and "reconstituted" in HA, and lubricins expressed in microgram quantities in early efforts to make it using recombinant DNA technology.

[0009] Previous attempts at recombinant production of full length lubricin at a scale suitable for commercial exploitation have not been successful. The very low, single or double-digit milligram per liter rate of production of human lubricin species expressed from CHO cells is considered too low to support a commercial product. One approach to solving this problem was to truncate the number of repeats in exon six, and therefore reduce the mass of glycosylation side chains while retaining at least some lubricating ability (see, e.g., U.S. Patent Nos. 7,642,236 and 7,893,029). This approach reportedly resulted in a gross productivity (before purification) of the truncated construct of three to four hundred milligrams per liter.

SUMMARY ~~OF THE INVENTION~~

[0010] It has now been discovered that the human PRG4 gene can be used to produce large, commercial quantities of a novel, highly glycosylated human-like form of lubricin, hereinafter referred to simply as "lubricin," multimeric lubricin, rhlubricin, or rhPRG4. This is accomplished as disclosed herein by transfecting the human PRG4 gene (hPRG4) into certain modified Chinese hamster ovary (CHO) cells which have been discovered to be competent to post-translationally glycosylate expressed proteins on a large scale, and then culturing the cells in commercial scale volumes of media, for example, at least 10 liters, more typically at least 50 liters, preferably at least 100 liters or at least 500 liters, and most preferably at 1,000 liters or more.

[0011] The lubricin of this ~~invention~~ present disclosure comprises polydisperse lubricin monomer units forming dimers and multimers and optionally free monomers. Each unit is heavily and variably glycosylated, with the glycosidic residue side chains contributing at least 30%, often 35% or 40%, and possibly as high as 45% or more of its molecular weight.

[0012] In native human lubricin, glycosylations consist of core 1 O-linked GalNAc-Gal (N-acetylgalactosamine-galactose) disaccharide, at least 60% of which is terminally substituted with a sialic acid, and also core 2 glycosylation involving addition to core 1 of GlcNac (N-acetylglucosamine) monosaccharides in various isomeric configurations. (See, *e.g.*, Estrella *et al.*, Biochem J., 429(2):359-67 (2010)).

[0013] The recombinant material produced as disclosed herein is enriched in core 1 glycans, as compared to native form human lubricin. Its glycosylation comprises at least 95% core 1 side chains, more likely more than 98% or 99%. Furthermore, the side chains often are sulfated to a degree not seen in native human lubricin. This distinguishes the rhPRG4 of this ~~invention~~ present disclosure from native hPRG4. The increased sulfation content is believed to add additional negative charges to the mucinous glycoprotein which may serve to enhance its ability to repel nearby biomolecules and thus increase its lubricity, and to stiffen the molecular structure, making it more rigid from a molecular standpoint, which can assist in its ability to function in reducing nanoscale and mesoscale friction.

[0014] The full length (non-truncated) lubricin monomer sequence (SEQ ID NO:1) comprises 1404 amino acids, or approximately 151 kDa in core protein. The signal sequence of human lubricin is residues 1-24 of SEQ ID NO:1. Accordingly, the mature form of human lubricin is residues 25-1404 of SEQ ID NO:1. Exhaustive reduction of the recombinant product produced as disclosed herein yields a monomeric species with an apparent molecular weight of about 300 kDa-460 kDa, as estimated by comparison to molecular weight standards in a number of molecular weight determination techniques including SDS tris-acetate 3-8% polyacrylamide gel electrophoresis. Glycosylation analysis using mass spectrometry techniques and other work in combination suggested that the true molecular weight of a glycosylated recombinant monomer (as opposed to inferred from gel mobility) likely is in the range of 220-280 kDa, and is unlikely to exceed about 300kDa. From a total of about 329 possible O-linkages (284 of which are threonines) potentially available as sites of O-linked glycosylation in the sequence of the lubricin monomer, a varying and unknown large number are substituted (100 to 150, perhaps as high as 200 or 220). Of the total glycosylation, about half comprise two sugar units (GalNAc-Gal), and half three sugar units (GalNAc-Gal-Sialic acid). The most abundant form is sulfated Gal-GalNac, the next most abundant is sialylated Gal-GalNac.

