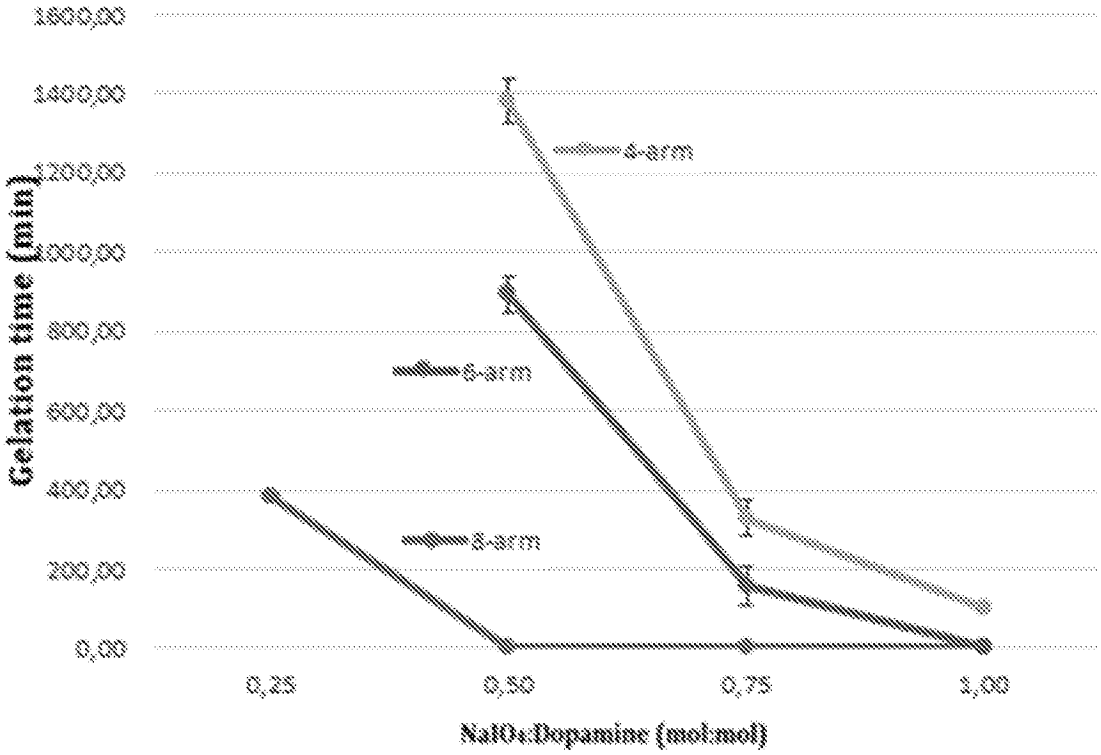

1800,00
1400,00
1200,00
1000,00
800,00
600,00
400,00
200,00
0,00
Gelation time (min)
4-arm
6-arm
8-arm
0,25
0,50
0,75
1,00
NaIO4:Dopamine (mol:mol)

Fig. 1

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	P122936NL00
Nederlands aanvraag nr. **2023358**	Indieningsdatum **21-06-2019**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Polyganics IP B.V., et al**	
Datum van het verzoek voor een onderzoek van internationaal type **31-08-2019**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN74348**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

Zie onderzoeksrapport

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**Zie onderzoeksrapport**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐	**GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐	**GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2023358

A. CLASSIFICATIE VAN HET ONDERWERP
INV. C08G18/48 A61L24/04 C08G18/77 C08G18/80 C08G18/86
C09J175/04 C09J175/08
ADD.
Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
C08G A61L C09J

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X,D	LIU ET AL.: "Injectable Dopamine-Modified Poly(ethylene glycol) Nanocomposite Hydrogel with Enhanced Adhesive Property and Bioactivity", ACS APPLIED MATERIALS AND INTERFACES, deel 6, 2014, bladzijden 16982-16992, XP055644724, in de aanvraag genoemd	1-3,5-8, 10,11
A	* het gehele document *	4,9, 12-15

X	DE 10 2014 226098 A1 (FRAUNHOFER-GESELLSCHAFT ZUR FÖRDERUNG DER ANGEWANDTEN FORSCHUNG E V [D]) 16 juni 2016 (2016-06-16)	1-3,5,8
A	* alineas [0001], [0011] - [0017], [0032] - [0044], [0058] * * voorbeelden 1,2 *	4,6,7, 9-15

	-/--	

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
4 maart 2020	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Neugebauer, Ute

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ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2023358

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	US 2013/345334 A1 (HOOGENBOOM RICHARD [NL] ET AL) 26 december 2013 (2013-12-26)	10,11
A	* alineas [0001], [0002], [0036] - [0040], [0109] * * voorbeelden 2,7 *	1-9, 12-15

A	US 2015/079151 A1 (BECKMAN ERIC J [US] ET AL) 19 maart 2015 (2015-03-19) * alineas [0002] - [0008] * * voorbeelden 1-3 *	1-15

