HYALINE CARTILAGE REPLACEMENT SUBSTANCE

Means for replacing hyaline cartilage that is capable of self-assembly in the absence of covalent cross-linking. Further, aggregates of the means are biphasic, containing both peptide and water. The means comprises one or more than one polypeptide having a sequence according to SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, or comprises a sequence where one or more than one amino acid is conservatively substituted, or where one more than one amino acid is substituted to promote cross-linking between individual polypeptides.
HYALINE CARTILAGE REPLACEMENT SUBSTANCE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States Government support under Cooperative Agreement DAMD17-97-2-7016, subcontract number 90-FY99-LLVARE-SANDBERG with the National Medical Test Bed, United States Department of the Army. The United States Government has certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATION

The present Application claims the benefit of United States provisional patent application 60/249,483, filed November 17, 2000, entitled “Scallop Abduction Peptides,” and claims the benefit of International Application PCT/US01/06066, filed February 26, 2001, entitled “Hyaline Cartilage Replacement Substance,” the contents of which are incorporated herein by reference in their entirety.

BACKGROUND

Osteoarthritis is the most prevalent form of arthritis and the leading cause of disability in the United States, affecting an estimated 20.7 million Americans. Osteoarthritis is characterized by degeneration of the hylaine cartilage that covers the articular surfaces of the joint, causing severe pain and loss of movement from bone-on-bone frictional wear.

Pathologically, osteoarthritis causes focal erosive lesions, cartilage destruction, subchondral sclerosis, cyst formation, and large osteophytes at the margins of the joints. At the cellular level, chondrocytes undergo cell death caused by upregulation of nitric oxide synthase. In addition, high levels of nitric oxide have also been shown to inhibit matrix production in chondrocytes. The underlying causes of osteoarthritis, however, are unknown.

In addition to osteoarthritis, there are many other diseases and conditions that damage hylaine cartilage in joints. Further, joints are a common site of direct injury, which often damages the hylaine cartilage in joints.

Treatment for osteoarthritis includes the administration of agents to control pain, agents to decrease inflammation at the site of joint damage, and agents that cause immunosuppression. However, all of these agents are of limited benefit. Additionally, joint replacement is used to restore function in patients with osteoarthritis and in patients with other joint diseases and injuries. However, joint replacement is associated with high cost and
a significant rate of morbidity.

There are currently no methods for the direct repair of damaged hyaline cartilage defects in joints. Additionally, there are no substances that can be used to directly repair cartilage defects in joints.

Therefore, it would be useful to have a method for the repair of cartilage defects in joints due to osteoarthritis and due to other diseases and injuries. Further, it would be useful to have a substance that can be used to directly repair cartilage defects in joints due to osteoarthritis and due to other diseases and injuries.

**SUMMARY**

In one embodiment, the present invention is a substance for replacing hyaline cartilage comprising a polypeptide having a sequence according to SEQ ID NO:1 repeated at least two times, where the two repeated segments are either consecutive or are separated by one or more than one additional amino acid residues. In another embodiment, the present invention is a substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:1 and having no more than about 100 amino acids residues in the entire sequence.

In another embodiment, the present invention is a substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:1, and water, or consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:1 and comprising no more than about 100 amino acids residues in the entire sequence, and water. The present invention also includes a composition for replacing hyaline cartilage comprising either of the two preceding substances.

In one embodiment, the present invention is substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2 or SEQ ID NO:3. In another embodiment, the present invention is substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2 or SEQ ID NO:3 and having no more than about 100 amino acids residues in the entire sequence.

In one embodiment, the present invention is a substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:2 or SEQ ID NO:3, and water. In another embodiment, the present invention is a substance for replacing hyaline cartilage consisting
essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:2 or SEQ ID NO:3 and comprising no more than about 100 amino acid residues in the entire sequence, and water. The present invention also includes a composition for replacing hyaline cartilage comprising either of the two preceding substances.

In a preferred embodiment, the present invention is a means for replacing hyaline cartilage according to the present invention. In a preferred embodiment, the means comprises any of the preceding substances, or comprises an aggregate of polypeptides and water.

