Title: HYALURONIC ACID-BASED DRUG DELIVERY SYSTEMS

Fig. 4

Abstract: The present invention relates to novel hyaluronic acid (HA) hydrogels comprising vesicles loaded with a drug or a protein or a nucleic acid. The new HA hydrogels provide sustain release formulations that are useful for several clinical and surgical applications, including but not limited to ophthalmology (e.g. glaucoma, corneal, ocular inflammatory, vitreoretinal and medical retinal diseases) and dermatological conditions.
Hyaluronic Acid-Based Drug Delivery Systems

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority under 35 U.S.C. §119(e) to United States Provisional Application No. 61/697,296 titled “Hyaluronic Acid-Based Drug Delivery Systems” filed on 6 September 2012 the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The invention relates to the use of hyaluronic acid (HA) hydrogel to deliver drugs such as small molecule or proteins or peptides or nucleic acids at a controlled rate for several clinical and surgical applications, including but not limited to ophthalmology (e.g. glaucoma, corneal, ocular inflammatory, vitreoretinal and medical retinal diseases) and dermatological conditions. In particular, the invention relates to a HA hydrogel comprising loaded vesicles dispersed in the HA hydrogel wherein the vesicles are loaded with one or more drugs, one or more proteins, or one or more nucleic acids. The drugs or proteins or nucleic acids are released in a controlled manner. In addition, the invention relates to various ways for preparing the HA hydrogel comprising the vesicles loaded with drugs or proteins or nucleic acids. The invention relates to a method for preparing the HA hydrogel comprising loaded vesicles dispersed therein.

BACKGROUND OF THE DISCLOSURE

[003] It is well known that hyaluronic acid (HA), a naturally-occurring glycosaminoglycan (GAG), plays a key role in wound healing. HA has a range of naturally occurring molecular sizes from 100 to 10,000,000 Da. HA is implicated in water homeostasis of tissues, in the regulation of permeability of other substances by steric exclusion phenomena and in the lubrication of joints. HA also binds specifically to proteins in extracellular matrix, on the cell surface, and within the cells cytosol, thereby having a role in cartilage matrix stabilization, cell motility, growth factor action, morphogenesis and embryonic development and inflammation. Unmodified HA has many important application in drug delivery and surgery. For example, it is used as an adjuvant for ophthalmic drug delivery. In addition, HA has
important application in the fields of visco-surgery, visco-supplementation and wound healing. HA is also a building-block for biocompatible and biodegradable polymers with application in drug delivery, tissue engineering and visco-supplementation.

[004] Hydrogels are formed by crosslinked polymers and are able to absorb high quantity of water without being dissolved. HA hydrogels are physically or covalently cross-linked HA gel. HA molecules are generally functionalized to allow reaction with a cross linker. Crosslinked HA hydrogels for example have been prepared by crosslinking with molecules such as di-epoxy-butane, ethylene-glycol di-glycidyl-ether (EGDGE) or poly-glycol diglycidyl-ether (PEGDE).

[005] HA hydrogels have been used for several application including drug delivery applications. They are able to provide sustained, local delivery of a variety of therapeutic agent. Use of HA as a scaffold material in hydrogel has been pursued due to the biocompatibility, low toxicity, lack of immune response and biodegradability of HA hydrogel.

[006] Although HA hydrogels have been studied for drug delivery applications, the delivery rates are difficult to control. If a hydrophilic drug is incorporated into the hydrogel, the incorporation is easy (large amounts can be loaded), but release is also rapid. On the other hand, it is difficult to get large amounts of hydrophobic drugs into such hydrogels, for solubility reasons. Any un-dissolved drug will migrate to the surface of the hydrogel and release in a burst (within a day or two).

[007] There is therefore the need to provide further and improved drug/protein/nucleic acid sustain delivery formulations that allow an efficient and localized controlled release of hydrophobic and hydrophilic drugs, proteins or nucleic acid, avoiding the above mentioned drawbacks.

SUMMARY OF THE INVENTION

[008] The present invention addresses this need by providing new and improved controlled release drugs or proteins or nucleic acids formulation and methods to prepare the same.

[009] In a first aspect, the present invention is directed to a hyaluronic acid (HA) hydrogel comprising loaded vesicles dispersed therein wherein the vesicles are loaded with drugs or proteins and peptides or nucleic acids ("loaded vesicles").

[010] The loaded vesicles are selected from micelles, liposomes and/or particles such as microparticles or nanoparticles. Preferably, the micelles are made of amphipilic self-
assembling molecules. Amphiphilic self-assembling molecules are preferably selected from polymers including block-copolymers and surfactants. Preferably, the particles are selected from chitosan nanoparticles or microparticles and poly(lactic-co-glycolic acid) (PLGA) nanoparticles or microparticles. Preferably, the amount of loaded vesicles is the 1% to the 40% by weight of the whole HA hydrogel system (HA hydrogel including the loaded vesicles). More preferably, 1-20% by weight of the whole HA hydrogel system.

[0011] The drug is preferably selected from a hydrophobic or hydrophilic drug, more preferably it is selected from an antibiotic drug such as ciprofloxacin, a chemio-therapeutic drug such as paclitaxel or doxorubicin or 5-fluorouracil, a drug for the treatment of glaucoma or ocular hypertension such as latanoprost and an anti-scaring drug as 5-fluorouracil.

[0012] Proteins are preferably selected from the group of monoclonal antibodies, such as bevacizumab (Avastin) and ranibizumab (Lucentis), or similar therapeutic proteins.

[0013] Peptides are preferably natriuretic peptides, including C-type natriuretic peptide (C-NP), A-type natriuretic peptide (A-NP), chimeric natriuretic peptide (CD-NP), mutant atrial natriuretic peptide (M-ANP).

[0014] Nucleic acids are preferably selected from the group of RNA, siRNA, DNA, cDNA and plasmid DNA.

