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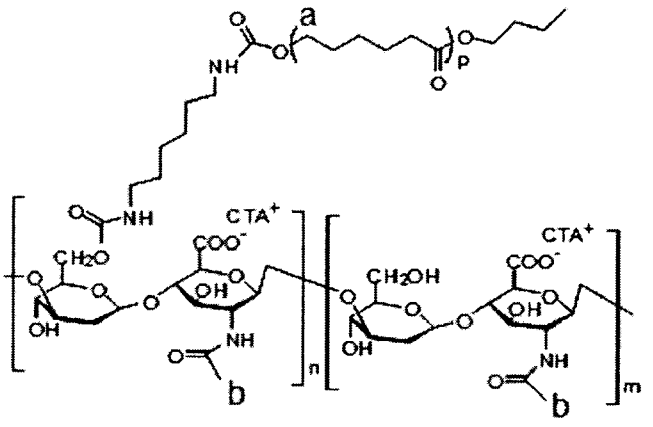
(54) **Title**: BIOMATERIALS WITH A SUSTAINABLE SURFACE

Figure 1.




(57) **Abstract**: Polymeric materials with improved (e.g. continuous) antifouling surfaces. The materials can be incorporated into a variety of materials, including medical devices, microspheres, nanoparticles, and containers. These materials combine amphiphilic copolymer conjugates having hyaluronan as the hydrophilic component, dispersed throughout bulk polymeric biomaterial. The hydrophobic component of the copolymer will have good compatibility with host bulk polymer, enabling excellent dispersion of the copolymer in the bulk, and anchorage for the hydrophilic component at the surface. The hydrophilic hyaluronan segment will spread on the surface of polymers and act as an antifouling layer in an aqueous environment; and the copolymer will dispersed uniformly in the polymer matrix and will not migrate to the surface because of its high molecular weight. Hence, as the bulk polymer degrades, the dispersed HA-amphiphile will become available to "coat" the dynamically evolving biointerface.

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# BIOMATERIALS WITH A SUSTAINABLE SURFACE

## Background of the Invention

With advances in surgery and in processing technology, polymeric biomaterials are increasingly being used as implants in human body in the last decades. The surface chemistry and morphology of a polymeric biomaterial are of critical importance when implanted in a living environment (see Ikada Y., *Biomateirals*, **1994,** *15*, 725-736). Generally, biomolecules in physiological fluids (such as proteins, oligopeptides, or polysaccharides, etc) and cells (such as platelets, macrophages, and fibroblasts) prefer to attach by specific receptor bindings, but they are also able to deposit on a material surface even without specific receptor recognition interactions (see Pasqui D, et al., *Biomacromolecules,* **2007**, *8*, 3531-3539). These biomolecules and cells that bind nonspecifically typically have hydrophobic character and are inclined to be adsorbed onto hydrophobic surfaces. The surfaces of most polymers used to prepare implants are hydrophobic. Such fouling at the surface will usually reduce the material's functionality and cause undesirable effects. Examples include background interference, protein accumulation onto biosensor surfaces, bacterial colonization of contact lenses and indwelling catheters, thrombosis in cardiovascular implants, and foreign body responses leading to isolation or rejection of implants and devices (see Banerjee I, et al., *Adv. Mater.*, **2011**, *23*, 690–718). Therefore, an interface between biomaterial and physiological fluid, which attenuates or completely prevents the absorption of nonspecific molecules and cells, is usually necessary and important for implants and devices.

In the late 1960s Baier demonstrated a correlation between relative adhesion of fouling organisms and the material's surface energy, called the Baier curve, which has been confirmed in several marine and biomedical environments (Baier RE., *J Mater Sci: Mater Med*, **2006**, *17*, 1057-1062). Based on the Baier curve, minimal fouling is typically achieved at a surface tension of 22-24 mM/m, which is lower than the surface energy of most polymeric biomaterials. The general principle of designing a bioresistant surface is to build a thin layer of hydrophilic material into the interface to decrease the surface energy of implants made from polymeric biomaterials. Whitesides and co-workers systematically varied the surface energy of self-assembled monolayers to test resistance to protein adsorption, and concluded that surfaces that are hydrophilic, electrically neutral and contain hydrogen bond acceptors are most effective at resisting protein adhesion (see Ostuni E, et al., *Langmuir*, **2001**, 17, 5605–5620). Well-packed zwitterionic compounds were found to be very efficient as stable nonfouling surfaces as well (see Chang Y, et al., *Langmuir*, **2008**, *24*, 5453–5458).

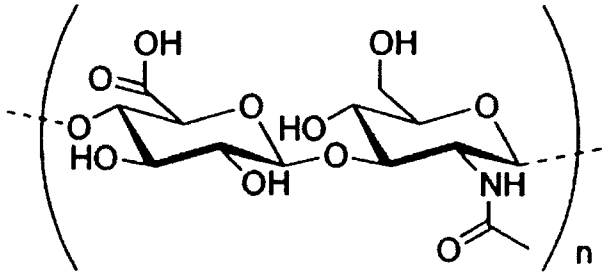
Peptidomimetic polymers were synthesized by Statz et al and showed excellent protein resistance and long lasting ability (several months) (see Statz AR, et al., *J Am Chem Soc*, **2005**, *127*, 7972-7973). Jiang et al developed and demonstrated that poly(zwitterions) act as nonfouling surfaces that can prevent protein adsorption from complex biological media, and also provide resistance to nonspecific protein adsorption from blood serum and plasma (see Jiang SY, et al., *Adv. Mater.*, **2010**, *22*, 920–932; and Yang W, et al., *Biomaterials*, **2009**, *30*, 5617–5621).

Among many antifouling coating and materials, grafted polyethylene glycol (PEG) and its oligomers on material surfaces are most commonly used because of low cost and easy processing. Several reviews can be consulted regarding antifouling surfaces (see Banerjee I, et al., *Adv. Mater.*, **2011**, *23*, 690–718; Caro A, et al., *J Phys Chem B*, **2009**, *113*, 2101-2109; Sharma S, et al., *Biosensors and Bioelectronics*, **2004**, *20*, 227-239; Dong B. Generation of Antifouling Layers from Polyethylene Glycol by Cold Plasma Techniques on Stainless Steel and Polymer Surfaces. University of Wisconsin, Madison, 2007; Magin CM, et al., *Materials Today*, **2010**, *13*, 36-44; and Dalsin JL, et al., *Acta Biomaterialia*, **2011**, *7*, 1550-1557).

While the antifouling modification of biomaterials through surface coating without altering the properties of bulk materials is good in the short term, there are concerns regarding their application in long term implants. Implants made from polymeric materials degrade over time (slowly or rapidly) when exposed to physiological fluids of the human body (see Syrett BC, Acharya A., *ASTM Committee F-4 on Medical and Surgical Materials and Devices,* ASTM Int, **1979**). Once the surface coating layer is destroyed or partially degraded, the antifouling effect is greatly reduced.

Biodegradable polymeric materials have been widely been used as surgical implants and as scaffolds for tissue engineering (see Gunatillake PA, Adhikari R., *European Cells and Materials*, **2003**, 5, 1-16). However, surface modification to provide persistent bioresistance remains challenging for numerous applications.

Hyaluronan (aka hyaluronic acid, HA) is a linear negatively charged polysaccharide consisting of repeating disaccharide units of N-acetyl-D-glucoamine and D-glucuronate, linked by β1-4 and β1-3 glycosidic bonds.



It is a polyelectrolyte and is highly water soluble. Along with its excellent biocompatibility, hyaluronan and its derivatives can be formed into hydrogels, particles, etc., which have been extensively studied as drug carriers or scaffolds for application in tissue engineering (see Leach JB, et al., *J Biomed Mater Res*, **2004**, *70A*, 74–82; and Lapcik L Jr, et al., *Biomaterials*, **2003,** *24*, 893-900). Some reports have also shown that hyaluronan-modified surfaces present significant resistance to protein absorption and cell adhesion (see Pavesio A, et al.,*Med Device Technol.*, **1997**, *8*: 20-1, 24-7; Cassinelli C, et al., *J. Biomater. Sci. Polymer Edn,* **2000**, *11*, 961– 977; and Morra M, Cassineli C., *J Biomater Sci Polym Ed.*, **1999**, *10*, 1107-24). Some products based on crosslinked HA gels or films can be found in the market for prevention of adhesions after surgery, such as the Seprafilm® and Sepracoat® product lines developed by Genzyme.

There is currently a need for new biodegradable polymeric materials with improved continuously and persistently antifouling surfaces. In particular there is a need for methods for preparation of polymeric biomaterials with antifouling surfaces that do not produce a significant negative affect on the bulk properties of polymers, such as thermal properties, mechanical properties, biocompatibility, etc. There is also a need for new biodegradable polymeric materials that have a long lasting antifouling effect and that are easy and simple to process, and that will not produce toxic molecules in the human body upon degradation.