The present invention also includes any substance according to the present invention where one or more than one amino acid of either SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 has been conservatively substituted, or has been substituted to promote cross-linking between individual polypeptides. Additionally, the present invention comprises a composition for replacing hyaline cartilage comprising any of the substances of the present invention and at least one other component such as a grow factor.

In a preferred embodiment, the present invention is a method for replacing hyaline cartilage in a patient with a hyaline cartilage defect. The method comprises or comprises the steps of, first, selecting a suitable patient. Then, a sufficient amount of hyaline cartilage replacement substance is provided. Next, the patient is prepped for surgery and the joint with the defect is exposed. Then, a hyaline cartilage replacement substance is placed into the defect. In a preferred embodiment, the hyaline cartilage replacement substance comprises a polypeptide comprising a sequence according to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3, or comprising a sequence according to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 where one or more than one amino acid of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides. In another preferred embodiment, the hyaline cartilage replacement substance comprises a pharmaceutical agent impregnated within the hyaline cartilage replacement substance.

**DESCRIPTION**

In one embodiment, the present invention is a substance that can be used to repair or replace hyaline cartilage defects in joints. The substance was derived from a natural
elastomer, referred to as "abductin," that is the major component of the elastic hinge ligament in marine scallops such as *Aequipecten irradians* and *Placopecten magellanicus*.

The primary structure of natural abductin has little if any sequence homology with other known natural elastomers. Functionally, abductin sustains recoil under compression while most other elastomers such as elastin sustain recoil under stretch conditions.

In the cross-linked state, natural abductin has an approximate elastic or Young's modulus of 3.0 MPa, nearly twice the Young's modulus of cartilage found in the anterior medial portion of the human tibia and the femoral head.

The cartilage replacement substance of the present invention can be used to repair hyaline cartilage defects due to several advantageous properties. First, individual molecules of the substance are capable of self-assembly in the absence of covalent cross-linking. Second, the matrices (aggregates) of the substance are biphasic, containing both peptide and water, like normal hyaline cartilage. The development and characterization of these substances will now be discussed in greater detail.

**A. Morphology of *Placopecten* Abductin**

*Placopecten magellanicus*, found off the coast of Nova Scotia, were obtained from the Canadian Department of Fisheries and Oceans. The scallops were shipped fresh on ice and were immediately sacrificed upon arrival. The dissected tissues were preserved in denaturing solution and frozen at -70°C. The abductor ligaments were dissected and stored at -20°C.

A *Placopecten* abductin sample fixed in Karnovsky's fixative and post-fixed with osmium tetroxide was subsequently embedded in epoxy resin and thin-sectioned for post-staining with uranyl acetate/Reynold's lead citrate and evaluated on a Zeiss Model 10 Transmission Electron Microscope at both low and high power.

Low power transmission electron microscopy revealed that *Placopecten* abductin is deposited in layers, much like the growth rings in trees. High power transmission electron microscopy of individual fibers suggested that the monomer aggregates were arranged similar to an intermittent string-of-beads with areas of poorly staining material possibly representing areas of glycosylation. The protein aggregates, 5-10 nm in diameter, were arranged linearly and attached to each other through some form of covalent cross-linking.

**B. Primary Structure of *Placopecten* Abductin**

The amino acid composition of abductin derived from *Placopecten magellanicus* was analyzed and revealed the prominent presence of four amino acids: glycine, aspartic acid,
methionine and phenylalanine. The results, expressed as residues per 100 are as follows:
Glycine 52, Methionine 12, Aspartate 10, Phenylalanine 6, Serine 4, Alanine 3, Glutamate 2,
Homocysteine 2, Lysine 2, Tyrosine 2, Arginine 1, Proline 1, Threonine 1 and Valine 1.
These amino acid composition results are unusual because of the high concentrations of the
two hydrophobic amino acids, phenylalanine and methionine. About 26% of the amino acid
residues in human elastin are hydrophobic as compared to 19% in abductin. Further, human
elastin contains about 3% phenylalanine compared to about 6% in abductin.

