**[0018]** In any event, the lubricin product of the 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 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, the 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 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, wherein at least 90% by weight of the glycosylation of the lubricin glycoprotein is core 1 glycosylation, at a concentration in the medium of at least 0.4 g/liter, and

purifying the lubricin glycoprotein from said medium.

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.

**[0025]** The glycoprotein produced according to the methods of the 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]** The 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, wherein at least 90% by weight of the glycosylation of the lubricin glycoprotein is core 1 glycosylation, 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.

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]** Another embodimentof the 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 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

**What is claimed is:**

1. 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, wherein at least 90% by weight of the glycosylation of the lubricin glycoprotein is core 1 glycosylation, at a concentration in the medium of at least 0.4 g/liter, and

purifying the lubricin glycoprotein from said medium.

2. The method of claim 1, wherein the CHO cells are CHO-M cells comprising a nucleic acid encoding the human PRG4 gene.

3. The method of claims 1 or 2, wherein the CHO cells are transfected with a first vector comprising a nucleic acid encoding a chromatin element and a second vector comprising a nucleic acid encoding the human PRG4 gene.

4. The method of claim 3, wherein the chromatin element is a boundary element, matrix attachment region, locus control region or a universal chromatin opening element.

5. The method of claim 4, wherein the chromatin element is a matrix attachment region.

6. 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 0.5 g/liter.

7. 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 0.8 g/liter.

8. 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 1.0 g/liter.

17. The method of any one of claims 1-16 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.

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

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

20. 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, wherein at least 90% by weight of the glycosylation of the lubricin glycoprotein is core 1 glycosylation, 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.

21. The composition of claim 20, 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.

22. The composition of claim 20, 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.

23. The composition of any of claims 20-22, wherein the lubricin glycoprotein comprises at least 35% by weight glycosidic residues.

24. The composition of any of claims 20-22, wherein the lubricin glycoprotein comprises at least 40% by weight glycosidic residues.

25. The composition of any of claims 20-24, wherein at least 95% by weight of glycosylation of the lubricin glycoprotein is core 1 glycosylation.

26. The composition of any of claims 20-24, wherein at least 99% by weight of glycosylation of the lubricin glycoprotein is core 1 glycosylation.

27. The composition of any of claims 20-26, wherein the glycosidic residues are enriched in sulfated saccharide side chains as compared with native human lubricin.

28. The composition of any of claims 20-27, further comprising a monomeric lubricin species admixed with multimeric lubricin species.

29. The composition of any of claims 20-28, comprising a dimeric lubricin species.

30. The composition of any of claims 20-29, 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.

31. The composition of any of claims 20-30, further comprising hyaluronic acid or a salt thereof in admixture with said lubricin glycoprotein.

32. Use of the composition of any of claims 20-31, for the preparation of a medicament for the treatment of an articular joint by viscosupplementation.

33. Use of the composition of any of claims 20-31, for the preparation of a medicament for topical application to a tissue surface.

34. Use of the composition of any of claims 20-31, for the preparation of a medicament for the treatment of dry eye disease.

35. Use of the composition of any of claims 20-31, for the preparation of a medicament for application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

36. Use of the composition of any of claims 20-31, for the preparation of a medicament for systemic injection to inhibit cell-cell adhesion or motility within the vasculature.

37. The composition of any of claims 20-31, for use in the treatment of an articular joint by viscosupplementation.

38. The composition of any of claims 20-31, for use in topical application to a tissue surface.

39. The composition of any of claims 20-31, for use in the treatment of dry eye disease.

40. The composition of any of claims 20-31, for use in the application to a body surface during surgery to inhibit subsequent formation of adhesions or fibrotic connective tissue.

41. The composition of any of claims 20-31, for use in systemic injection to inhibit cell-cell adhesion or motility within the vasculature.