## Summary of the Invention

Applicant has discovered new polymeric materials with improved (e.g., continuous) antifouling surfaces. These materials combine amphiphilic copolymer conjugates having hyaluronan as the hydrophilic component, dispersed throughout bulk polymeric biomaterial. Accordingly, without being bound by theory, it is believed that the hydrophobic component of the copolymer will have good compatibility with host bulk polymer, enabling excellent dispersion of the copolymer in the bulk, and anchorage for the hydrophilic component at the surface. Thus, the hydrophilic

hyaluronan segment will spread on the surface of polymers and act as an antifouling layer in an aqueous environment; and the copolymer will dispersed uniformly in the polymer matrix and will not migrate to the surface because of its high molecular weight. Hence, as the bulk polymer degrades, the dispersed HA-amphiphile will become available to "coat" the dynamically evolving biointerface.

Accordingly the invention provides a composition comprising 1) from about 0.1 to about 30 weight percent of the composition as one or more POLYMER conjugates that comprise A HYDROPHILIC POLYMER SEGMENT linked to one or more HYDROPHOBIC POLYMER SEGMENTS, and 2) at least about 70 weight percent of the composition as BULK POLYMERIC BIOMATERIAL. In one embodiment the invention provides a composition comprising 1) from about 0.1 to about 30 weight percent of the composition as a hyaluronan copolymer conjugate, which conjugate comprises hyaluronan linked to one or more polycaprolactone segments, and 2) at least about 70 weight percent of the composition as bulk polycaprolactone.

The compositions of the invention can be incorporated into a wide variety of materials and devices, including but not limited to medical, dental, and pharmaceutical implants, surgical devices and accessories, scaffolds for tissue engineering, container linings, antibacterial and antiseptic surfaces for households, and construction materials. The compositions of the invention can also be incorporated into microspheres and nanoparticles that can be used in diagnostic, biosensing, and drug/protein/gene delivery systems.

## Brief Description of the Figures

Figure 1. Shows the structure and the proton NMR spectra of CTA-HA-g-PCL and CTA-HA.

Figure 2. DLS results of HA-g-PCL nanoaggregates in water and chloroform at different time points.

Figure 3. TEM images of HA-g-PCL nano-aggregates in water and chloroform.

Figure 4. XPS spectra of the PCL/HA-g-PCL nanocomposite films and pristine PCL film. X-axis=Counts and Y-axis= Binding Energy (ev).

Figure 5. Static water contact angle of the nanocomposite films and pristine PCL film.

Figure 6. Static water contact angle of the nanocomposite films polished from 1 mm to 0.5, 0.4, and 0.2 mm thick, compared with the pristine PCL film.

Figure 7. Illustrated data from Example 1 showing antifouling property of a PCL/HA-g-PCL nanocomposite film.

Figure 8. Quantification of adherent cells on the surfaces of nanocomposite and pristine PCL films.

Figure 9. Quantification of adherent cells on the surfaces of nanocomposite films polished to different depth, in comparison with the surfaces of pristine PCL and tissue cultured plate (TCP).

## Detailed Description

### HYDROPHILIC POLYMER SEGMENT

The compositions of the invention comprise POLYMER conjugates that comprise A HYDROPHILIC POLYMER SEGMENT linked to one or more HYDROPHOBIC POLYMER SEGMENTS. The HYDROPHILIC POLYMER SEGMENT can be a water-soluble polymer known to the field, synthetic or natural, charged or non-charged, degradable or nondegradable, having at least one functional group (such as for example COOH, OH, or amine, or SH) through which the "hydrophobic segments" can be chemically conjugated. These include but not limited to glycosaminoglycans such as hyaluronan (HA), heparan sulfate, chondroitin sulfate, keratan sulfate; other natural polymers such as alginate, dextran, starch, chitosan and chitosan derivatives, carboxymethyl cellulose and other water-soluble cellulose derivatives; synthetic hydrophilic polymers such as PEG, polyvinylpyrrolidone, poly(alkyl)acrylic acid and their derivatives and copolymers containing carboxylic acid groups in the side-chains, polysialic acid, polyglutamic acid, poly(alkyl)arylates or poly(alkyl)acrylamides and their derivatives and copolymers containing amino groups in the side-chains.

In one embodiment of the invention the HYDROPHILIC POLYMER SEGMENT is hyaluronan. The hyaluran that is incorporated into the compositions of the invention can be a linear polysaccharide consisting of repeating disaccharide units of N-acetyl-D-glucoamine and D-glucuronate, linked by β1-4 and β1-3 glycosidic bonds. Typically the hyaluronan has an average molecular weight in the range of from about 1,000 to about 2,000,000. In one embodiment of the invention the hyaluronan has an average molecular weight in the range of from about 10,000 to about 1,000,000. In another embodiment of the invention the hyaluronan has an average molecular weight in the range of from about 700,000 to about 900,000. Hyaluronan is readily available from a number of commercial suppliers in a variety of molecular weight ranges and grades.

In another embodiment of the invention the HYDROPHILIC POLYMER SEGMENT is alginate.

HYDROPHOBIC POLYMER SEGMENTS

The compositions of the invention comprise POLYMER conjugates that comprise A HYDROPHILIC POLYMER SEGMENT linked to one or more HYDROPHOBIC POLYMER SEGMENTS. The HYDROPHOBIC POLYMER SEGMENTS are typically biodegradable, poorly water-soluble polymers known to the field, having at least one functional group (such as COOH, OH, amine, SH) through which the "hydrophilic component" can be covalently conjugated. These include but are not limited to polylactide, polyglycolide, polydioxanone, poly(trimethylene carbonate), polyanhydride, poly(ester anhydride) poly(anhydride-co-imide), poly(ortho ester), polyacetal, polyketal, tyrosine-derived polycarbonate, polyhydroxylalkanoates, polycaprolactone (PCL), polyurethane, poly(ester amide), poly(propylene fumarate), Pseudo poly(amino acids), poly(amino acids), poly(alkyl cyanoacrylate), polyphosphazene, polyphosphoester. These include both homopolymers and copolymers of the families above. In one embodiment of the invention the HYDROPHOBIC POLYMER SEGMENT is polylactic acid (PLA). In one embodiment of the invention the HYDROPHOBIC POLYMER SEGMENT is a co-polymer of polylactic acid and poly glycolic acid (PLGA). In one embodiment of the invention the HYDROPHOBIC POLYMER SEGMENT is a co-polymer of polylactic acid and polycaprolactone (PLCA). Methods for preparing and derivatizing HYDROPHOBIC POLYMER SEGMENT are described in C.G. Pitt, "Poly-ε-caprolactone and its copolymers," in Biodegrable Polymers as Drug Delivery Systems, edited by M. Chasin and R. Langer, Dekker, New York, 1990, and in references cited therein.

In one embodiment of the invention the HYDROPHOBIC POLYMER SEGMENT is polycaprolactone. Typically each polycaprolactone segment has an average molecular weight in the range of from about 500 to about 10,000. In one embodiment of the invention each polycaprolactone segment has an average molecular weight in the range of from about 500 to about 5,000. In another embodiment of the invention each polycaprolactone segment has an average molecular weight in the range of from about 500 to about 1,000.

The PCL segments can be attached to the HYDROPHILIC POLYMER SEGMENT through any acceptable means that does not interfere with the function of the copolymer conjugates in the compositions of the invention. For example, the PCL segments can be attached through one or more of the hydroxy groups on the HYDROPHILIC POLYMER SEGMENT through ether, ester, carbonate, or carbamate bonds. In one embodiment the PCL segments can be attached through one or more of the hydroxy groups on the HYDROPHILIC POLYMER SEGMENT through ester bonds.

In another embodiment the PCL segments can be attached through one or more of the hydroxy groups on the HYDROPHILIC POLYMER SEGMENT through carbamate bonds. Additionally, a linker group can be disposed between the PCL segment and the hydroxy oxygen of the HYDROPHILIC POLYMER SEGMENT. The nature of the linker group is not critical provided the linker group does not interfere with the function of the hyaluronan copolymer conjugates in the compositions of the invention. In one embodiment of the invention the linker can comprise a linear, branched, or cyclic alkyl group or a 6-10 membered monocyclic or bicyclic aromatic group, or a combination thereof. In another embodiment the linear or branched alkyl group comprises 2-18 carbon atoms and the cyclic alkyl group can comprise 3-18 carbon atoms. In another embodiment the linker is derivable from an diisocyanate that comprises from 2-18 carbon atoms. In another embodiment the linker is derivable from hexamethylene -1,6-diisocyanate.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 200 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 100 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 50 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 25 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 10 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to up to about 5 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to 1 polycaprolactone segment.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to at least about 100 polycaprolactone segments.

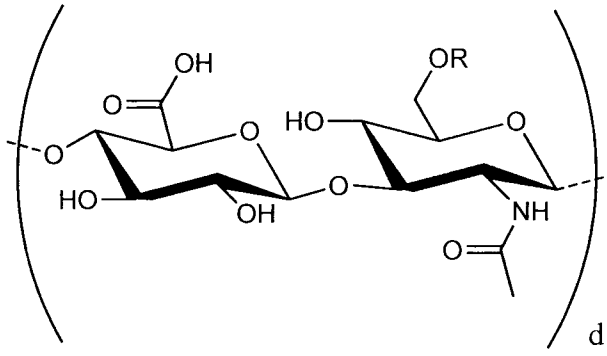
In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to at least about 50 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to at least about 25 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to at least about 10 polycaprolactone segments.