Peptide mapping of abductin derived from *Placopesten magellanicus* revealed a
repeating pentapeptide Phe-Gly-Gly-Met-Gly (FGGMG), residues 1-5 of SEQ ID NO:1, that
is also found in *Argopesten irradians* abductin:

MNAYICLAAC LIAAVSAAGY GGGAGSGMGT GGMGGGMNAG GFGGMGGGMGG
GKGGFGGIGG FGGMGGGPG GFGGMGGFGG MAAKGFGGGM GSGMGFFGGM
GGGNAGFGGGM GGGNAGFGGGM GGGGGFGGGK Y, SEQ ID NO:4. A protein sequence
homology search indicated that FGGMG, residues 1-5 of SEQ ID NO:1, occurs a maximum
of eight times within the published amino acid sequences for abductin derived from
*Argopesten irradians*. It was also observed that FGGMG, residues 1-5 of SEQ ID NO:1, is
largely found to occur at an ordered frequency such that each pentapeptide is flanked by five
amino acids on each end. The conservation of this repeating sequence between two species in
the family *Pectinidae* indicated that structural domains containing FGGMG, residues 1-5 of
SEQ ID NO:1 have an important functional role.

C. Secondary Structure of Hyaline Cartilage Replacement Peptides

To further elucidate the role of the peptide FGGMG, residues 1-5 of SEQ ID NO:1,
within the abductin peptide, a 25 amino acid portion of the abductin peptide,
FGGMGGGNAGFGGMGGGNAGFGGMG, SEQ ID NO:1, was synthesized via solid phase
peptide synthesis. SEQ ID NO:1 contains the largest repeating unit in *Argopesten* abductin,
the decapeptide FGGMGGNAG, residues 1-10 of SEQ ID NO:1, and is formed by two
GGNAG repeats, residues 5-10 of SEQ ID NO:1, flanked on either side by FGGMG,
residues 1-5 of SEQ ID NO:1.

Further, in order to study the effects of hydrophobic interaction, a modified version of
SEQ ID NO:1 was also synthesized, AGGMGGGNAGAGGMGGGNAGAGGMG, SEQ ID
NO:2, having Alanine residues substituted for each Phenylalanine residue. Both SEQ ID
NO:1 and SEQ ID NO:2 were studied using circular dichroism (CD) spectroscopy.
The secondary structures of both SEQ ID NO:1 and SEQ ID NO:2 were evaluated at 25°C and 45°C in hexafluoroisopropanol instead of TFE because both SEQ ID NO:1 and SEQ ID NO:2 were soluble only in HFIP. However, HFIP has similar helix inducing properties as TFE. Spectra for both SEQ ID NO:1 and SEQ ID NO:2 were taken from 190 nm to 250 nm with a pathlength of 0.1 cm.

The CD spectra revealed that SEQ ID NO:1 adopted a random coil conformation at 25°C and a polyproline II conformation at 45°C, where SEQ ID NO:2 remained in a random coil conformation at both 25°C and 45°C. These results indicate that SEQ ID NO:1 undergoes an inverse temperature transition much like the human elastin sequence Poly(VPGVG). Since SEQ ID NO:2 did not undergo a reverse temperature transition, it appears that the increased hydrophobicity provided by phenylalanine residues in SEQ ID NO:1 is responsible for the inverse temperature transition.

D. Biophysics of Hyaline Cartilage Replacement Peptides

Moisture analysis was performed on 73 mg of SEQ ID NO:1 and 71 mg of SEQ ID NO:2 after 22 hours of air drying at room temperature using a Denver Instruments model IR-200 moisture analyzer. Moisture analysis was carried out at 40°C with a preset cutoff moisture loss rate of 0.05 % moisture loss over 10 min. The temperature, weight and percent moisture were printed out every 60 seconds in order to monitor the kinetics of moisture loss for each peptide. After 24 hours of air drying, 83 mg of SEQ ID NO:1 and 80 mg of SEQ ID NO:2 were subjected to moisture analysis at 30°C using the same methods.