[0015] The HA hydrogel according to the present invention is preferably an HA hydrogel which is a covalently crosslinked HA hydrogel. The HA moieties (backbone) that form the hydrogel are functionalized with a functional group to be linked to a crosslinker. The crosslinker is any molecule suitable to act as a crosslinker in HA hydrogel preparation. The crosslinking is performed in the presence of loaded vesicles to provide the HA hydrogel of the invention. The functional group is any molecule or moiety or group that is attached, preferably covalently attached to the HA and is able to react with a crosslinker molecule to crosslink the functionalized HA and for the HA hydrogel. Alternatively, the functional group is any molecule or moiety or group that is attached, preferably covalently attached to HA and is able itself to act as a crosslinker to form HA hydrogel. Preferably, HA is functionalized with methacrylic acid or anhydride to give methacrylate-HA (HA-MA), HA is functionalized with adipic acid dihydrazide (ADH) to give HA-ADH, or HA is functionalized with lactic acid to give MeLAHA or with caprolactone to give MeCLHA. Preferably, the crosslinker molecule is polyethylene glycol diglycidyl ether (PEGDE). The crosslinking of HA-MA with PEGDE provides, in the presence of loaded vesicles, an HA hydrogel comprising the loaded vesicles dispersed therein according to the invention. Alternatively, the crosslinking of HA-ADH with
PEGDE provides, in the presence of loaded vesicles, an HA hydrogel comprising loaded vesicles dispersed therein according to the invention.

[0016] Alternatively, in the functionalized HA the functionalizing group is the crosslinker. Preferably, the functionalized HA is selected from HA-MA and HA-ADH. The functional group MA may act as a crosslinker to form HA-MA hydrogel. The functional group ADH may act as a crosslinker to form HA-ADH hydrogel.

[0017] Preferably, the HA hydrogel is selected from an HA-MA hydrogel, HA-ADH hydrogel, HA-ADH crosslinked with PEGDE-hydrogel, HA-MA crosslinked with PEGDE-hydrogel.

[0018] Preferably, the molar ratio of effectively functionalized HA to crosslinker (wherein the crosslinker is not the functional group itself) is in the range of 1:20 to 1:1, preferably 1:10, to 1:2, more preferably 1:5 to 1:2. Hence, preferably the amount of crosslinker added is from 2 to 10 times the moles of HA effectively functionalized. For example, for a system such as HA-ADH system a crosslinker (which is not ADH itself) is used. For example with HA-ADH the crosslinker PEGDE is used. Generally, PEGDE is used in a ratio of 2 to 10 times the moles of HA-ADH effectively functionalized. For example, if the degree of functionalization of HA-ADH is of 50% (i.e. 50% of the whole HA molecules have ADH) then 2 to 10 times this amount of PEGDE can be added.

[0019] In a second aspect, the present invention provides methods for preparing the HA hydrogel comprising the loaded vesicles dispersed therein according to the present invention.

[0020] The method comprises a) providing a functionalized HA with a functional group/moiety suitable to be linked to a crosslinker and b) crosslinking the functionalized HA moiety optionally in the presence of a crosslinker, wherein the crosslinking occurs in the presence of loaded vesicles and wherein when the crosslinker is not present the functional group/moiety on the HA acts as a crosslinker. Preferably, the functionalized HA is selected from HA-MA or HA-ADH. Preferably, the crosslinker is selected from PEGDE. Preferably, when the functional group act as crosslinker, the functional group is MA.

[0021] According to the method of the invention, the loaded vesicles are mixed with the functionalized HA to form a mixture. The crosslinker is then added to said mixture and let crosslink to provide the HA hydrogel comprising the loaded vesicles dispersed therein. Alternatively, the loaded vesicles are mixed with the crosslinker and the functionalized HA is added subsequently.

[0022] In the embodiment wherein the crosslinker is not required, because the functional group/moiety of the HA functionalized moiety acts as crosslinker the mixture comprising the
HA functionalized and the loaded vesicles is let crosslink. The functionalized HA can be added as freeze-dried functionalized HA to the solution comprising the loaded vesicles. The concentration of the functionalized HA in the solution comprising the loaded vesicles is preferably of 1% to 20% (w/v), more preferably 2% to 4% (w/v).

[0023] The crosslinking is a chemical reaction or a radical polymerization (crosslinking) preferably a photo-crosslinking. Preferably the photo-crosslinking is a UV photo-crosslinking that optionally occurs in the presence of a photo-initiator. An initiator such as Irgacure 2959 is used to start the crosslinking. The amount of initiator is of 3% to 15% by weight of the functionalized HA preferably 10% by weight of the functionalized HA. For example, the amount of initiator is of 3% to 15% by weight of HA-MA, preferably 10% by weight of HA-MA when HA-MA is used to prepare HA-MA hydrogel.

[0024] In a third aspect, the present invention is directed to an HA hydrogel comprising loaded vesicles dispersed therein obtainable with any of the methods of the present invention.

[0025] In a fourth aspect, the present invention is directed to an HA hydrogel comprising loaded vesicles dispersed therein as defined above for use as a medicament.

[0026] In a fifth aspect the present invention is directed to a pharmaceutical formulation comprising the HA hydrogel comprising loaded vesicles dispersed therein as defined above. The pharmaceutical formulation is for oral, topical, intravenous, subcutaneous, intraocular or intramuscular administration.

**DESCRIPTION OF THE DRAWINGS**

[0027] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0028] Fig. 1 shows a scheme of formation of micelles of PEG/PPG/PEG block copolymers and the load of a hydrophobic drug.

[0029] Fig. 2 shows a scheme of formation of micelles of PEG/PLA block copolymers and the load of a hydrophobic drug.

[0030] Fig. 3a is a picture of the phospholipid structure, the building block of liposomes. Fig. 3b shows the section of a liposome. It shows that a hydrophilic drug can be loaded in the liposome core and a hydrophobic drug can reside in the bilayer of the liposome.

[0031] Fig. 4 is a plot illustrating the % cumulative latanoprost (Ltp) release in HA hydrogel with and without Ltp loaded in Egg liposomes.
[0032] Fig. 5 is a scheme of the process for preparing HA hydrogel with dispersed loaded vesicles using photocrosslinkable HA-MA.

[0033] Fig. 6 shows the % cumulative drug release over 15 days of a HA-MA hydrogel at 4% wherein the Ltp is directly loaded (no vesicles) vs. HA-MA hydrogel 4% according to the present invention i.e. with Ltp loaded liposomes.