In one embodiment of the invention each HYDROPHILIC POLYMER SEGMENT is linked to at least about 5 polycaprolactone segments.

In one embodiment of the invention the POLYMER conjugate has the formula:




wherein:

d is an integer selected from 5-5000;

each R is independently H, -C(=O)N(H)-PCL, or -C(=O)N(H)-Y-N(H)C(=O)O-PCL; wherein at least one R is -C(=O)N(H)-PCL, or -C(=O)N(H)-Y-N(H)C(=O)O-PCL.

each PCL is independently a polycaprolactone chain having a molecular weight of from about 500 to 10,000; and

Y is a suitable linker group.

BULK POLYMERIC BIOMATERIAL

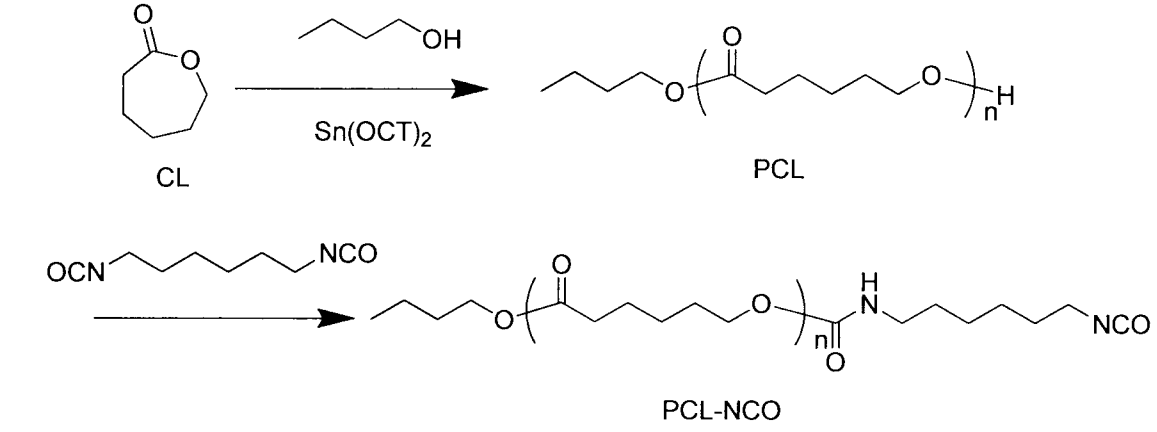
The compositions of the invention comprise POLYMER conjugates that comprise a HYDROPHILIC POLYMER SEGMENT linked to one or more HYDROPHOBIC POLYMER SEGMENTS, and 2) at least about 70 weight percent of the composition as BULK POLYMERIC BIOMATERIAL. The BULK POLYMERIC BIOMATERIAL is typically a biodegradable, poorly water-soluble polymer known to the field. These include but not limited to polylactide, polyglycolide, polydioxanone, poly(trimethylene carbonate), polyanhydride, poly(ester anhydride) poly(anhydride-co-imide), poly(ortho ester), polyacetal, polyketal, tyrosine-derived polycarbonate, polyhydroxylalkanoates, polycaprolactone (PCL), polyurethane, poly(ester amide), poly(propylene fumarate), Pseudo poly(amino acids), poly(amino acids), poly(alkyl cyanoacrylate), polyphosphazene, polyphosphoester. These include both homopolymers and copolymers of the families above.

In one embodiment of the invention the BULK POLYMERIC BIOMATERIAL can be polycaprolactone (PCL). PCL is available from a number of commercial sources and it can be prepared as described in C.G. Pitt, "Poly-ε-caprolactone and its copolymers," in Biodegrable Polymers as Drug Delivery Systems, edited by M. Chasin and R. Langer, Dekker, New York, 1990. Chapter 2, pp 71-120.

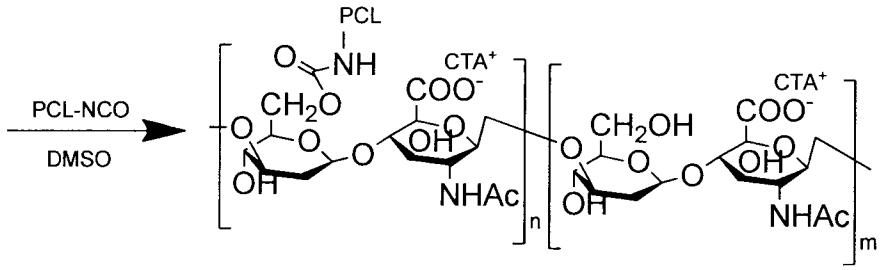
Typically the bulk polycaprolactone has an average molecular weight in the range of from about 2,000 to about 1,000,000. In one embodiment of the invention the bulk polycaprolactone has an average molecular weight in the range of from about 5,000 to about 200,000. In another embodiment of the invention the bulk polycaprolactone has an average molecular weight in the range of from about 10,000 to about 20,000.

Typically, for compositions of the invention, if the hydrophilic component is an anionic polymer or neutral polymer, then the surface coating will be antifouling, antiadhesive, and lubricious; and if the hydrophilic component is a cationic polymer, then the surface coating will be bioadhesive, and potentially antimicrobial.

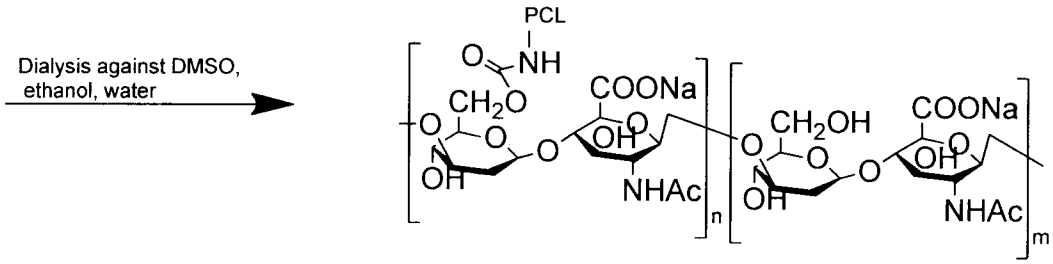
Processes for preparing copolymer conjugates and compositions described herein are provided as further embodiments of the invention and are illustrated in Scheme 1.




HA-NA ($M_n$: 880,000. N: 2220)




CTA-HA-g-PCL




HA-g-PCL (Degree of grafting: 1.4%)
(about 31 PCL grafts per HA chain)

Scheme 1. Chemical synthesis of the HA conjugate with PCL (HA-g-PCL).

The invention will now be illustrated by the following non-limiting Examples.

## Examples

Example 1.

*Materials*

Medical grade hyaluronan sodium salt (HA-Na) was purchased from Lifecore Biomedical (Chaska, MN) with an average molecular weight of 8.88 $10^6$ g/mol. Cetyltrimethyl ammonium bromide (CTAB), ε-caprolactone, 1-butanol, stannous octoate, dibutyltin dilaurate (DBTDL), toluene, and hexamethylene -1,6-diisocyanate (HDI) were all purchased from Sigma Aldrich.

*Synthesis of cetyltrimethyl ammonium HA salt (CTA-HA).*

The method was adapted from the work of Laurent Pravata, et al. (see Pravata L, et al., *New amphiphilic lactic acid oligomer-hyaluronan conjugates: synthesis and physicochemical characterization*, Biomacromolecules **2008**, *9*, 340-348). Briefly, 0.55 g of CTAB was dissolved in 7.5ml of distilled water at 40°C. This solution was added dropwise to aqueous solution of 0.6 g HA-Na (1 wt %) at 40°C. The white precipitate that formed was collected and washed three times with hot water and then dried under vacuum.

*Synthesis of poly(ε-caprolactone) (PCL) with hydroxyl as end group*

Typically, 10 g of ε-caprolactone monomer and 0.11 g of 1-butanol were added into a dry flask, and then 20ml of toluene and 0.1g of stannous octoate were injected into the reaction system. After thorough mixing, the flask was placed in an oil bath with constant temperature at 130 °C, and the reaction was continued for 24 h under magnetic stirring. Product was cooled down and poured into 200 ml of ethanol; the precipitation was collected and dried under vacuum.

*Synthesis of poly(ε-caprolactone) with isocyanate as end group (PCL-NCO)*

Synthesized hydroxyl-ended PCL (5 g ) and HDI (0.4 g) were added into a dry flask, followed by the addition of 25 ml toluene as solvent and a drop of DBTDL in toluene as catalyst. The flask was placed in an oil bath with temperature kept at 60°C under magnetic stirring. After 4 h, product was precipitated into 100 ml of diethyl ether, and the precipitate was collected and dried under vacuum.

*Synthesis of CTA-HA conjugate with PCL (named: CTA-HA-g-PCL)*

0.3 g of PCL-NCO and 0.3g of CTA-HA were added into a dry flask with 20 ml of DMSO as solvent, and a few drop of DBTDL in toluene as catalyst. The reaction was conducted in 60°C for 4 h. Following that, product was poured into 80 ml of acetone. The precipitate was collected and washed in acetone three times, then dried under vacuum.