The relative gel forming abilities of both SEQ ID NO:1 and SEQ ID NO:2 and their gel stability were measured as a function of moisture loss per unit time and percent moisture retention at a predetermined moisture loss cutoff slope. Moisture loss was induced by increasing the temperature to 30°C and 40°C respectively and held constant using infrared heat until the rate of moisture loss was less than 0.05% over ten minutes (moisture loss slope cutoff parameter).

Moisture loss kinetics for SEQ ID NO:1 and SEQ ID NO:2 indicated that SEQ ID NO:1 retained more moisture than SEQ ID NO:2 at both 30°C and 40°C although SEQ ID NO:1 lost moisture faster than SEQ ID NO:2 at 40°C. At 30°C, SEQ ID NO:2 had the higher rate of moisture loss. At this temperature, both SEQ ID NO:1 and SEQ ID NO:2 assume a random coil conformation. At 40°C, however, SEQ ID NO:1 had the higher rate of moisture loss. At this temperature, SEQ ID NO:1 assumes a polyproline II conformation.
while SEQ ID NO:2 remains in a largely random coil conformation. Further, in going from 30°C to 40°C, the rate of moisture loss for SEQ ID NO:1 increased exponentially while the rate of moisture loss for SEQ ID NO:2 increased linearly, indicating that the inverse temperature transition is responsible for the exponential increase in the rate of moisture loss for SEQ ID NO:1.

Moisture retention was also related to the inverse temperature transition. Moisture content analysis indicated that SEQ ID NO:1 and SEQ ID NO:2 contained 79.52% and 88.75% moisture, respectively, at 30°C, and 84.93% and 94.37% moisture, respectively, at 40°C. Therefore, at both 30°C and 40°C SEQ ID NO:1 retains more moisture than SEQ ID NO:2. These results show the maximum amount of moisture evaporated from each peptide sample within the slope cutoff parameter and indicates that SEQ ID NO:1 and SEQ ID NO:2 gel matrices retard the rate of moisture loss such that their final moisture contents are not absolute but rather a measure of percent moisture retention within the moisture loss cutoff slope. The fact that SEQ ID NO:1 retains more moisture at 40°C than SEQ ID NO:2 may be due to its ability to undergo an inverse temperature transition to a more ordered structural conformation. In addition, the CD spectrum of SEQ ID NO:2 at 45°C shows signs of denaturing that may also explain why SEQ ID NO:2 retains less moisture than SEQ ID NO:1 at 40°C.

Temperature-dependent turbidimetry studies were performed on both SEQ ID NO:1 and SEQ ID NO:2. The results indicated that both SEQ ID NO:1 and SEQ ID NO:2 aggregate or self-assemble between 23°C and 30°C. The maximum absorbance for SEQ ID NO:1 was roughly 0.09 optical density units larger than the maximum absorbance for SEQ ID NO:2, indicating that SEQ ID NO:1 self-assembles to a greater extent than SEQ ID NO:2, probably due to the phenylalanine residues present in SEQ ID NO:1. In addition, SEQ ID NO:1 had a lower critical solution temperature, the temperature below which polymers are soluble and above which polymers form insoluble aggregates, of 23°C while SEQ ID NO:2 had a lower critical solution temperature of 24°C.

E. Morphology of Hyaline Cartilage Replacement Peptide Matrices

Environmental scanning electron microscopy of SEQ ID NO:1 and SEQ ID NO:2 shows that both peptides form biphasic (protein + water) fibers also indicative of self-assembly and gel formation. However, the SEQ ID NO:2 fibers are significantly smaller than SEQ ID NO:1 fibers measuring roughly 4 μm and 20 μm in diameter, respectively.
Both peptides also form larger networks of fibers. The larger fibers of SEQ ID NO:1 appear to form pores that are about twice as large as pores formed by SEQ ID NO:2 fibers. This pore size difference can be accounted for by the data showing that SEQ ID NO:1 retains roughly 9% more moisture than SEQ ID NO:2 at 30°C. Hence SEQ ID NO:1 retains more water because of the larger capacity of its porous gel matrix compared to the capacity of the porous gel matrix formed by SEQ ID NO:2.