[0034] Fig. 7 shows a SEM image of PLGA microparticles loaded with 5-FU showing the particle size and the sphericity.

[0035] Fig. 8 shows the release profile of 5-FU from PLGA microparticles of batches 10, 11 and 6 in HA hydrogel.

[0036] Fig. 9 shows the comparison of % cumulative release of 1) HA-MA+5FU sol, 2) HA-MA+5-FU gel and 3) HA-MA+ PLGA/5 FU batch 6 microparticles.

**DETAILED DESCRIPTION**

[0037] The present inventors have found that the incorporation of vesicles loaded with hydrophobic and/or hydrophilic drug or proteins or nucleic acids into HA hydrogel provides formulations that have a control release profile and do not present the drawback of the direct incorporation of hydrophilic/hydrophobic drug or protein or nucleic acid into an hydrogel (respectively: rapid release and release in a burst within a day or two).

[0038] Advantageously, the present inventors have discovered that a HA hydrogel comprising loaded vesicles dispersed therein provides a system with an improved controlled release profile when compared to a system wherein the drug or the protein or the nucleic acid is directly loaded in the HA hydrogel and when compared to a system wherein the drug or the protein or the nucleic acid is loaded in the vesicles and released from the vesicle alone (no HA hydrogel is present).

[0039] Hence, in an aspect the present invention is directed to HA hydrogel comprising loaded vesicles dispersed therein wherein the vesicles are loaded with one or more drugs, one or more proteins or one or more nucleic acids.

[0040] “Vesicles” are as defined herein small sack/bubble able to contain a drug/protein/peptide/nucleic acid and to release it at the desired physiological conditions. Preferably, vesicles according to the present invention are selected from liposomes or micelles or particles selected from nanoparticles and microparticles. The vesicles are prepared such that they are loaded with hydrophobic or hydrophilic drug, protein, peptide or nucleic acid. Preferably, the loaded vesicles are prepared before their incorporation into the HA hydrogel.
"Micelles" are made of self-assembling amphipilic molecules. Self-assembling amphipilic molecules spontaneously exist in a unique structure (micelles) beyond a certain concentration. This concentration is known as the "critical micelle concentration" (CMC). Self-assembling amphipilic molecules can be for example polymers and surfactants. Examples include surfactants such as C_{10}-C_{22} alkyl sulphate, C_{10}-C_{22} alkyl betaine, C_{10}-C_{22} alkyl trimethyl ammonium salts and C_{10}-C_{22} alkyl glucosides and polymers, preferably block copolymers such as copolymers of PLGA (poly(lactide-co-glycolide) and PEG (polyethylene glycol), copolymers of polycaprolactone (PCL) and PEG, copolymers of polyethylene glycol-polyactic acid (PEG/PLA), copolymers of polyethylene glycol (PEG) and polypropylene glycol (PPG) (also known as polyethylene and polypropylene oxides). Copolymers of polyethylene glycol (PEG) and polypropylene glycol (PPG) are generally sold under the brand names of Pluronic®.

As said, a defined property of self-assembling amphipilic molecules such as Pluronic® is the ability of the molecules called "unimers" to self-assemble into micelles in aqueous solution. These "unimers" form a molecular dispersion in water at the molecule concentration below the CMC. At a concentration above the CMC, the unimers aggregate, forming micelles through a process called "micellization". For example with block copolymer, the driving force of the micellization is the hydrophobic interaction of the hydrophobic block (such as the PO block in Pluronic®). The hydrophobic block self-assembles into the inner core of the micelles covered by the hydrophilic corona of the hydrophilic block (such as the EO block in Pluronic®). Pluronic® micelles are for example pictured as spheres composed by a PO core and an EO corona (Fig. 1). Additional micelle morphology including lamelles or rods can be formed. Generally, the micelles in dependence of the block polymer type used have different average hydrodynamic diameter. For example in dependence of the Pluronic® type used, the micelles can have an average hydrodynamic diameter ranging from 20 to about 80 nm. The number of block polymer forming one micelle is referred as the "aggregation number".

The center of the micelles is hydrophobic. If the hydrophobic drug or molecule is mixed into such polymers, the drug will reside in the hydrophobic core, as shown in the Fig. 1. The process of transfer of hydrophobic drug into the hydrophobic core (such as PO core) of the micellar solution is referred as "solubilization". The released of the drug occurs then by drug delivery mechanism.

Polyethylene glycol-polyactic acid (PEG/PLA) copolymers are another example of copolymer forming micelles in an aqueous solution in a concentration above CMC. (Fig.
2). The present inventors have found that PEG/PLA copolymers are able to form micelles that incorporate hydrophobic drugs such as paclitaxel. The present inventor have found that to extract a greater level of release control, as well as overall drug loading, a significant amount of such micelles can be incorporated into HA hydrogels (~10-20% by weight), thus extending duration of drug release from a few days to a few months.

[0045] In an aspect of the invention, the micelles are made of self-assembling amphiphilic molecules. Self-assembling amphiphilic molecules can be selected from surfactants such as C₁₀₋C₂₂ alkyl sulphate, C₁₀₋C₂₂ alkyl betaine, C₁₀₋C₂₂ alkyl trimethyl ammonium salts and C₁₀₋C₂₂ alkyl glucosides or polymer, such as block copolymers. Preferably, the self-assembling amphiphilic molecules according to the present invention are block copolymers. Preferably self-assembling amphiphilic molecules are selected from copolymers of polyethylene glycol (PEG) and polypropylene glycol (PPG) such as Pluronic® and PEG/PLA; more preferably, Pluronic® is Pluronic® 127.

[0046] “Liposomes” are vesicles composed by a lipid bilayer. Liposomes are often composed of phosphatidylcholine-enriched phospholipids. (Fig. 3). Both hydrophilic and hydrophobic drugs can be loaded into a liposome. Hydrophobic drugs are incorporated into the bilayer of the lipid vesicles, while hydrophilic drug are loaded into the “core” of the liposome (Fig. 3). For example, the present inventors have incorporated in a liposomal core the antibiotic ciprofloxacin a hydrophilic drug with a good loading and control release. A further release control has been achieved when such loaded liposomes were mixed with HA hydrogel precursors and crosslinked. Additionally, the present inventors have incorporated in the liposomal bilayer hydrophilic drug such as latanoprost. The loaded liposomes were further incorporated in the HA hydrogel precursors prior to the HA hydrogel crosslinking. The hydrogel precursors were let forming the HA hydrogel. The incorporation of liposomes into HA hydrogel further increase the control release of the drug.