*Synthesis of HA conjugated with PCL (named: HA-g-PCL)*

The method of remove the CTA group from CTA-HA-g-PCL is adapted from to Pravata et al. (see Pravata L, et al., *New amphiphilic lactic acid oligomer-hyaluronan conjugates: synthesis and physicochemical characterization*, Biomacromolecules **2008**, *9*, 340-348). Briefly, 0.3 g of CTA-HA-g-PCL was dissolved in 30 ml of a 2:1 (v/v) phosphate buffer (0.3 M, pH 7.4) : DMSO mixture and dialyzed (dialysis bag MW cutoff = 3500) against DMSO (1 day), ethanol (1 day), water (1 day), ethanol (1 day), and finally water (3 days). The product was recovered by freeze-drying.

*Preparation of PCL/HA-g-PCL blends*

PCL was dissolved in $CHCl_3$. A certain amount of HA-g-PCL was dispersed in $CHCl_3$ with the aid of ultrasonication. The two solutions were mixed and poured into a glass Petri dish, followed by evaporation of solvent to harvest a polymer film. A smooth PCL/HA-g-PCL blend film was prepared by further compression molding at 100°C.

*Measurements*

$^1H$ NMR spectra were recorded on a Varian Unity spectrometer (300 MHz) with $CDCl_3$ or DMSO-$d_6$ as solvent. Fourier transform infrared spectroscopy (FTIR) was conducted using a Nicolet Series II Magna-IR System 750 with OMNIC® software for data collection and analysis.

Particle sizes of the HA-g-PCL in both water and chloroform were measured by transmission electron microscopy (TEM: JEOL JEM-1210) with phosphotungstic acid as a staining agent. Dynamic laser scattering (DLS) was also used to measure the particle size of HA-g-PCL aggregates in water and chloroform. Samples were prepared as follows: 1 mg of HA-g-PCL were added to 20 g of $H_2O$ ($CHCl_3$), followed by ultrasonication (Sonic Dismembrator, model 100, Fisher Scientific) for 2 hours.

Differential Scanning Calorimetry (DSC) was carried out over a temperature range of -100°C to 100°C using a TA Q100 DS calorimeter purged with nitrogen. The heating or cooling rate was 10 °C/min. Static mechanical tensile stress-strain measurements were performed on the samples using Rheometrics Minimat equipment and a MTS Testworks 4 computer software package for automatic control of test sequences and data acquisition and analysis. Dumbbell-shaped test specimens were cut from the synthesized films and tested at room temperature with a crosshead speed of 20 mm/min according to the ASTM D882-88 standard method.

Contact angle was measured through an image analysis contact angle meter, MCA-3, from Kyowa Interface Science Co. (Japan). X-ray Photoelectron Spectra were collected from Surface Science SSX-100 equipment.

*Protein adsorption*

Fluorescein-labeled bovine serum albumin (FITC-BSA) was dissolved in PBS at 10 mg/mL. PCL and PCL/HA-*g*-PCL films (5 5 1mm, L W H) were soaked in the FITC -BSA/PBS solution and placed in a 37°C incubator. At specified time points, polymer films were removed and washed three times with DI water. Fluorescence micrographs of sample surfaces were recorded using an Olympus IX70 inverted microscope equipped with an Olympus DP72 camera and CellSens software. All sample images were acquired using the same exposure time (10 s). The amount of protein adsorbed onto the surfaces was approximated by the green fluorescence intensity of each image.

*Cell Adhesion*

Films of PCL/HA-g-PCL blends were sterilized before placing into cell media as follows: samples were placed in PBS buffer for 12 h, and dried and exposed to UV for another 3 h. Mouse fibroblast cells (NIH3T3) were seeded into 24-well plates (0.5 mL in each well, 20,000 cells/well) followed by the addition of sterilized PCL/HA-g-PCL films (5mm 5mm 1mm, L W H). The pla te was incubated at 37°C and 0.5 % $CO_2$ for 24 h. After 24 h, samples were taken out of the plate after rinsing with fresh PBS and transferred into a new 24-well plate with 0.5 ml fresh PBS in each well. Cells attached on the sample surfaces were stained with calcein AM. After 30 min of incubation with calcein AM at 37°C under $CO_2$, images of cells attached on the samples surfaces were taken with an Olympus fluorescence microscope.

**Results and discussions**

*The synthesis and characterization of HA-g-PCL*

First, PCL end-capped with hydroxyl group was synthesized through ring-opening polymerization with a yield of 90%. The number average molecular weight (Mn) of PCL synthesized was 7300 Da calculated from the proton NMR spectrum with a yield of 90%. The PCL end-capped with isocyanate was confirmed by FTIR. The peak at 2200 $cm^{-1}$ corresponding to the vibration of isocyanate functional group clearly confirmed the success of end-capping reaction.

The synthesized CTA-HA-g-PCL was characterized by $^{1}$H-NMR with DMSO-$d_6$ as solvent, shown in Figure 1. The degree of grafting of PCL was calculated from the following formula:

$$\text{Degree of grafting} = 3I_a/(2I_b\ 66)\ \ 100\%$$

where $I_a$ is the peak area of a and $I_b$ is the peak area of b. In this case, the degree of grafting of PCL is 1.4%., and correspondingly there is an average of 31 PCL chains conjugated to one HA chain. The general preparation route of HA-g-PCL is shown in Scheme 1.

*Self-assembly and particle size of HA-g-PCL in different solvents*

The particle size distribution of HA-g-PCL was measured by DLS in both water and chloroform, and the colloidal stability of these particles is shown as a function of incubation time (Figure 2). This measurement was to confirm that self assembly of the HA-PCL conjugate can happen in both solvents. The average effective diameter of the particles is a little larger in chloroform (565) than in water (487) and does not change much over 4 h (Figure 2), whereas unconjugated HA could not be dispersed and be colloidally stable. This demonstrates that the amphiphilic HA conjugate can form stable nano-aggregates in both water and nonpolar solvent such as chloroform.

The particles size measured by TEM is around 50 nm in water and 60 nm in chloroform (Figure 3). Compared with the sizes measured by DLS, they are much smaller. The possible reason is that DLS measures the particle size in solution, with particles swelled by solvent, while TEM measures the size of dry particles.

*Preparation of PCL/HA-g-PCL composites (or blends)*

Two kinds of PCL/HA-g-PCL composites consisting of bulk PCL and HA conjugates were prepared, of which the weight content of HA conjugate HA-g-PCL was 1 wt% and 3 wt%.

*Thermal and mechanical properties of the PCL/HA-g-PCL composites*

The thermal properties of pristine PCL and PCL/HA-g-PCL composites were characterized by DSC. A melting point at 55 °C for the pristine PCL was observed, and it didn't change much after incorporation of HA-g-PCL. The degree of crystallinity of the PCL composites was also calculated; it decreased slightly with the addition of HA-g-PCL, from 58% for the pure PCL to 5% for the PCL/HA-g-PCL-1% and and 50% for PCL/HA-g-PCL-3%. Detailed data are provided in Table 1.

Table 1. Thermal and mechanical properties of PCL/HA-g-PCL nanocomposite films.

| | Tensile Strength (MPa) | Modulus (MPa) | Elongation at break (%) | Melting Point (°C) | Crystallinity (%) |
|---|---|---|---|---|---|
| PCL | 18±5 | 310±25 | 950±50 | 55 | 58 |
| PCL/1% HA-g-PCL | 20±7 | 330±43 | 880±41 | 56 | 55 |
| PCL/3% HA-g-PCL | 21±4 | 350±60 | 790±54 | 56 | 50 |

Clearly, the thermal property of PCL did not change much with addition of HA-g-PCL. Both the tensile strength and modulus showed a slight increase after incorporation of HA-g-PCL, but not significant; the elongation at break decreased a little at the same time. Overall, we conclude that the incorporation of a minute amount of HA-g-PCL (1% and 3%) into bulk PCL didn't change the bulk properties of PCL.

*Surface characterization of PCL/HA-g-PCL nanocomposite films*

XPS was used to explore the surface of the nanocomposite films for evidence that the HA was located on the surface. The 1s spectra of C, O, and N are shown in Figure 4. There was no nitrogen element detected on the surface of pristine PCL; small peaks at 392 eV corresponding to nitrogen was observed for the PCL/HA-g-PCL nanocomposites, which increased with higher HA-g-PCL content. The quantified data was shown in Table 2. Because only HA contains nitrogen element, this result confirms the presence of HA on the surface of the nanocomposite films.

Table 2. Surface chemical composition of PCL/HA-g-PCL nanocomposite films and pristine PCL film.

| | C(%) | O(%) | N(%) |
|---|---|---|---|
| PCL | 85.2 | 14.8 | 0 |
| PCL/1% HA-g-PCL | 84.4 | 15.4 | 0.2 |
| PCL/3% HA-g-PCL | 82.7 | 16.8 | 0.5 |

Pristine PCL film is highly hydrophobic and HA is hydrophilic, so the measurement of water contact angle of the nanocomposite films will give us clues on whether there is any HA on the surface. For the pristine PCL, the contact angle is around 80 °C, which dropped significantly to 58 °C after incorporation of HA-g-PCL, indicating that the HA on the surface of the nanocomposite films made the surface significantly hydrophilic. To show that PCL segment of the HA-g-PCL conjugate is a reliable anchor and that HA will not be lost with time in water, we measured the time dependence of contact angle of PCL/HA-g-PCL nanocomposite films shown in Figure 5. After 4 weeks of immersion in water, the contact angles of PCL/HA-g-PCL films are stable with little change, meaning the HA persists on the surface of the nanocomposites.