[0015] The lubricin expression product is resistant, although not immune, to breakdown into monomeric or dimeric lubricin species. The results of exhaustive reduction imply that it comprises disulfide cross-links between and within monomer units. Also, treatment with denaturing buffers without reduction can result in lower molecular weight products, suggesting higher order quaternary structures where the chains also are held together by hydrophobic

interaction, hydrogen bonding, physical entanglement and/or other non-covalent associations allowing for self-assembly. The dimers and multimers are polydisperse. Molecular species within it typically have molecular weights of at least about 450-600 kDa, and multimeric species frequently 2,000 kDa or more. Typically, some species of the non-reduced complex essentially do not enter the 3-8% SDS-PAGE gel in an electrophoresis experiment. The larger species of the complex is believed to comprise between three and five, and perhaps as many as 20 monomer units.

[0016] Without wishing to be bound by theory, it is believed such larger supramolecular components are formed as a function of monomer/dimer concentration. Currently, a concentration of at least about 0.5 mg/ml monomer/dimer is believed to be optimal for spontaneous formation of larger complex. Concentrations well below this, *e.g.*, less than about 0.1 mg/ml, comprise monomers and dimers, and only a minor amount of complex; concentrations well above can form aggregates visible with the naked eye as a cloudy or hazy solution. Surfactants, preferably physiologically compatible nonionic surfactants that are generally regarded as safe, *e.g.*, polyoxyethylene-based surfactants, or excipients may be used to prevent large aggregate formation while permitting formation of the complex, which appears always to be present together with dimeric species.

[0017] Testing of preparations comprising the lubricin of the ~~invention~~ present disclosure shows that its lubricating and tissue protection properties under load may exceed that of recombinant lubricin heretofore known in the art. Without wishing to be bound by theory, the inventors hereof hypothesize that while stick-slip phenomenon occurs during movement of unlubricated interfacing tissues under load, a coating of the lubricin of the ~~invention~~ present disclosure can transfer shear away from the underlying cartilage surface to layers within the coating of polydisperse lubricin. That is, the inventors believe that under load and reciprocating motion, the underlying surface experiences less shear, preserving its integrity, as molecules of lubricin within the coating slip over one another, then likely rearrange when the load is removed. (See, *e.g.*, Lee *et al.*, PNAS, 110(7):E567-574 (2013)). The authors state that joint wear is not directly related to the friction coefficient, but more directly related to stick-slip sliding, and that the different molecular components of the joint work synergistically to prevent wear.

[0018] In any event, the lubricin product of the ~~invention~~present disclosure, when tested, exhibits outstanding lubricating properties, resulting in coefficients of friction (both static and dynamic) often within 150%, 120%, 110% or essentially equal to the coefficients of purified, native bovine lubricin, as measured by cartilage-on-cartilage lubrication testing as disclosed herein. In these tests, the human-like glycoprotein of the ~~invention~~ present disclosure achieves static coefficients of friction at or below 0.5 and lower than 0.2 (depending on test conditions as disclosed herein), and kinetic coefficients of friction often at or below about 0.1, both as measured by depressurized cartilage upon cartilage bearings in vitro, with a stationary area of contact. When combined with hyaluronic acid (HA), these values improve to below about 0.3 and less than 0.1 for the certain static measurement (depending on dwell time) and less than 0.1 for the kinetic measurement, quite close to the accepted value for synovial fluid. Accordingly, such compositions can dramatically reduce joint wear.

[0019] Accordingly, ~~one aspect of~~ the ~~invention~~ present disclosure comprises a method for the commercial production of lubricin. In one embodiment, the method includes the steps of culturing, in a medium, Chinese hamster ovary (CHO) cells transfected with and which express the human PRG4 gene and post-translationally glycosylate the expression product for a time and under culture conditions sufficient to produce a lubricin glycoprotein, and purifying the lubricin glycoprotein from said medium. For example, in some embodiments, the lubricin glycoprotein is separated from host cell proteins and other contaminants in the extracellular broth to at least partially purify it. The recombinant protein need only be enriched from the culture medium, rather than purified to homogeneity in order to be purified for the purposes of the method of the ~~invention~~present disclosure. The method is sufficient to produce a lubricin glycoprotein having at least 30% by weight glycosidic residues at a concentration in the medium of at least 0.4g/L.

[0019a] In one aspect of the present disclosure, there is provided a method of manufacture of a recombinant lubricin glycoprotein comprising the steps of:

culturing, in a medium, Chinese hamster ovary (CHO) cells transfected with and which express the human PRG4 gene and post translationally glycosylate the expression product for a time and under culture conditions sufficient to produce a lubricin glycoprotein comprising at least 30% by weight glycosidic residues at a concentration in the medium of at least 0.4 g/liter, and

purifying the lubricin glycoprotein from said medium.