[0047] In another embodiment, both hydrophobic and hydrophilic drugs can be loaded into the same liposome. The hydrophobic drug is in the bilayer part of the liposome by passive loading and the hydrophilic drug is in the core of the liposome by active loading.

[0048] Liposomes are preferably selected from EPC (or EggPC) liposomes, 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) based liposome and 1,2-dimyristoyl(d54)-sn-glycero-3-phosphocholine (DMPC) based liposome.

[0049] Particles according to the present invention are non-self-assembling particles in the size (diameter) range of 5nm to 50µm. Particles are defined as nanoparticles or microparticles
in dependence of their size (diameter) range. Generally, nanoparticles have a size range from 5nm to 250nm. Microparticles have a size range >250nm to 50μm. Nanoparticles and microparticles are prepared by forming a complex between a drug, preferably a hydrophilic drug, with anionic or cationic species and then forming the nanoparticles. Anionic species used to prepare nanoparticles and microparticles are for example poly styrene sulfonate or poly-acrylic acid, cationic species are for example chitosan.

[0050] By way of a non-limiting example: drugs such 5-Fluorouracil (5-FU)

![Chemical structure of 5-Fluorouracil](image)

will dissolve in NH₄OH and exists as negative ions at high pH and as positive ions at lower pH. Therefore, such drugs may be complexed with anionic species (e.g., poly styrene sulfonate or poly-acrylic acid) at low pH to form complexes that can be more efficiently loaded into hydrogels compared to pure drug. At a higher pH, these drugs can be complexed with cations such as chitosan.

[0051] Hence, for hydrophilic drug, the present invention also comprises the preparation of complexed drug, for example with chitosan. The complexation using chitosan is followed by nano- or microparticles formation. The formed nanoparticles or microparticles are mixed into the HA hydrogel precursors which are crosslinked. The release control is optimized. Drug types that can be loaded in chitosan nanoparticles or microparticles include 5-Fluorouracil (5-FU) used as a chemotherapeutic and anti-scarring agent. Nucleic acids such as siRNA; plasmid DNA can be loaded in nanoparticles or microparticles.

[0052] Nanoparticles or microparticles according to the invention are also poly(lactic-glycolic acid) (PLGA)-nanoparticles (NPs) or PLGA-microparticles (PLGA-MPs). PLGA-NPs or PLGA-MPs are loaded with a drug or protein or polypeptide or nucleic acid. The loaded PLGA-NPs or PLGA-MPs are then embedded in the HA hydrogel via polymerization of the HA hydrogel precursors. PLGA-NPs or PLGA-MPs may be prepared by double emulsion solvent evaporation. Double emulsion solvent evaporation method is disclosed for

[0053] Nanoparticles according to the present invention have a diameter ranging from 5nm to 250 nm, preferable 40 to 100nm. Microparticles according to the present invention have a diameter ranging from > 250nm to 50µm.

[0054] HA is an anionic, non-sulfated glycosaminoglycan. “HA hydrogel” as defined herein is a crosslinked HA networks. The crosslinking is a preferably a covalent crosslinking i.e. HA is covalently bonded to crosslinker molecules so as to create a polymer network. HA contains a number of hydrophilic groups that have affinity for water. HA hydrogels are prevented from dissolving due to the bonds formed between the HA chains via the crosslinkers. Hence, HA or HA chemically modified can form an HA hydrogels via polymerization (cross-linking).

[0055] HA molecule has carboxyl groups, acetamido groups and OH group that can be functionalized with a chemical group suitable for crosslinking. According to the present invention, functionalized HA is an “HA hydrogel precursor”. The crosslinker is the other “HA hydrogel precursor”. When the functionalized HA is crosslinked optionally in the presence of crosslinker molecules it forms a HA hydrogel. Examples of functionalized HA are adipic acid dihydrazide HA (HA-ADH), methacrylated HA (HA-MA), thiolated HA (HA-SH). HA-ADH can be synthesized and used for the preparation of HA-MA by reaction with methacrylic anhydride. HA-ADH can be used for the preparation of thiolated HA (HA-SH) by reaction with with Traut’s reagent (iminothiolane). Other functionalized HA are MeLAHA and MeCLHA. Preferred functionalized HA are HA-MA and HA-ADH.

[0056] “Crosslinkers” or crosslinking reagents/agents are molecules that contain two or more reactive ends capable or chemically attaching to specific functional groups of HA or functionalized HA. Non limitative examples of crosslinkers that can be used for preparing the HA hydrogel according to the present invention are: butylene glycol diglycidyl ether (BDG), butanediol diglycidyl ether (BDDGE) or poly(ethylene glycol) diglycidyl ether (PEGDE). Preferably, the crosslinker is PEGDE, more preferably is PEGDE of formula:

\[
\begin{align*}
\text{O} & \quad \text{[O]} \quad \text{O} \\
\text{O} & \quad \text{[O]} \quad \text{O} \\
\text{n} & \\
\end{align*}
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wherein \(n=1\) to 50, preferably \(n=3-6\).
[0057] The functionalized HA may be crosslinked in the presence of a crosslinker. As an illustrative example of this embodiment of the invention, HA-ADH and PEGDE or HA-MA and PEGDE in the presence of loaded vesicles may be crosslinked to give the HA hydrogel of the invention. The functionalized HA may be crosslinked in the presence of loaded vesicles and in the absence of a crosslinker as the functional group acts as crosslinker. As illustrative example of this embodiment of the invention, in the presence of loaded vesicles, HA-MA may be crosslinked, preferably is photo-crosslinked, to give a HA hydrogel (HA-MA hydrogel) having dispersed therein the loaded vesicles. Alternative as an illustrative example of this embodiment of the invention, in the presence of loaded vesicles, HA-ADH may be chemically crosslinked to give a HA hydrogel (HA-ADH hydrogel) having dispersed therein the loaded vesicles.