Given the known antifouling property of HA (for example, see review by Marco Morra, "Engineering of biomaterials surfaces by hyaluronan" Biomacromolecules 2005, 6, 1205-1223), the invention provides a sustainable antifouling surface for the medical devices made from biodegradable polymeric materials; that is to say, the antifouling surface is renewable despite the degradation of the bulk polymer. Since the degradation of PCL film is slow in pH 7.4 buffer solution, the surfaces of PCL/HA-g-PCL nanocomposite films were polished to reveal the interior of the film at different depth. This may not be exactly the same as the degradation process, but it gives some clues as to how the materials would behave once the surface layer degraded and eroded away. The contact angle of PCL/HA-g-PCL nanocomposite films at different depth was measured and shown in Figure 6. No difference was observed comparing with the surface and interior of PCL/HA-g-PCL nanocomposite films based on the contact angle results, showing that the distribution of the HA-g-PCL nanoaggregates throughout the bulk of the nanocomposite film is uniform and that the HA-covered surface persists regardless of the loss (or erosion) of the nanocomposite film.

*Protein adsorption*

FITC-BSA was used as a model protein to test the antifouling properties of the materials. With unmodified PCL as control, PCL/HA-*g*-PCL composites showed an obviously improvement of anti-absorption of BSA protein up to 32 h, as shown in Figure 7.

*Cell culture*

The antifouling property of the PCL/HA-g-PCL nanocomposite films was tested with mouse fibroblast NIH3T3 cells. The results after the films were immersed in water for 1 week and 4 weeks were obtained using light microscopy and the quantification of cells adhering to the surfaces is shown in Figure 7. The anti-adhesion effect was very significant at both time points; there were almost no cells observed on the surfaces of PCL/HA-g-PCL composites compared with the large amount of cells attached and spread on the surfaces of pristine PCL and bare tissue culture plate (TCP) controls. The polished samples were also tested for cell adhesion also with PCL/HA-g-PCL-3% as an example. A very similar result was obtained with at most a very small amount of cells found attached on the sample surfaces at different depths of polishing (Figure 10). Therefore, we conclude that with even a minute amount of inclusion of the HA conjugate into the bulk PCL, the nanocomposite films are extremely effective in resisting nonspecific adhesion of cells. The cell-resistant property of these surfaces is due to the presence and persistence of HA and is maintained regardless of the loss of bulk nanocomposite films.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

## CLAIMS

What is claimed is:

1. A composition comprising 1) from about 0.1 to about 30 weight percent of the composition as one or more POLYMER conjugates that comprise A HYDROPHILIC POLYMER SEGMENT linked to one or more HYDROPHOBIC POLYMER SEGMENTS, and 2) at least about 70 weight percent of the composition as BULK POLYMERIC BIOMATERIAL.

2. The composition of claim 1 wherein each HYDROPHILIC POLYMER SEGMENT is selected from glycosaminoglycans, heparan sulfate, chondroitin sulfate, keratan sulfate, alginate, dextran, starch, chitosan, carboxymethyl cellulose, PEG, polyvinylpyrrolidone, poly(alkyl)acrylic acid, polysialic acid, polyglutamic acid, poly(alkyl)acrylates, poly(alkyl)acrylamides and hyaluronan.

3. The composition of claim 1 or 2 wherein each HYDROPHOBIC POLYMER SEGMENT is selected from polylactide, polyglycolide, polydioxanone, poly(trimethylene carbonate), polyanhydride, poly(ester anhydride), poly(anhydride-co-imide), poly(ortho ester), polyacetal, polyketal, tyrosine-derived polycarbonate, polyhydroxylalkanoates, polyurethane, poly(ester amide), poly(propylene fumarate), pseudo poly(amino acids), poly(amino acids), poly(alkyl cyanoacrylate), polyphosphazene, polyphosphoester, and polycaprolactone.

4. The composition of any one of claims 1-3 wherein the BULK POLYMERIC BIOMATERIAL comprises polylactide, polyglycolide, polydioxanone, poly(trimethylene carbonate), polyanhydride, poly(ester anhydride) poly(anhydride-co-imide), poly(ortho ester), polyacetal, polyketal, tyrosine-derived polycarbonate, polyhydroxylalkanoates, polyurethane, poly(ester amide), poly(propylene fumarate), Pseudo poly(amino acids), poly(amino acids), poly(alkyl cyanoacrylate), polyphosphazene, polyphosphoester and polycaprolactone.

5. The composition of claim 1 wherein each HYDROPHILIC POLYMER SEGMENT is hyaluronan.

6. The composition of claim 1 or 2 wherein each HYDROPHOBIC POLYMER SEGMENT is polycaprolactone.

7. The composition of any one of claims 1-3 wherein the BULK POLYMERIC BIOMATERIAL comprises polycaprolactone.

8. The composition of claim 1 which is a composition comprising 1) from about 0.1 to about 30 weight percent of the composition as one or more hyaluronan conjugates that comprise hyaluronan linked to one or more polycaprolactone segments, and 2) at least about 70 weight percent of the composition as bulk polycaprolactone.

9. The composition of claim 8 wherein each hyaluronan is linked to up to about 200 polycaprolactone segments.

10. The composition of claim 8 wherein each hyaluronan is linked to up to about 100 polycaprolactone segments.

11. The composition of claim 8 wherein each hyaluronan is linked to up to about 50 polycaprolactone segments.

12. The composition of claim 8 wherein each hyaluronan is linked to up to about 25 polycaprolactone segments.

13. The composition of claim 8 wherein each hyaluronan is linked to up to about 10 polycaprolactone segments.

14. The composition of claim 8 wherein each hyaluronan is linked to up to about 5 polycaprolactone segments.

15. The composition of claim 8 wherein each hyaluronan is linked to 1 polycaprolactone segments.

16. The composition of claim 8 wherein each hyaluronan is linked to at least about 100 polycaprolactone segments.

17. The composition of claim 8 wherein each hyaluronan is linked to at least about 50 polycaprolactone segments.

18. The composition of claim 8 wherein each hyaluronan is linked to at least about 25 polycaprolactone segments.

19. The composition of claim 8 wherein each hyaluronan is linked to at least about 10 polycaprolactone segments.

20. The composition of claim 8 wherein each hyaluronan is linked to at least about 5 polycaprolactone segments.

21. The composition of any one of claims 8-20 wherein the hyaluronan has an average molecular weight in the range of from about 1,000 to about 2,000,000.

22. The composition of any one of claims 8-20 wherein the hyaluronan has an average molecular weight in the range of from about 10,000 to about 1,000,000.

23. The composition of any one of claims 8-20 wherein the hyaluronan has an average molecular weight in the range of from about 700,000 to about 900,000.

24. The composition of any one of claims 8-23 wherein each polycaprolactone segment has an average molecular weight in the range of from about 500 to about 10,000.

25. The composition of any one of claims 8-23 wherein each polycaprolactone segment has an average molecular weight in the range of from about 500 to about 5,000.

26. The composition of any one of claims 8-23 wherein each polycaprolactone segment has an

average molecular weight in the range of from about 500 to about 1,000.

27. The composition of any one of claims 8-26 wherein the bulk polycaprolactone has an average molecular weight in the range of from about 2,000 to about 1,000,000.

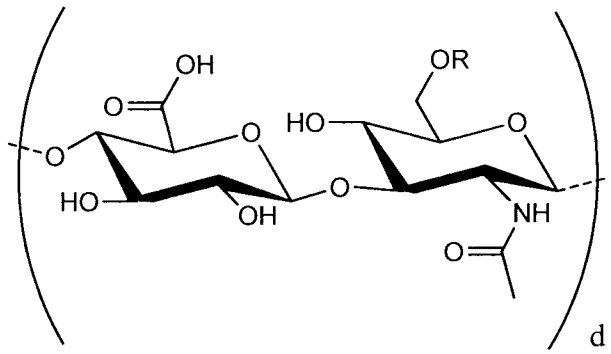
28. The composition of any one of claims 8-26 wherein the bulk polycaprolactone has an average molecular weight in the range of from about 5,000 to about 200,000.

29. The composition of any one of claims 8-26 wherein the bulk polycaprolactone has an average molecular weight in the range of from about 10,000 to about 20,000.

30. The composition of any one of claims 8-29 wherein the hyaluronan is linked to the one or more polycaprolactone segments through amide, ether, ester, carbonate, or carbamate bonds.

31. The composition of any one of claims 8-29 wherein the hyaluronan is linked to the one or more polycaprolactone segments through a carbamate bond.

32. The composition of any one of claims 8-29 wherein the Polymer conjugate has the formula:




wherein:

d is an integer selected from 3-5000;

each R is independently H, -C(=O)N(H)-PCL, or -C(=O)N(H)-Y-N(H)C(=O)O-PCL; wherein at least one R is -C(=O)N(H)-PCL, or -C(=O)N(H)-Y-N(H)C(=O)O-PCL.

each PCL is independently a polycaprolactone chain having a molecular weight of from about 500 to 10,000; and

Y is a suitable linker group.