**F. Design and Synthesis of an additional Hyaline Cartilage Replacement Peptide**

Next, a synthetic peptide was designed that could be covalently cross-linked into a stable three-dimensional matrix. The peptide incorporated FGGMGGGNAGFGGMMGNNAGFGGGMG, SEQ ID NO:1, and two partial human Factor XIII substrate sequences, TIGEGQHHHLG, residues 1-12 of SEQ ID NO:3, and GAKDV, residues 38-42. Residues 1-12 of SEQ ID NO:3 were incorporated on the N-terminus of SEQ ID NO:1 and residues 38-42 of SEQ ID NO:3 were incorporated twice consecutively on the C-terminus end of SEQ ID NO:1 to produce TIGEGQHHHLGFGGMMGNNAGFGGGMMGNNAGFGGGMMGAKDVGAKDV, SEQ ID NO:3

SEQ ID NO:3 was synthesized via standard solid phase FMOC chemistry on a preloaded resin. After synthesis, 100 mg of peptide resin were cleaved using a standard TFA cleavage protocol and the peptide was immediately precipitated in ether to remove the hydrophobic protecting groups. The precipitated peptide was then spun down and dried under a gentle stream of nitrogen overnight.

Though designed to be covalently cross-linked, the dry peptide SEQ ID NO:3 surprisingly formed a gel-like, nearly translucent, yellowish pellet that was rubbery to the touch, even though it was not covalently cross-linked. The initial aggregate was held together by a combination of peptide monomer “entanglement” and intermolecular hydrophobic interactions or “hydrophobic cross-links” in the absence of covalent cross-linking. SEQ ID NO:3 monomer “entanglement” probably occurred between the amorphous regions of the peptide when exposed to ether. Additionally, hydrophobic cross-linking provided enough structural stability to perform mechanical property measurements on SEQ ID NO:3 aggregate that was not covalently cross-linked. Further, the biphasic (protein + water) properties of this material suggested that there is some water sequestered within that most likely contributes to the elastic properties. SEQ ID NO:3 gave some fluorescence upon
exposure to ultraviolet light and was only marginally soluble in water due to its hydrophobic F and M residues.

G. Mechanical Properties and Biophysics of the Additional Hyaline Cartilage Replacement Peptide

The resistance to compressive load of the cartilage substitute, SEQ ID NO:3, was experimentally determined using the Finkin’s indentation method on a nickel size aggregate of SEQ ID NO:3. This method allowed for the computation of the Young’s modulus from the indentation of a thin elastomeric layer supported by a rigid half-space.

The calculated Young’s modulus for SEQ ID NO:3 was approximately 0.08 MPa (± 0.04). Although this Young’s modulus is about 19 times lower than the Young’s modulus for knee or hip hyaline cartilage, the ability of SEQ ID NO:3 to form an aggregate without covalent cross-linking at all was unexpected and these data prove that hydrophobic interactions alone can account for at least partial elastomeric stability.

Moisture loss analysis was performed to determine the relative gel forming ability of SEQ ID NO:3 and gel stability was measured as a function of moisture loss per unit time and percent moisture retention at a predetermined moisture loss cutoff slope. Moisture loss was induced by increasing the temperature to 30°C and 40°C respectively and held constant using infrared heat until the rate of moisture loss was less than 0.05% over ten minutes (moisture loss cutoff slope). Similar tests were performed with SEQ ID NO:1 and SEQ ID NO:2 for comparison.

At 30°C, SEQ ID NO:2 had the fastest initial rate of moisture loss followed by SEQ ID NO:3 and SEQ ID NO:1. At 40°C, SEQ ID NO:1 had the fastest initial rate of moisture loss followed by SEQ ID NO:2 and SEQ ID NO:3. As stated above, at 40°C, SEQ ID NO:1 assumes a polyproline II structure while SEQ ID NO:2 remains in a random coil conformation. Interestingly, SEQ ID NO:3 behaves similarly to SEQ ID NO:2 with regard to initial rate of moisture loss. The initial rate of moisture loss at 40°C for SEQ ID NO:1 is 4.4 times as fast as its initial rate of moisture loss at 30°C whereas the initial rates of moisture loss at 40°C for SEQ ID NO:2 and SEQ ID NO:3 are 1.9 and 1.8 times as fast as their respective initial rates of moisture loss.