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2023358

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
DE 102014226098 A1	16-06-2016	DE 102014226098 A1	16-06-2016
		WO 2016097040 A1	23-06-2016
US 2013345334 A1	26-12-2013	AU 2012202543 A1	01-08-2013
		BR 112013017140 A2	20-09-2016
		CA 2824471 A1	03-05-2012
		CN 103429268 A	04-12-2013
		EP 2661283 A2	13-11-2013
		EP 3446721 A1	27-02-2019
		ES 2729787 T3	06-11-2019
		JP 5960165 B2	02-08-2016
		JP 6529931 B2	12-06-2019
		JP 2014503017 A	06-02-2014
		JP 2017008315 A	12-01-2017
		KR 20130132916 A	05-12-2013
		RU 2013136382 A	10-02-2015
		TR 201909167 T4	22-07-2019
		US 2013345334 A1	26-12-2013
		US 2017266337 A1	21-09-2017
		US 2019231923 A1	01-08-2019
		WO 2012057628 A2	03-05-2012
US 2015079151 A1	19-03-2015	AU 2011329147 A1	04-07-2013
		BR 112013011958 A2	24-10-2017
		CA 2817916 A1	24-05-2012
		CN 103429692 A	04-12-2013
		EP 2640797 A2	25-09-2013
		IL 226303 A	31-05-2016
		JP 5993862 B2	14-09-2016
		JP 2014502297 A	30-01-2014
		KR 20130119944 A	01-11-2013
		NZ 611998 A	28-11-2014
		RU 2013127243 A	27-12-2014
		US 2012121686 A1	17-05-2012
		US 2015079151 A1	19-03-2015
		WO 2012068052 A2	24-05-2012
		ZA 201304375 B	27-08-2014

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN74348	21.06.2019		NL2023358

International Patent Classification (IPC)
INV. C08G18/48 A61L24/04 C08G18/77 C08G18/80 C08G18/86 C09J175/04 C09J175/08

Applicant
Polyganics IP B.V., et al

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

	Examiner
	Neugebauer, Ute

Application number

WRITTEN OPINION

NL2023358

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	4, 9, 12-15
	No:	Claims	1-3, 5-8, 10, 11
Inventive step	Yes:	Claims	4, 9, 12-15
	No:	Claims	1-3, 5-8, 10, 11
Industrial applicability	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Box No. VIII Certain observations on the application

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: LIU ET AL.: "Injectable Dopamine-Modified Poly(ethylene glycol) Nanocomposite Hydrogel with Enhanced Adhesive Property and Bioactivity",ACS APPLIED MATERIALS AND INTERFACES, deel 6, 2014, bladzijden 16982-16992, XP055644724, in de aanvraag genoemd

D2: DE 10 2014 226098 A1

D3: US 2013/345334 A1

Novelty/ Inventive Step

The following is stated as far as the present claims could be understood (see item clarity, below):

Document D1 discloses an *injectable hydrogel* which is based on dopamine-functionalized four-arm PEG, containing *catechol* units and an *amide* group, i.e. dihydroxyphenyl units, and nanosilicate. suitable as tissue adhesive, which shows high lap shear adhesion (D1: especially see the experimental section including schemes 1 and 2, and the results and discussion section).

Document D2 discloses a hydrogel as tissue adhesive being prepared from polypeptide (thus, amide groups in polymer chain) containing *dihydroxylphenyl* groups and 4-arm-PEG-SH (D2: especially see paragraphs 1, 11-16 and examples 1,2).

Document D3 discloses a tissue adhesive being a *hydrogel* based on copolymers of oxazoline derivatives and having a burst pressure of 23 mm/Hg, being > 0.03 bar, respectively, 0.99 bar (D3: especially see paragraph 109 and example 7).

The present application does not meet the criteria of patentability, because the subject-matter of claims 1-3, 5-8, 10 and 11 is not new.

Documents D1 to D3 are silent with respect to the structure III of the polymer with Z being a (thio)urethane and/or urea bond , a kit (claim 9) and caprolactam-blocked hydroxy-functional compounds, process of preparation and functionalized material as claimed in claims 12-15.

Re Item VIII

Certain observations on the application

Clarity

1. Independent claim **10** relates to an injectable hydrogel which is characterized solely by its parameters of lap shear adhesion and burst pressure, as the technical feature of the tissue-adhesive polymer according to claims 1 to 8 is merely preferred.

However, it is clear from claim 1 and at least the examples of the description that the injectable hydrogel is a based on a tissue-adhesive polymer, containing at least one polymer chain which is functionalized with an ArOH group being a hydroxylsubstituted aromatic group which is bonded to said polymer chain by an amide, urethane, thiourethane or urea bond, according to claims 1 to 8, being oxidized.

Said feature is essential to the definition of the invention as any independent claim must contain all the technical features essential to the definition of the invention.

The same applies to claim 11 (option of unspecified injectable hydrogel).

2. The subject-matter of independent claim **11** covers a mixture of two different categories of claims, firstly, either a specified tissue-adhesive polymer or an unspecified injectable hydrogel (physical entity) and secondly, the use of said polymer or hydrogel in medical treatment (activity). As there are only two basic kinds of claims, either to a physical entity such as a product or to an activity such as a use said mixing of categories is not allowed.

3. The "incorporation by reference" of the entire contents of other documents could lead to doubt as to the extent of protection sought by the claims. This phrase should therefore be deleted (cf. page 26 in the description).