33. The composition of claim 32 wherein each Y comprises a linear, branched, or cyclic alkyl group or a 6-10 membered monocyclic or bicyclic aromatic group, or a combination thereof.

34. The composition of claim 32 wherein each Y comprises a linear or branched alkyl group that comprises 2-18 carbon atoms.

35. The composition of claim 32 wherein each Y comprises a cyclic alkyl group that comprises about 3-18 carbon atoms.

36. The composition of claim 32 wherein each Y is derivable from an diisocyanate that comproses from 2-18 carbon atoms.

37. The composition of claim 32 wherein each Y is derivable from hexamethylene -1,6-diisocyanate.

38. The composition of any one of claims 32-37 wherein d is an integer selected from 25-2500.

39. The composition of any one of claims 32-37 wherein d is an integer selected from 1750-2250.

40. The composition of any one of claims 32-39 wherein each PCL is independently a polycaprolactone chain having a molecular weight of from about 500 to 10,000.

41. The composition of claim 1 wherein the HYDROPHILIC POLYMER SEGMENT is hyaluronan; the HYDROPHOBIC POLYMER SEGMENT is polycaprolactone; and the BULK POLYMERIC BIOMATERIAL is polycaprolactone.

42. The composition of claim 1 wherein the HYDROPHILIC POLYMER SEGMENT is hyaluronan; the HYDROPHOBIC POLYMER SEGMENT is polylactic acid; and the BULK POLYMERIC BIOMATERIAL is polylactic acid.

43. The composition of claim 1 wherein the HYDROPHILIC POLYMER SEGMENT is PEG; the HYDROPHOBIC POLYMER SEGMENT is polycaprolactone; and the BULK POLYMERIC BIOMATERIAL is polycaprolactone.

44. The composition of claim 1 wherein the HYDROPHILIC POLYMER SEGMENT is chitosan; the HYDROPHOBIC POLYMER SEGMENT is polycaprolactone; and the BULK POLYMERIC BIOMATERIAL is polycaprolactone.

45. The composition of claim 1 wherein the HYDROPHILIC POLYMER SEGMENT is alginate.

46. The composition of claim 1or 45 wherein the HYDROPHOBIC POLYMER SEGMENT is polylactic acid.

47. The composition of claim 1 or 45 wherein the HYDROPHOBIC POLYMER SEGMENT is a co-polymer of polylactic acid and poly glycolic acid.

48. The composition of claim 1 or 45 wherein the HYDROPHOBIC POLYMER SEGMENT is a co-polymer of polylactic acid and polycaprolactone.

49. A medical device comprising the composition of any one of claims 1-48.

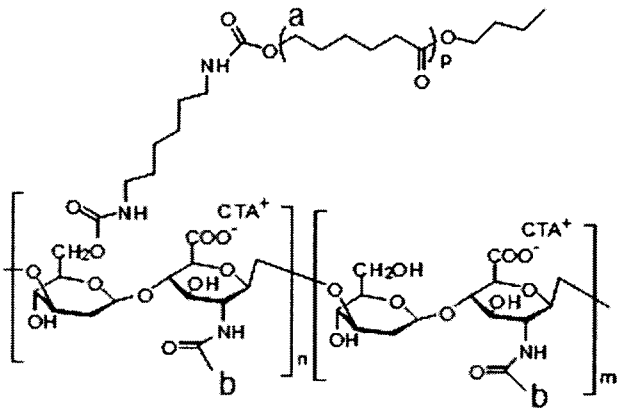
50. The medical device of claim 49 wherein the medical device comprises a coating that comprises the composition of any one of claims 1-48.

51. The medical device of claim 46 which is a scaffold for tissue engineering.

52. A microsphere or nanoparticle that comprises the composition of any one of claims 1-48.

53. A container having a surface and a coating on the surface that comprises the composition of any one of claims 1-48.

Figure 1.


a
p
NH
CTA+
COO-
CH2O
OH
NH
b
n
CH2OH
m

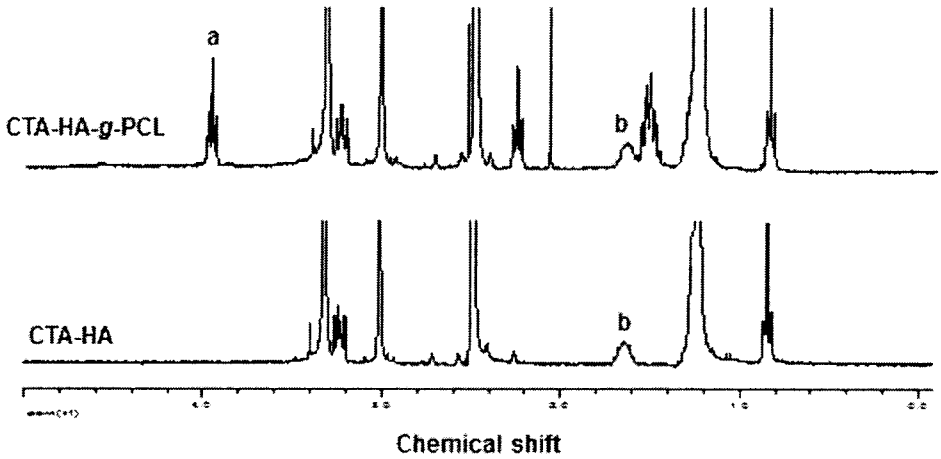

CTA-HA-g-PCL
a
b
CTA-HA
b
Chemical shift

Figure 2.

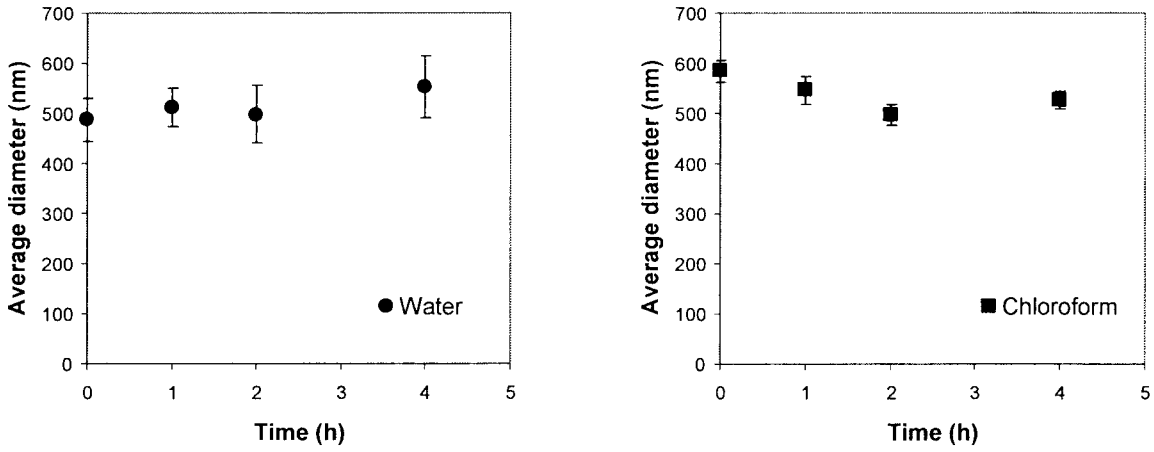
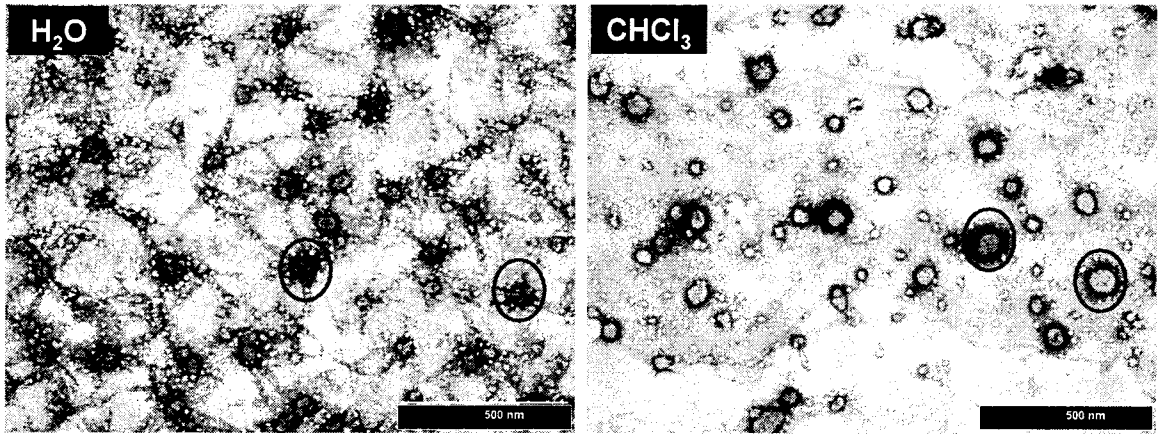

Average diameter (nm)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Time (h)
Water
Average diameter (nm)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Time (h)
Chloroform

Figure 3.




Average Size: ~50nm

Average size: ~60nm

Figure 4.