The moisture loss curve for SEQ ID NO:1 and SEQ ID NO:2 showed a 5.41% and 5.62% decrease, respectively, in moisture retention in going from 30°C to 40°C while SEQ ID NO:3 surprisingly showed a gain in moisture retention of 6.08% in going from 30°C to
40°C. This finding indicated that the SEQ ID NO:3 gel matrix actually becomes more stable at 40°C than it was at 30°C and that SEQ ID NO:3 should self-assemble to an even greater extent than SEQ ID NO:1 and SEQ ID NO:2, thus providing added stability to the SEQ ID NO:3 gel matrix at 40°C.

In order to further find a trend in relative gel stability for SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3, all three peptides were also subjected to moisture loss analysis at 50°C. At this temperature, the initial rates of moisture loss for SEQ ID NO:1 (16.18 %/min. ± 1.09) and SEQ ID NO:3 (15.46 %/min. ± 1.66) have essentially converged having no significant difference while SEQ ID NO:2 (28.39 %/min. ± 3.78) had the highest rate of moisture loss. With regard to moisture content, SEQ ID NO:1 retains 2.44% more moisture than SEQ ID NO:2 while SEQ ID NO:3 retains 7.31% more moisture than SEQ ID NO:1.

Initial rate of moisture loss plotted against temperature for each peptide, as a measure of moisture loss kinetics, gives a polynomial, linear and logarithmic curve for SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3, respectively. Additionally, percent moisture content plotted against temperature for each peptide gives a linear, logarithmic and polynomial curve for SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3 respectively. In both cases, SEQ ID NO:3 appears to have superior gel stability at all three temperatures, most notably at 40°C.

At 40°C, the initial rate of moisture loss for SEQ ID NO:3 is far lower than the initial moisture loss rates for SEQ ID NO:1 and SEQ ID NO:2. Moisture retention is also dramatically higher for SEQ ID NO:3 at 40°C as evidenced by a drop in moisture content. Thus, SEQ ID NO:3 undergoes some form of temperature dependent structural changes and self-assembly based on its moisture loss kinetics at 40°C.

H. Conservative Substitutions of Hyaline Cartilage Replacement Substance

In a preferred embodiment, the present invention also comprises one or more than one substance based on SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, where one or more than one amino acid in one of these sequences is conservatively substituted with another amino acid. Conservative substitution means the replacement of a first amino acid residue with a second amino acid residue where the second amino acid residue does not substantially affect the advantageous properties of the substance. Such substitutions are well known in the art and include substituting a tryptophan, leucine or isoleucine for a phenylalanine, substituting a valine for a methionine and substituting a glutamate for an aspartate.

I. Production of Hyaline Cartilage Replacement Substance with Covalent Cross-linking
In another preferred embodiment, the present invention also comprises one or more than one substance based on SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 in aggregates where at least some of the individual polypeptides are covalently cross-linked. These covalently cross-linked aggregates of hyaline cartilage replacement substance can be used for replacement of cartilage defects as can the non-covalently cross-linked aggregates disclosed in this disclosure.

In one embodiment, the covalent cross-linking is accomplished by substituting one or more than one cysteine or homocysteine or residue for one or more than one methionine residue, as will be understood by those with skill in the art with reference to this disclosure.

In another preferred embodiment, the covalent cross-linking can be accomplished by exposing an aggregate of a hyaline cartilage replace substance according to the present invention to gamma irradiation, as will be understood by those with skill in the art with reference to this disclosure.