[0058] The amount of the loaded vesicles in the HA hydrogel may be in the range from 1% to 60% by weight of the whole HA hydrogel system (HA hydrogel plus loaded vesicles). Preferably, the amount of loaded vesicles is from 2% to 30% by weight of the whole HA hydrogel system, more preferably from 4% to 20% by weight of the whole HA hydrogel system.

[0059] The percentage/degree of functionalization on the HA backbone may depend on the functionalizing group. Typically, the percentage of functionalization is of 5 to 80% (i.e., 5 to 80 of the HA molecules have a functionalization/bear a functional group). Preferably the degree of functionalization of HA-ADH is of 20 to 80%, preferably the degree of functionalization of HA-MA is of 5% to 20%, more preferably 10%; preferably the degree of functionalization of HA-ADH is 45% to 50%.

[0060] Hence, the languages “functionalized HA” or “HA-ADH” or “HA-MA” etc. according to the present invention indicate a composition of HA comprising both functionalized and non-functionalized HA. The degree of functionalization is indicated by the percentage as disclosed above. The language “HA effectively functionalized” refers to the part of the “functionalized HA” which effectively bear the functional group. As an explanatory non limiting example, HA-MA with a 50% degree of functionalization means a composition wherein half molecules of HA are functionalized with MA and half molecules of HA are not functionalized. 50% of the HA molecules are effectively functionalized with MA. As an additional explanatory non limiting example, HA-MA with a 60% degree of functionalization means a composition wherein 60% of molecules of HA are functionalized with MA and 40%
of molecules of HA are not functionalized i.e. are HA. 60% of the HA molecules are “effectively” functionalized with MA.

[0061] In a second aspect, the present invention is directed to a method for the preparation of HA hydrogel having loaded vesicles dispersed therein. The method comprises:

a) providing functionalized HA with a functional moiety wherein the functional moiety is for crosslinking;

b) crosslinking the functionalized HA optionally in the presence of a crosslinker;

wherein

the crosslinking occurs in the presence of loaded vesicles; and

wherein

when the crosslinker is not present the functional moiety acts as a crosslinker.

[0062] The functionalized HA is a HA hydrogel precursor. In the method of the invention the functionalized HA may be any functionalized HA suitable for the preparation of HA hydrogel. Functionalized HA are for example HA-MA, HA-ADH, HA-SH, MeLAHA and MeCLHA. Preferably, functionalized HA are selected from HA-ADH and HA-MA.

[0063] A crosslinker molecule is a HA hydrogel precursor. “Crosslinkers” or “crosslinking reagents” are molecules that contain two or more reactive ends capable or chemically attaching to specific functional groups of HA or functionalized HA: non limitative examples of crosslinkers that can be used for preparing the HA hydrogel according to the present invention are: butylene glycol diglycidyl ether (BDG), butanediol diglycidyl ether (BDDGE) or poly(ethylene glycol) diglycidyl ether (PEGDE). Preferably the crosslinker is PEGDE, more preferably is PEGDE of formula:
wherein $n=50$ to 400 wherein $n=1$ to 50, preferably $n=3-6$.

[0064] Vesicles, including micelles, liposomes and particles selected from nanoparticles and microparticles as disclosed above and loaded with a drug or protein or peptide or nucleic acids are used in the method of the invention to prepare the HA hydrogel comprising loaded vesicles dispersed therein.

[0065] The crosslinking reaction can be a chemical crosslinking for example via condensation or addition reaction or a radical polymerization wherein the polymerization (crosslinking) involves the formation of radical through some initiator source such as light, temperature or redox-reaction. Preferably, the radical polymerization (crosslinking) occurs via photo-polymerization (photo-crosslinking). Typically, UV radiation is used for photo-crosslinking. Typically, a photo initiator, such as Irgacure 2959, is present in the solution containing the HA hydrogel precursors and the loaded vesicles when the HA hydrogel is formed via photo-crosslinking.

[0066] The conversion of an OH group to aldehyde of HA using adipic acid dihydrazide (ADH) followed by reaction with ethylene dicarbodiimide (EDC) to yield a reactive functional group in the HA backbone (HA-ADH) can be used in the method of the invention. The hydrogel is then form by crosslinking in the presence of a crosslinker such as PEDGE according to the following scheme:
HA-ADH HYDROGEL
SYNTHESIS ROUTE

HYALURONIC ACID + ADIPIC ACID DIHYDRAZIDE
EDC (CARBOXYL ACTIVATING AGENT)

HA-ADH

HA-ADH + POLYETHYLENE GLYCOL DIGLYCIDYL ETHER
CROSSLINKED HYDROGEL

EPOXY AMINE REACTION
[0067] The gel precursors (in this case the HA-ADH and the PEGDE) can be mixed with loaded vesicles such loaded liposomes/loaded micelles/loaded nanoparticles as disclosed above and then crosslinked. The crosslinking process is performed via chemical- or photo crosslinking. The cross-linking process is performed at any suitable temperature. Preferably, it is performed at ambient temperature. Preferably, it is performed at a temperature ranging from 20 °C to 25 °C. The advantage of this feature is that the proteins/nucleic loaded in the vesicles are not denatured.

[0068] Vesicles according to the invention can be liposomes, micelles preferably of self-assembling amphipilic molecules or particles selected from nanoparticles and microparticles as disclosed above.

[0069] The loaded vesicles may be provided in a solution or in a suspension. The solvent may be selected in dependence of the vesicles. Solvent may be for example water or ethanol. Preferably, the solvent is water.

[0070] The HA used according to the present invention is a HA in a form suitable to form a hydrogel. It can be named as HA hydrogel precursor. Hence, the HA hydrogel precursor may be a functionalized HA. In an aspect, any HA functionalized for the purpose to be crosslinked can be used in the process of the present invention. Preferably, HA-ADH, HA-MA, HA-SH are used in the method of the invention. The cross-linkable functionalized HA can be added to the solution/suspension of the loaded vesicles as freeze dried functionalized HA or as a solution.