[0020] In some embodiments, the CHO cells are CHO-M cells comprising a nucleic acid encoding the human PRG4 gene. In other embodiments the CHO cells are transfected with a first vector comprising a nucleic acid encoding a chromatin element and are transfected with a second vector comprising a nucleic acid encoding the human PRG4 gene. The chromatin element may be a boundary element, a matrix attachment region, a locus control region, or a universal chromatin opening element. In a preferred embodiment, the chromatin element is a matrix attachment region.

a mixture of recombinant lubricin that includes monomeric, dimeric, and multimeric lubricin species. In some embodiments, the lubricin glycoprotein comprises at least five disulfide-bonded or non-covalently associated individual glycosylated amino acid chains and has a molecular weight of at least 1200 kDa.

[0024a] In another aspect of the present disclosure, there is provided a lubricin glycoprotein produced by the method as described herein.

[0025] The glycoprotein produced according to the methods of the ~~invention~~present disclosure, when tested using the protocol outlined below, produces a coefficient of friction approaching the lowest values ever observed for purified native mammalian lubricin. For example, in some embodiments, the recombinant lubricin glycoprotein is a multimeric protein that produces a static coefficient of friction no greater than 150% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test. In other embodiments, the recombinant lubricin glycoprotein is a multimeric protein that produces a static coefficient of friction no greater than 120% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test. In yet other embodiments, the recombinant lubricin glycoprotein is a multimeric protein that produces a static coefficient of friction no greater than 110% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

[0026] ~~Another aspect of the~~ The ~~invention~~ present disclosure is also directed to compositions of a recombinant, multimeric, lubricin glycoprotein expressed from the human PRG4 gene in a host cell culture. The recombinant lubricin glycoprotein is at least 30% by weight glycosidic residues and produces a dynamic coefficient of friction no greater than 150% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

[0026a] In yet another aspect of the present disclosure, there is provided a composition of matter comprising:
a recombinant multimeric lubricin glycoprotein expressed from the human PRG4 gene in a host cell culture, comprising at least 30% by weight glycosidic residues, and producing a dynamic coefficient of friction no greater than 150% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

[0027] In some embodiments, the recombinant lubricin glycoprotein is at least 35%, at least 40% or at least 45% by weight glycosidic residues.

[0028] In some embodiments, the recombinant lubricin glycoprotein produces a dynamic coefficient of friction no greater than 110% or no greater than 120% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

[0029] In some embodiments, the glycosidic residues of the recombinant lubricin may differ from those of native human lubricin as the glycosylation of the recombinant lubricin is at least 90%, at least 95%, or at least 99% by weight core 1 glycosylation. Also, in some

embodiments, the glycosidic residues of the recombinant lubricin are enriched in sulfated monosaccharides as compared with native human lubricin.

[0030] In some embodiments, the recombinant lubricin is a mixture of monomeric, dimeric and multimeric species. In some embodiments, the lubricin includes monomeric species. In some embodiments, the lubricin includes dimeric species. In some embodiments, the lubricin includes multimeric species. In some embodiments, the lubricin is a mixture of multimeric and monomeric species.

[0031] In some embodiments, the lubricin glycoprotein comprises at least five disulfide-bonded or non-covalently associated individual glycosylated amino acid chains and has a molecular weight of at least 1200 kDa.

[0032] In some embodiments, the composition of recombinant lubricin glycoprotein further includes hyaluronic acid or a salt thereof in admixture with the lubricin.

[0033] ~~In a~~Another ~~embodiment~~aspect of~~,~~ the ~~invention~~ present disclosure is directed to a composition comprising a solution comprising 100 grams of human lubricin where the glycosylation of the lubricin is at least 99% by weight core 1 glycosylation. In one embodiment, the lubricin is recombinant human lubricin. In yet another embodiment, the concentration of lubricin in the solution is at least 0.5 g/L. In yet another embodiment, the solution is a cell culture medium.