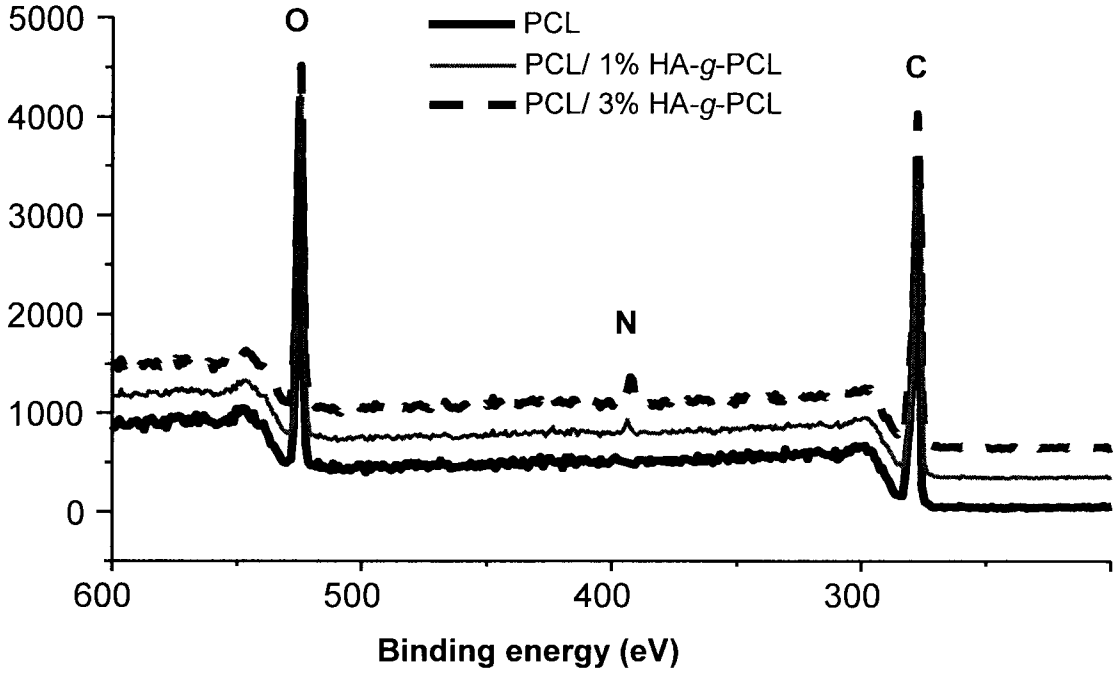

5000
4000
3000
2000
1000
0
600
500
400
300
O
N
C
PCL
PCL/ 1% HA-g-PCL
PCL/ 3% HA-g-PCL
Binding energy (eV)

Figure 5.

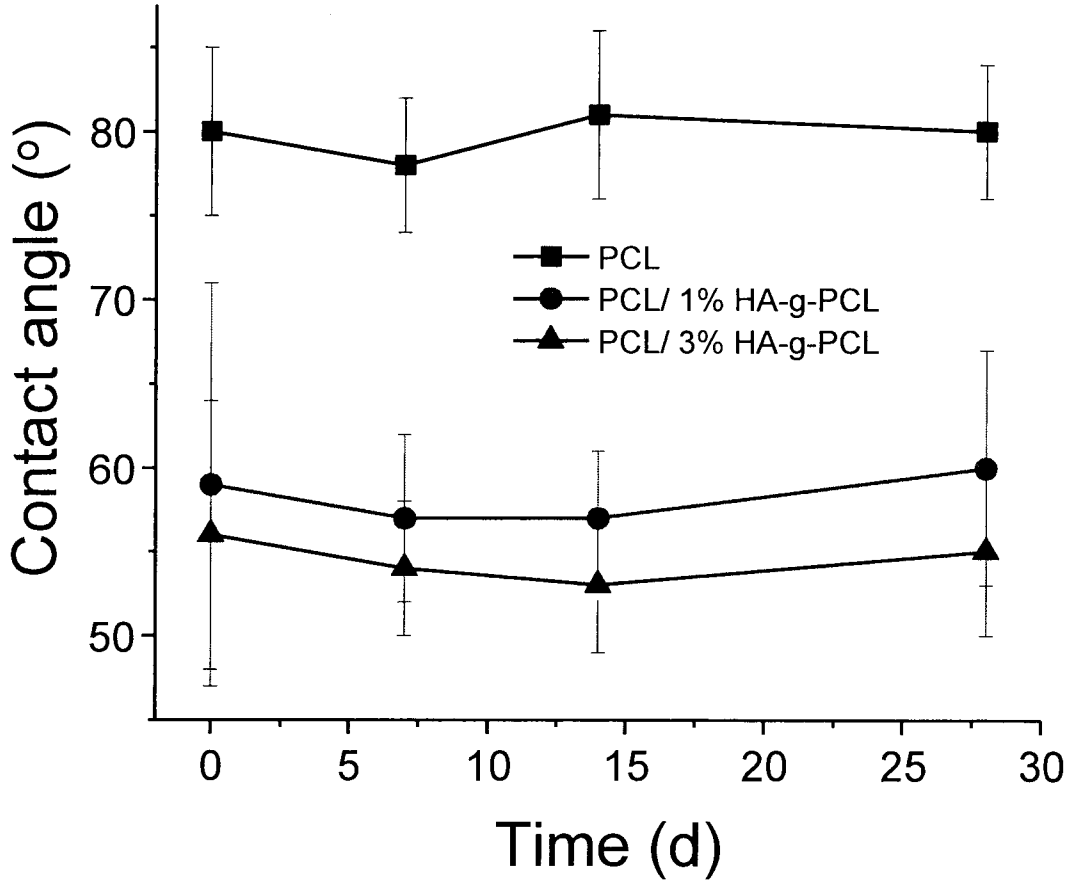

Contact angle (°)
80
70
60
50
0
5
10
15
20
25
30
Time (d)
PCL
PCL/ 1% HA-g-PCL
PCL/ 3% HA-g-PCL

Figure 6.

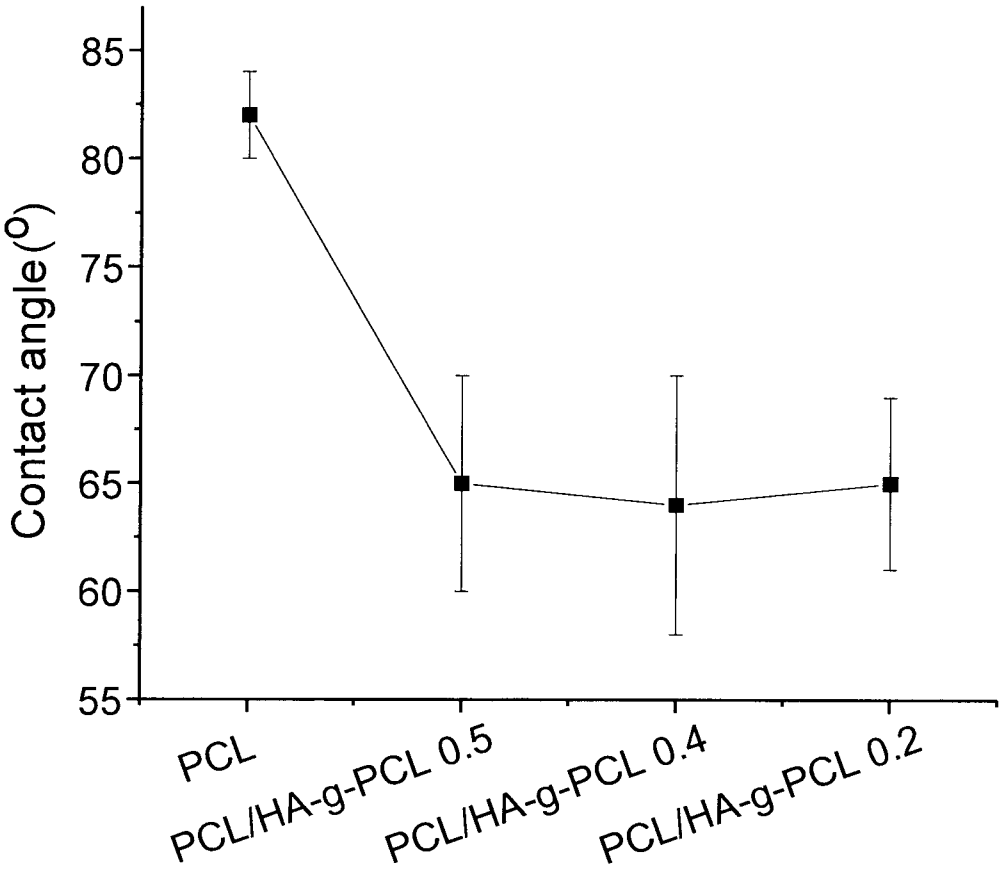
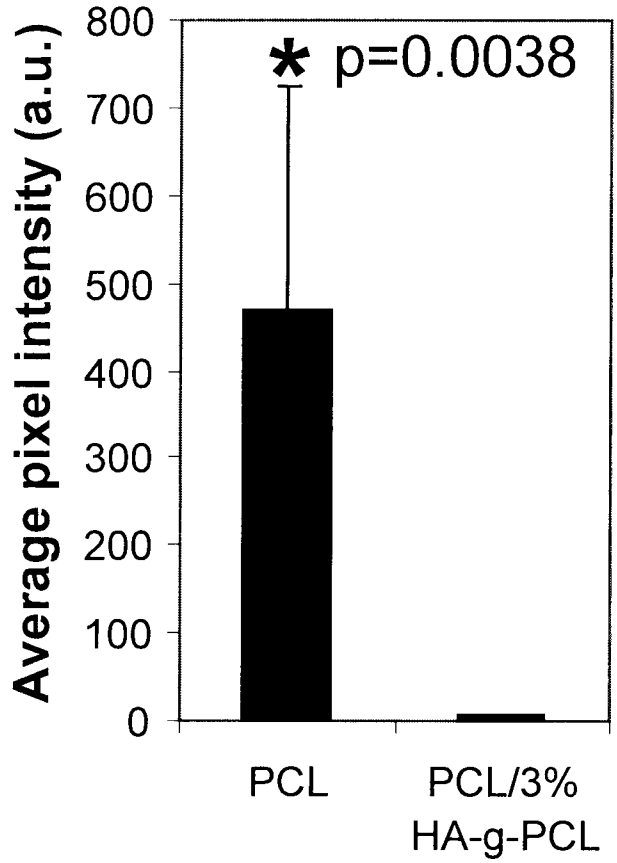