[0071] Crosslinkers suitable for the preparation of a HA hydrogel may be added to the solution/suspension containing the loaded vesicles and the functionalized HA. The method of the invention envisages the use of any crosslinkers suitable for the preparation of HA hydrogel. Preferred cross-linkers are (BDG), butanediol diglycidyl ether (BDDGE) or poly(ethylene glycol) diglycidyl ether (PEDGE). More preferred cross-linker id PEGDE.

[0072] Once the solution/suspension comprising the loaded vesicles is mixed with the HA hydrogel precursors, the crosslinking reaction is induced. The crosslinking reaction can be a chemical crosslinking reaction such as a condensation or addition reaction or a radical polymerization (crosslinking). Radical polymerization may be initiated by light (photo-crosslinking), temperature or redox-reaction, preferably the radical polymerization is a photo crosslinking. Preferably, the photo crosslinking is a UV crosslinking reaction.
[0073] Typically, the preparation of HA hydrogel in the presence of PEDGE and HA-ADH is a chemical crosslinking reaction. Typically, the preparation of HA hydrogel in the presence HA-MA and no additional crosslinker is a photo-crosslinking reaction. The photo-crosslinking may require the presence of a photo initiator. The photo initiator may be mixed to the HA-hydrogel precursor before adding it the solution/suspension of the loaded vesicles. Alternatively, the HA hydrogel precursors and the photo-initiator may be added sequentially or simultaneously to the solution comprising the loaded vesicles. The photo crosslinking reaction is then started by exposing the solution/suspension comprising the loaded vesicles, the HA hydrogel precursor and the photo-initiator to a suitable radiation. Photo-initiators according to the present invention is preferably Irgacure 2959 of formula

![Irgacure 2959](image)

[0074] An exemplificative scheme of the photo-crosslinking HA hydrogel preparation using HA-MA is reported in Fig. 5.

[0075] Method of administration

[0076] Pharmaceutical formulations comprising the HA hydrogel of the invention can be prepared accordingly with the suitable excipient(s) in dependence of the method of administration. Suitable pharmaceutical formulations can be in the form of tablet, capsules, solution such as injectable solution, suspension, cream.

[0077] The HA hydrogel formulation discussed above can be administered by injection, and more specifically by subcutaneous, intradermal, intraocular or intramuscular injection, orally or topically. They may also be delivered locally (e.g. intraspinal or intratumoral) in the treatment of cancer. The HA hydrogel of the invention contains an effective amount of drug, protein or peptide or nucleic acid. Exact dosages will vary depending on patient factors such as age, sex, general condition, and the like. Those of skill in the art can readily take these factors into account and use them to establish effective therapeutic concentrations without resort to undue experimentation.

[0078] HA hydrogel according to the invention can be advantageously prepared in situ. In other words, the crosslinking can occur just before the application of the hydrogel on the site of interest.
[0079] The hyaluronic acid (HA) hydrogel according to the invention and as disclosed above delivers drugs or proteins or peptides or nucleic acids at a controlled rate for several clinical and surgical applications, including but not limited to ophthalmology (e.g. glaucoma, corneal, ocular inflammatory, vitreoretinal and medical retinal diseases) and dermatological conditions.

DEFINITIONS

[0080] The term "C_{10}-C_{22} alkyl" as used herein, refer to saturated, straight- or branched-chain hydrocarbon radicals containing between 10 and 22 carbon atoms, or the like, respectively. Examples of C_{10}-C_{22} alkyl radicals include, but are not limited to C_{10}, C_{12} such laureryl radicals, C_{14}, C_{16} radicals.

[0081] **Examples**

[0082] Example 1: Preparation of latanoprost-loaded liposomes: Extending the duration of release of an anti-glaucoma drug, latanoprost (Ltp)

[0083] Ltp is a hydrophobic drug. To make Ltp loaded EPC liposomes, 0.5mM concentration of Ltp and 10mm EPC were dissolved in a solvent mixture of 2:1 (v/v) ratio of chloroform:methanol in a round bottom flask (drug to lipid ratio of 0.05). The drug-lipid solution was manually mixed and the solvents were removed from the flask using a rotary evaporator maintained at 40°C water bath for 2hrs. A thin, dried drug-lipid film was obtained and this film was hydrated using PBS (pH7.4) buffer. The film was hydrated completely by manual shaking in a water bath maintained at 60°C for 10-15mins to form multilamellar vesicles (MLVs). MLVs suspension was extruded 15times through polycarbonate filters of size 0.2μm and 0.08μm fitted on to a bench top extruder to obtain Ltp loaded large unilamellar vesicles (LUVs) of EPC (size~100nm).

[0084] Example 2: Preparation of methacrylate HA (HA-MA) + Ltp loaded EPC solution

[0085] 20mg or 40mg of freeze-dried HA-MA was taken and dissolved directly in 1ml of Ltp loaded EPC liposome suspension and allowed to stir overnight at room temperature to prepare 2% (w/v) or 4% HA-MA solution respectively.

[0086] Example 3: Preparation of adipic dihydrazide HA (HA-ADH) + Ltp loaded EPC solution
40mg of freeze dried HA-ADH was taken and dissolved directly in 1ml of Ltp loaded EPC liposome suspension and allowed to stir overnight at room temperature to prepare 4% (w/v) HA-ADH solution.

Example 4: Preparation of Chitosan/ 5-FU Nanoparticles by Ionotropic Gelation

Materials: Chitosan 100 kDa (US sample), Chitosan (Sigma: 20-200 cps), Sodium tripolyphosphate (TPP), hydrophilic drug (5-FU)

Equipments: IKA overhead stirrer, Magnetic stirrer, Thermo Centrifuge, Ultracentrifuge

Protocol I:
§ Prepare 1 mg/mL chitosan-5FU (hydrophilic drug) solutions in 1 % acetic acid and filter using 0.22 μm filter (100 mL)
§ Prepare Triphenylphosphine (TPP) concentration 0.5 mg/mL in ultra pure water and filter using 0.22 μm filter (50 mL)
§ Adjust the pH of chitosan solution to 4.6 to 4.8 by adding 5M NaOH
§ Add TPP solution drop wise to chitosan solution under stirring at 1200 rpm using IKA overhead stirrer in 30 min and continue stirring for 30 min.
§ Centrifuge the solution at 25000 rpm, re-disperse the pellet and measure the size using Malvern zeta sizer.