[0034] Compositions of the ~~invention~~ present disclosure may be used for the preparation of a medicament for any known or hereafter discovered medical or other use of PRG4 glycoprotein, including as a coating for various devices intended for contact with the body (see, *e.g.*, U.S. Patent Application Publication Nos. 2009/0068247 and 2011/0142908); for the treatment of an articular joint in a human or animal by enhancement of joint lubrication (U.S. Patent Application Publication No. 2004/0229804) or viscosupplementation (U.S. Patent Application Publication No. 2008/0287369); for topical application to a tissue surface, e.g., during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue (U.S. Patent Application Publication No. 2004/0229804); for the treatment of dry eye disease (U.S. Patent Application Publication No. 2011/0059902); for treatment of dry mouth disease (U.S. Patent Application Publication No. 2013/0039865); for treatment of interstitial cystitis (U.S. Patent Application Publication No. 2012/0321693); as a vaginal lubricant (U.S. Patent Application

Publication No. 2012/0052077); for a contact lens care and storage solution (U.S. Patent Application Publication No. 2012/0321611) or for systemic injection to, for example, inhibit cell-cell adhesions or motility within the vasculature (see, e.g., US provisional application 61/908,959 filed November 26, 2013).

[0034a] In yet another aspect of the present disclosure, there is provided a use of the composition as described herein, for the preparation of a medicament for the treatment of an articular joint by viscosupplementation.

[0034b] In a further aspect of the present disclosure, there is provided a use of the composition as described herein, for the preparation of a medicament for topical application to a tissue surface.

[0034c] In another aspect of the present disclosure, there is provided a use of the composition as described herein, for the preparation of a medicament for the treatment of dry eye disease.

[0034d] In yet another aspect of the present disclosure, there is provided a use of the composition as described herein, for the preparation of a medicament for application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

[0034e] In a further aspect of the present disclosure, there is provided a use of the composition as described herein, for the preparation of a medicament for systemic injection to inhibit cell-cell adhesion or motility within the vasculature.

[0034f] In another aspect of the present disclosure, there is provided a composition as described herein, for use in the treatment of an articular joint by viscosupplementation.

[0034g] In yet another aspect of the present disclosure, there is provided a composition as described herein, for use in topical application to a tissue surface.

[0034h] In a further aspect of the present disclosure, there is provided a composition as described herein, for use in the treatment of dry eye disease.

[0034i] In another aspect of the present disclosure, there is provided a composition as described herein, for use in the application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

[0034j] In yet another aspect of the present disclosure, there is provided a composition as described herein, for use in systemic injection to inhibit cell-cell adhesion or motility within the vasculature.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIGS. 1 and 2 are plasmid maps of vectors used in development of the lubricin-expressing CHO-M clone used in the process of the invention and encoding the full length sequence of hPRG4.

[0036] FIGS. 3 and 4 depict polyacrylamide gels useful in assessing the structure of the rhlubricin of the invention.

[0037] FIG. 5 is a graphical depiction of the productivity of one lubricin production run measuring micrograms of lubricin produced over time per liter of culture of transfected CHO-M cells. There are three bars at each date of harvest. The bar at the left is "Std Curve 24 Jun 14." The bar in the middle is "Std Curve 24 Jul 14," and the bar at the right is "Std Curve 25 Jul 14."

[0038] FIG. 6 is a diagram showing core 1 glycans of rhlubricin of the invention.

[0039] FIG. 7 is a chromatograph, with peaks labeled, showing the relative abundance of the various di- and tri-saccharides pendent from SER and THR residues in the rhlubricin of the invention.

[0040] FIG. 8 is a diagram showing the larger range of glycans extending into the core 2 structures on native lubricin extracted from human synovial fluid.

[0041] FIG. 9 is a chromatograph, with peaks labeled, showing the relative abundance of the various sugar residues in native human lubricin.

[0042] FIGS. 10A, 10B, and 10C are plots of surface tension vs. rhPRG4 and/or polyoxyethylene surfactant concentrations showing the reduction in surface tension with increased concentration of rhPRG4.

~~9. The method of any one of claims 1-5, wherein the cells are cultured for a time and under culture conditions sufficient to produce said lubricin glycoprotein at a concentration in the medium of at least 2.0 g/liter.~~

~~10~~9. The method of any one of claims 1-~~9~~8 wherein at least 95% by weight of glycosylation of the lubricin glycoprotein is core 1 glycosylation.

~~11~~10. The method of any one of claims 1-~~9~~8 wherein at least 99% by weight of glycosylation of the lubricin glycoprotein is core 1 glycosylation.

~~12~~11. The method of any one of claims 1-~~11~~10 wherein the glycosidic residues are enriched in sulfated saccharide side chains as compared with native human lubricin.