**J. Method for Replacing Hyaline Cartilage**

In one embodiment, the present invention is a method for replacing hyaline cartilage in a patient with a hyaline cartilage defect. The method includes, first, selecting a suitable patient to undergo the procedure. A suitable patient has a hyaline cartilage defect in the articular surface of a joint, whether from disease or from injury, and who is one of sufficient health to undergo the procedure with an acceptable risk of morbidity and mortality.

Screening patients for these criteria is well within the level of skill of health care practitioners of ordinary skill in the art

Next, a sufficient amount of hyaline cartilage replacement substance is prepared for the procedure. The hyaline cartilage replacement can be any of the substances according to the present invention, and can comprise more than one substance according to the present invention. In a preferred embodiment, the substance comprises SEQ ID NO:3. Preferably, the substance is sterilized in a manner that does not affect its advantageous physical properties. For example, sterilization can be effected through radiation, rather than through heat.

Then, the patient is prepped for surgery in a manner suitable for operating on the joint with the hyaline cartilage defect, as will be understood by those with skill in the art with reference to this disclosure. The joint is then exposed and cleaned of damaged hyaline
cartilage remnants. The hyaline cartilage replacement substance is then placed into the defect in a sufficient amount to restore the articular surface. The joint is then restored and closed.

In a preferred embodiment, the hyaline cartilage replacement substance comprises human chondrocyte cells within the aggregate. The aggregate seeded with chondrocyte cells are produced by growing chondrocyte cells on the aggregate before implantation, preferably by using chondrocyte growth factors, as will be understood by those with skill in the art with reference to this disclosure.

In another preferred embodiment, hyaline cartilage replacement substance comprises one or more than one pharmaceutical agents impregnated within the substance, such as cartilage growth factors. The one or more than one pharmaceutical agents is impregnated within the substance by rehydrating partially dehydrated aggregates of the substance in a mixture of water and the one or more than one pharmaceutical agent.

Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure.
WHAT IS CLAIMED IS:

1. A substance for replacing hyaline cartilage comprising a polypeptide having a sequence according to SEQ ID NO:1 repeated at least two times, where the two repeated segments are either consecutive or are separated by one or more than one additional amino acid residues.

2. The substance of claim 1, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

3. A substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:1 and having no more than about 100 amino acids residues in the entire sequence.

4. The substance of claim 3, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

5. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:1, and water.

6. The substance of claim 5, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

7. A composition for replacing hyaline cartilage comprising the substance of claim 5.

8. The substance of claim 7, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

9. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:1 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

10. The substance of claim 9, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

12. The substance of claim 10, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

13. A substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2.

14. The substance of claim 13, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

15. A substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2 and having no more than about 100 amino acids residues in the entire sequence.

16. The substance of claim 15, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

17. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:2, and water.

18. The substance of claim 17, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

19. A composition for replacing hyaline cartilage comprising the substance of claim 17.

20. The substance of claim 19, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

21. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:2 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

23. The substance of claim 21, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

24. A substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:3.

25. The substance of claim 24, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

26. A substance for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:3 and having no more than about 100 amino acids residues in the entire sequence.

27. The substance of claim 26, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

28. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:3, and water.

29. The substance of claim 28, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.


31. The substance of claim 30, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

32. A substance for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:3 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

33. A composition for replacing hyaline cartilage comprising the substance of claim 32.
34. The substance of claim 32, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

35. Means for replacing hyaline cartilage comprising a polypeptide having a sequence according to SEQ ID NO:1 repeated at least two times, where the two repeated segments are either consecutive or are separated by one or more than one additional amino acid residues.

36. The means of claim 35, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

37. Means for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:1 and having no more than about 100 amino acids residues in the entire sequence.

38. The means of claim 37, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

39. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:1, and water.

40. The means of claim 39, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

41. Means for replacing hyaline cartilage comprising the means of claim 39.

42. The means of claim 41, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

43. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:1 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

44. The means of claim 43, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

45. Means for replacing hyaline cartilage comprising the means of claim 43.
46. The means of claim 44, where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

47. Means for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2.

48. The means of claim 47, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

49. Means for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:2 and having no more than about 100 amino acids residues in the entire sequence.