Observations: In all the batches prepare following the above protocol around 90 % particles have the size range 50 to 140 nm.

Example 5: HA Hydrogel with and without Ltp-EggPC liposome-chemical crosslinking

EggPC is a lipid that forms liposomes. Latanoprost (Ltp) was loaded into eggPCs using standard methods described in the literature. These liposomes were then mixed with HA-ADH and the epoxy crosslinker PEGDE, and allowed to set overnight. Release of latanoprost was then quantified. HA Hydrogel with a load of Latanoprost (no liposome) was prepared. Release of latanoprost was then quantified.
[0096] The cumulative Ltp release from the HA-hydrogel with and without liposome was measured. The results are reported in Fig. 4.

[0097] Clearly, the latanoprost-loaded liposomes release drug more slowly than the drug directly dispersed in the hydrogel.

[0098] **Example 6: PLGA microparticles based system for sub-conjuntival controlled release of 5-FU.**

[0099] PLGA microparticles loaded with 5-FU were prepared by double emulsion technique. The microparticles were then lyophilized and dispersed in a HA precursors solution (HA-MA). Irgacure was used as initiator. UV crosslinking followed to give HA hydrogel with 5-FU loaded PLGA microparticles dispersed therein. The Batches formulations are disclosed in Table 1:

<table>
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<tr>
<th>Batch Formulation</th>
<th>IV</th>
<th>pH Of PVA</th>
<th>% Yield</th>
<th>% Loading</th>
<th>% EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 11 - 400mg PLGA + 20mg 5Fu (40% PLGA)</td>
<td>0.4E</td>
<td></td>
<td>51.25</td>
<td>2.0</td>
<td>39.4</td>
</tr>
<tr>
<td>Batch 10 - 300mg PLGA + 20mg 5Fu (30% PLGA)</td>
<td>0.4E</td>
<td>1.7</td>
<td>41.67</td>
<td>3.5</td>
<td>52.2</td>
</tr>
<tr>
<td>Batch 6 - 200mg PLGA + 20mg 5Fu (20% PLGA)</td>
<td>0.4</td>
<td>20</td>
<td>3.7</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

[00100] Fig. 7 report the SEM images of the microparticles obtained.

[00101] Fig. 8 shows the release profile of the three batches over a period of 15 days.

[00102] Batch 6 hydrogel was then compared with a formulation of 5-FU dispersed in HA-MA hydrogel (5-FU free/no particle) and 5-FU dispersed in HA-MA sol. It can be clearly seen that the incorporation of the PLGA microparticles loaded with 5-FU retard the release of the drug. (Fig. 9) In particular, Fig. 9 shows the complete release of 5-FU in a couple of days if loaded into sol (no X-link) or from the crosslinked gel.

[00103] **Example 7: UV crosslinkable hydrogels.**
[00104] The hydrophobic drug was incorporated into the gel prior to crosslinking, and its release profile measured. In addition, liposomes containing the same hydrophobic drug were also incorporated into the gel precursors prior to crosslinking, and the consequent release profile also quantified. In particular latanoprost was used as a drug and HA-MA was used as HA functionalized moiety. The hydrogel is a 4% (w/v) HA-MA hydrogel. The data are shown in Fig. 6.

[00105] It can be clearly seen that incorporation of liposomes into the hydrogel retards the release of the drug, and leads to an almost linear (ideal) release profile over 2 weeks. This principle can be applied to hydrophobic drugs incorporated first into liposome/micelle and then into the hydrogel.

[00106] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by exemplary embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00107] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00108] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.
CLAIMS

1. A hyaluronic acid (HA) hydrogel comprising loaded vesicles dispersed therein wherein the loaded vesicles are loaded with one or more drugs, one or more proteins, or one or more nucleic acids.

2. The HA hydrogel of claim 1, wherein the loaded vesicles are selected from micelles, liposomes and/or particles selected from nanoparticles and microparticles.

3. The HA hydrogel according to claims 1-2, wherein the micelles are made of amphipilic self-assembling molecules.

4. The HA hydrogel according to claims 1-2, wherein the vesicles are liposomes.

5. The HA hydrogel according to claims 1-4 wherein the self-assembling molecules are selected from polymers and surfactants, preferably block copolymer, more preferably Pluronic block copolymer.

6. The HA hydrogel according to claims 1-5 wherein the particles are selected from chitosan nanoparticles, chitosan microparticles, poly(lactic-co-glycolic acid) (PLGA) nanoparticles and poly(lactic-co-glycolic acid) (PLGA) microparticles.

7. The HA hydrogel according to claims 1-6, wherein the drug is selected from a hydrophobic or hydrophilic drug, preferably is selected from an antibiotic drug, a chemio-therapeutic drug, a drug for the treatment of glaucoma or ocular hypertension such as latanoprost or 5-fluorouracil (5-FU), the protein is selected from therapeutic peptide or protein or monoclonal antibodies, anti-VEGF antibodies such as bevacizumab and ranibizumab, the nucleic acid is selected from siRNA and plasmid DNA.

8. The HA hydrogel according to claims 1-7, wherein the HA hydrogel is a covalently crosslinked hydrogel.

9. The HA hydrogel according to claims 1-8 wherein in the HA hydrogel
a) the functionalized HAs functionalized for crosslinking are interconnected by crosslinkers; or

wherein

b) the functionalized HAs, the functionalizing group is the crosslinker.

10. The HA hydrogel according to claims 1-9 wherein the functionalized HA is selected from methacrylate–HA (HA-MA) or adipic acid dihydrazide HA (HA-ADH).

11. The HA hydrogel according to claims 1-10 wherein the crosslinker is poly(ethylenglycol) diglycidyl ether (PEGDE).

12. The HA hydrogel according to claims 1-10 wherein the HA hydrogel is selected from a HA-MA hydrogel, HA-ADH hydrogel, HA-ADH crosslinked with PEGDE-hydrogel, HA-MA crosslinked with PEGDE hydrogel.