Contact angle(°)
85
80
75
70
65
60
55
PCL
PCL/HA-g-PCL 0.5
PCL/HA-g-PCL 0.4
PCL/HA-g-PCL 0.2

Figure 7.


Average pixel intensity (a.u.)
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700
600
500
400
300
200
100
0
* p=0.0038
PCL
PCL/3%
HA-g-PCL

Figure 8.

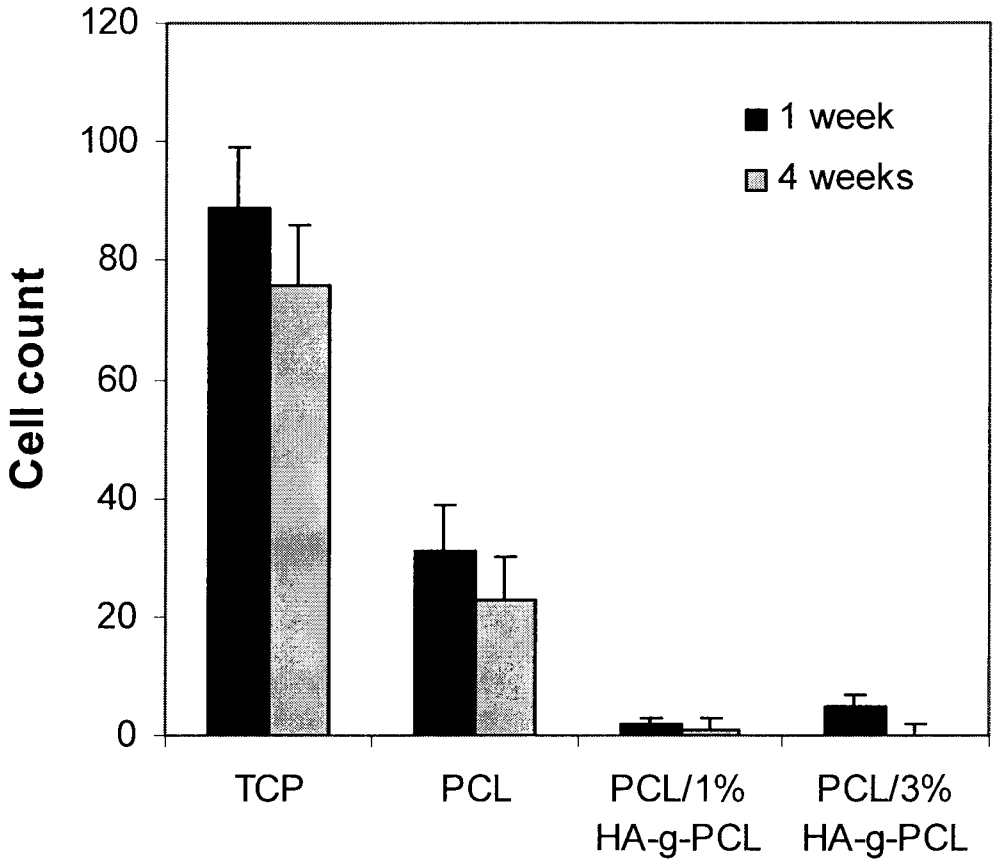
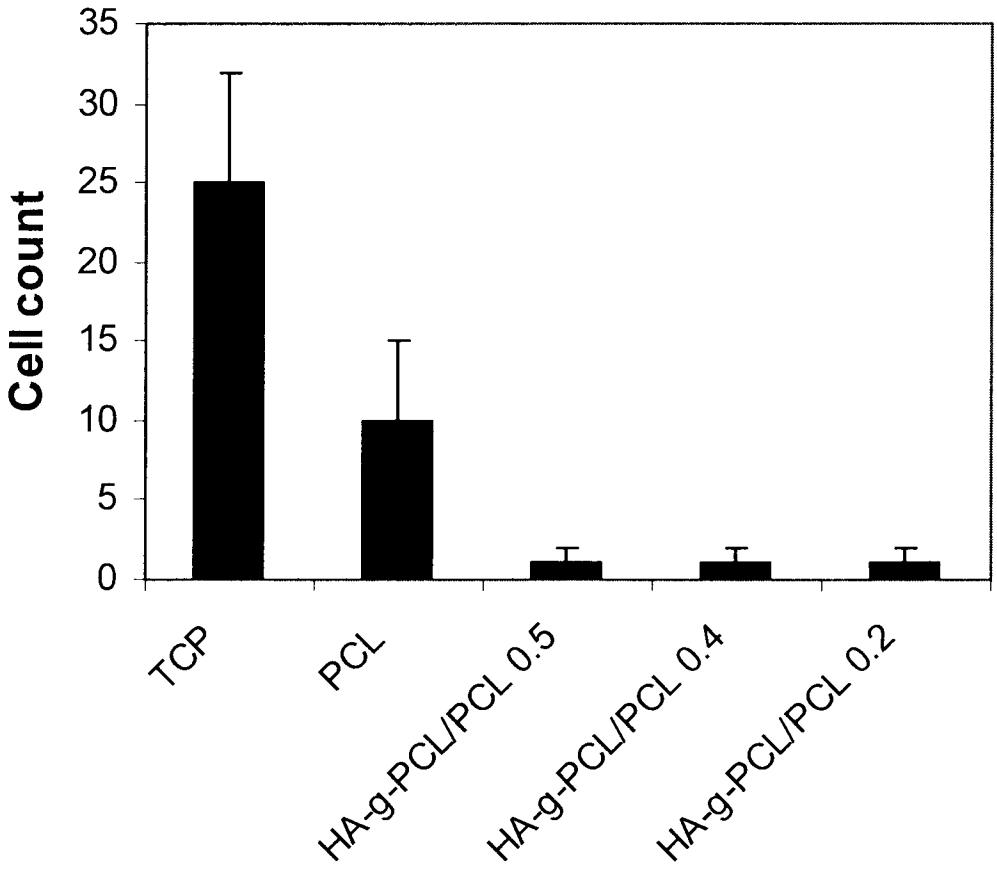

120
100
80
60
40
20
0
Cell count
1 week
4 weeks
TCP
PCL
PCL/1% HA-g-PCL
PCL/3% HA-g-PCL

Figure 9.


Cell count
35
30
25
20
15
10
5
0
TCP
PCL
HA-g-PCL/PCL 0.5
HA-g-PCL/PCL 0.4
HA-g-PCL/PCL 0.2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/30543

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 25/34, C08B 37/08, C08L 67/04 (2013.01)
USPC - 424/404, 514/54, 526/123
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8)- A01N 25/34, C08B 37/08, C08L 67/04 (2013.01);
USPC- 424/404, 514/54, 526/123

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, PatBase (USPTO, EPO, JPO, WIPO, PCT), GoogleScholar (PL, NPL), FreePatentsOnline (USPTO, EPO, JPO, WIPO, NPL); search terms: polymer, copolymer, hyaluronan, hyaluronic, hyaluronate, polycaprolactone, caprolactone, lactide, lactic, polylactic, polylactide, glycolic, polyglycolic, segment, block

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2009/0035350 A1 (STANKUS et al.) 05 February 2009 (05.02.2009), para [0009], [0010], [0014], [0017], [0025], [0102], [0105], [0109], [0112], [0114], [0134], [0157], [0158], [0164], [0167], [0196]-[0198], [0236] | 1-3, 5, 6, 8-23, 41-48 |
| Y | US 2011/0136722 A1 (KWON) 09 June 2011 (09.06.2011), para [0010]-[0104] | 1-3, 5, 6, 8-23, 41-48 |
| Y | US 2007/0098736 A1 (CLELAND et al.) 03 May 2007 (03.05.2007), para [0007]-[0071] | 1-3, 5, 6, 8-23, 41-48 |
| Y | US 2005/0244363 A1 (HOSSAINY et al.) 03 November 2005 (03.11.2005), para [0009]-[0073] | 1-3, 5, 6, 8-23, 41-48 |
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