~~13~~12. The method of any one of claims 1-~~12~~11 wherein the lubricin glycoprotein comprises a multimeric protein producing a static coefficient of friction no greater than 150% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~14~~13. The method of any one of claims 1-~~12~~11 wherein the lubricin glycoprotein comprises a multimeric protein producing a static coefficient of friction no greater than 120% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~15~~14. The method of any one of claims 1-~~12~~11 wherein the lubricin glycoprotein comprises a multimeric protein producing a static coefficient of friction no greater than 110% of the static coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~16~~15. The method of any one of claims 1-~~15~~14 wherein the lubricin glycoprotein comprises a monomeric lubricin species co-purified from said culture medium and in admixture with a multimeric lubricin species.

~~17~~16. The method of any of claims ~~13-15~~12-14, wherein the lubricin glycoprotein comprises a dimeric lubricin species.

~~18~~17. The method of any one of claims 1-~~17~~16 wherein the lubricin glycoprotein comprises at least five disulfide-bonded or non-covalently associated individual glycosylated amino acid chains and has a molecular weight of at least 1200 kDa.

~~19~~18. The method of any one of claims 1-~~18~~17 wherein the cells are cultured in at least 10, 50, or 100 liters of medium.

~~20~~19. The method of any one of claims 1-~~19~~18 wherein the lubricin glycoprotein comprises at least 35% by weight glycosidic residues.

~~21~~20. The lubricin glycoprotein produced by the method of any one of claims 1-~~20~~19.

~~22~~21. A composition of matter comprising:

a recombinant multimeric lubricin glycoprotein expressed from the human PRG4 gene in a host cell culture, comprising at least 30% by weight glycosidic residues, and producing a dynamic coefficient of friction no greater than 150% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~23~~22. The composition of claim ~~22~~21, wherein the lubricin glycoprotein is characterized as producing a dynamic coefficient of friction no greater than 120% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~24~~23. The composition of claim ~~22~~21, wherein the lubricin glycoprotein is characterized as producing a dynamic coefficient of friction no greater than 110% of the dynamic coefficient of friction of purified native bovine lubricin as measured in a cartilage on cartilage friction test.

~~25~~24. The composition of any of claims ~~22-24~~21-23, wherein the lubricin glycoprotein comprises at least 35% by weight glycosidic residues.

~~26~~25. The composition of any of claims ~~22-24~~21-23, wherein the lubricin glycoprotein comprises at least 40% by weight glycosidic residues.

~~27~~26. The composition of any of claims ~~22-26~~21-25, wherein at least ~~99~~95% by weight of glycosylation of the lubricin glycoprotein is core 1 glycosylation.

~~28~~27. The composition of any of claims ~~22-27~~21-26, wherein the glycosidic residues are enriched in sulfated saccharide side chains as compared with native human lubricin.

2928. The composition of any of claims 22-2821-27, further comprising a monomeric lubricin species admixed with multimeric lubricin species.

3029. The composition of any of claims 22-2921-28, comprising a dimeric lubricin species.

3130. The composition of any of claims 22-3021-29, comprising a lubricin species comprising at least five disulfide-bonded or non-covalently associated individual glycosylated amino acid chains having a molecular weight of at least 1200 kDa.

3231. The composition of any of claims 22-3121-30, further comprising hyaluronic acid or a salt thereof in admixture with said lubricin glycoprotein.

3332. Use of the The composition of any of claims 22-3221-31, for the preparation of a medicament for the treatment of an articular joint by viscosupplementation.

3433. Use of the The composition of any of claims 22-3221-31, for the preparation of a medicament for topical application to a tissue surface.

3534. Use of the The composition of any of claims 22-3221-31, for the preparation of a medicament for the treatment of dry eye disease.

3635. Use of the The composition of any of claims 22-3221-31, for the preparation of a medicament for application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

3736. Use of the The composition of any of claims 22-3221-31, for the preparation of a medicament for systemic injection to inhibit cell-cell adhesion or motility within the vasculature.

3837. A composition comprising a solution comprising 100g of human lubricin, wherein the lubricin comprises glycosylation that is at least 99% by weight core 1 glycosylation.

3938. The composition of claim 3837, wherein the human lubricin is recombinant human lubricin.

4039. The composition of claim 38-37 or 3938, wherein the concentration of lubricin in the solution is at least 0.5 g/L.

40. The composition of any of claims 21-31, for use in the treatment of an articular joint by viscosupplementation.

41. The composition of any of claims 21-31, for use in topical application to a tissue surface.

42. The composition of any of claims 21-31, for use in the treatment of dry eye disease.

43. The composition of any of claims 21-31, for use in the application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

44. The composition of any of claims 21-31, for use in systemic injection to inhibit cell-cell adhesion or motility within the vasculature.