13. The HA hydrogel according to any of the preceding claims wherein the amount of loaded vesicles dispersed in the HA hydrogel is the 1% to the 40 % by weight of the whole HA hydrogel.

14. The HA hydrogel according to claim 9 a) wherein the moles of crosslinker are 2 to 10 times the mole of effectively functionalized HA.

15. The HA hydrogel of any of the preceding claims wherein the vesicles are selected from PGLA nanoparticles loaded with 5-FU, PGLA microparticles loaded with 5-FU or EggPC liposomes loaded with latanoprost, chitosan nanoparticles loaded with 5-FU, chitosan microparticles loaded with 5-FU, PEG/PLA micelles loaded with paclitaxel, liposomes loaded with doxorubicin, liposomes loaded with siRNA, liposome loaded with plasmid DNA.

16. A method for preparing the HA hydrogel as defined in any of the preceding claims comprising:

   a) providing functionalized HA with a functional moiety wherein the functional moiety is for crosslinking;
b) crosslinking the functionalized HA optionally in the presence of a crosslinker

wherein

the crosslinking occurs in the presence of loaded vesicles and

wherein

when the crosslinker is not present the functional moiety acts as a crosslinker.

17. The method of claim 16 wherein the functionalized HA is selected from HA-MA or HA-ADH.

18. The method of claims 16-17 wherein the crosslinkers is selected from PEGDE.

19. The method of claims 16-18 wherein the loaded vesicles are mixed with the functionalized HA to form a mixture and then the crosslinker is added to said mixture.

20. The method of claims 19 wherein the functionalized HA is freeze-dried HA-MA.

21. The method of claims 16-20 wherein the effectively functionalized HA and the crosslinker are present in a ratio 1:1 to 1:20.

22. The method of claims 16-21, wherein the crosslinking occurs at a T of 20 °C to 25°C.

23. The method of claims 16-22 wherein the crosslinking is a photo-crosslinking.

24. The method of claim 23 wherein the photo-crosslinking is a UV photo-crosslinking.


26. A HA hydrogel comprising loaded vesicles dispersed therein as defined in claims 1-15 and 25 for use as a medicament.

27. A pharmaceutical formulation comprising the HA hydrogel as defined in claims 1-15 and 25-26.

28. The pharmaceutical formulation of claims 27 for oral, topical, intravenous, subcutaneous or intramuscular administration.
29. The method of claims 16 to 25 wherein the functionalized HA has a degree of functionalization ranging from 5 to 80%.
Fig. 1
Fig. 2
Phospholipid

Fig. 3a
Fig. 4

Ltp release - 2% HA with and without Ltp loaded EggPC liposomes

% Cumulative Ltp Release

Release time (days)
STIR FOR 2H AT RT, pH 8

20 MOLAR EXCESS OF METHACRYLIC ANYHYDRIDE

SOME OF THE PRIMARY ALCOHOLS ARE METHACRYLATED TO FORM PHOTOCROSSLINKABLE HA-MA

REACTION MIXTURE IS DIALYSED AGAINST A 0.1M NaCl SOLUTION FOR AT LEAST 48H, CHANGING THE SOLUTION AT SUITABLE TIMEPOINTS, AND THEN LYOPHILIZED

REACTION MIXTURE IS KEPT AT 4 °C FOR 24H

SOLUTIONS OF DIFFERENT HA-MA CONCENTRATIONS, i.e. 1%, 2% & 4%, WERE PREPARED. 1 ML OF EACH SOLUTION WAS INJECTED INTO METAL WELLS WITH A DIAMETER OF 17MM

1% IRGACURE 2959, 365nm UV WAVELENGTH, 10 MIN EXPOSURE

HYDROGELS FORMED

Fig. 5
Fig. 6
Fig. 8
5Fu release profile

- HA-MA+5Fu sol
- HA-MA+5Fu gel
- HA-MA+Batch5 PLGA gel

% Cumulative release vs Release period, days

Fig. 9
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/711 (2006.01)  A61P 27/06 (2006.01)

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)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, MEDLINE, CA, BIOSIS, and PATENTLENS: keywords: Hyaluronan, hyaluronic acid, vesicles, micelles, liposomes, particles, chitosan, PLGA, dispersion, encapsulation, latanaprost, glaucoma, RNA, DNA, and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>Documents are listed in the continuation of Box C</td>
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X Further documents are listed in the continuation of Box C  X See patent family annex

* Special categories of cited documents:
  *A* document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
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  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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  "&" document member of the same patent family

Date of the actual completion of the international search: 9 December 2013
Date of mailing of the international search report: 09 December 2013

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Telephone No. 0262832083
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<td>X</td>
<td>US 2010/0285113 A1 (SHOICHER, M. S., ET AL.) 11 November 2010 See, in particular: Abstract, paragraphs [0040], [0056], [0062], [0074]–[0092], [0114]–[0120]; and Table 1.</td>
<td>1-11, 13, 14, and 26-28</td>
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<td>X</td>
<td>LEACH, J.B., ET AL., “Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds,” Biomaterials, 2005, Vol. 26, pages 125-135 See, in particular: Abstract and page 127-129, sections 2.1, 2.5, and 2.6.</td>
<td>1, 2, 6, 8-14, and 16-29</td>
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<td>X</td>
<td>US 2009/0226531 A1 (LYONS, R.T., ET AL.) 10 September 2009 See, in particular: Paragraphs [0010]; [0027]; [0039]; [0043]; and [0110].</td>
<td>1, 2, 6, 7, 13, and 26-28</td>
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<tr>
<td>X</td>
<td>WO 2002/087563 A2 (ANGIOTECH PHARMACEUTICALS INC.) 07 November 2002 See, in particular: Abstract; page 22-23, bridging paragraph; page 42, lines 22-30; page 45, lines 3-6; and Examples 1-3, 5, and 15.</td>
<td>1-5, 7, 15, and 26-28</td>
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This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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<td>US 2010/0285113 A1</td>
<td>11 Nov 2010</td>
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<tr>
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<tr>
<td>WO 2002/087563 A2</td>
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End of Annex