50. The means of claim 49, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

51. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:2, and water.

52. The means of claim 51, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

53. Means for replacing hyaline cartilage comprising the means of claim 51.

54. The means of claim 53, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

55. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:2 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

56. Means for replacing hyaline cartilage comprising the means of claim 55.

57. The means of claim 55, where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.
58. Means for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:3.

59. The means of claim 58, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

60. Means for replacing hyaline cartilage comprising a polypeptide comprising a sequence according to SEQ ID NO:3 and having no more than about 100 amino acids residues in the entire sequence.

61. The means of claim 60, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

62. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide consisting essentially of a sequence according to SEQ ID NO:3, and water.

63. The means of claim 62, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

64. Means for replacing hyaline cartilage comprising the means of claim 62.

65. The means of claim 64, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

66. Means for replacing hyaline cartilage consisting essentially of an aggregate of polypeptides, each polypeptide comprising a sequence according to SEQ ID NO:3 and comprising no more than about 100 amino acids residues in the entire sequence, and water.

67. Means for replacing hyaline cartilage comprising the means of claim 66.

68. The means of claim 66, where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

69. Means for replacing hyaline cartilage.

70. Means for replacing hyaline cartilage comprising an aggregate of polypeptides and water.
71. A method for replacing hyaline cartilage in a patient with a hyaline cartilage
defect, comprising:
   a) selecting a suitable patient;
   b) providing a sufficient amount of hyaline cartilage replacement substance;
   c) prepping the patient for surgery;
   d) exposing the joint with the defect; and
   e) placing a hyaline cartilage replacement substance into the defect.

72. The method of claim 71, where the hyaline cartilage replacement substance
comprises a polypeptide comprising a sequence according to SEQ ID NO:1, or comprising a
sequence according to SEQ ID NO:1 where one or more than one amino acid of SEQ ID
NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID
NO:1 is substituted to promote cross-linking between individual polypeptides.

73. The method of claim 71, where the hyaline cartilage replacement substance
comprises a polypeptide comprising a sequence according to SEQ ID NO:2, or comprising a
sequence according to SEQ ID NO:2 where one or more than one amino acid of SEQ ID
NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID
NO:2 is substituted to promote cross-linking between individual polypeptides.

74. The method of claim 71, where the hyaline cartilage replacement substance
comprises a polypeptide comprising a sequence according to SEQ ID NO:3, or comprising a
sequence according to SEQ ID NO:3 where one or more than one amino acid of SEQ ID
NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID
NO:3 is substituted to promote cross-linking between individual polypeptides.

75. The method of claim 71, where the hyaline cartilage replacement substance
comprises a pharmaceutical agent impregnated within the hyaline cartilage replacement
substance.

76. A method for replacing hyaline cartilage in a patient with a hyaline cartilage
defect, comprising the steps of:
   a) selecting a suitable patient;
   b) providing a sufficient amount of hyaline cartilage replacement substance;
   c) prepping the patient for surgery;
   d) exposing the joint with the defect; and
   e) placing a hyaline cartilage replacement substance into the defect.
77. The method of claim 76, where the hyaline cartilage replacement substance comprises a polypeptide comprising a sequence according to SEQ ID NO:1, or comprising a sequence according to SEQ ID NO:1 where one or more than one amino acid of SEQ ID NO:1 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:1 is substituted to promote cross-linking between individual polypeptides.

78. The method of claim 76, where the hyaline cartilage replacement substance comprises a polypeptide comprising a sequence according to SEQ ID NO:2, or comprising a sequence according to SEQ ID NO:2 where one or more than one amino acid of SEQ ID NO:2 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:2 is substituted to promote cross-linking between individual polypeptides.

79. The method of claim 76, where the hyaline cartilage replacement substance comprises a polypeptide comprising a sequence according to SEQ ID NO:3, or comprising a sequence according to SEQ ID NO:3 where one or more than one amino acid of SEQ ID NO:3 is conservatively substituted, or where one or more than one amino acid of SEQ ID NO:3 is substituted to promote cross-linking between individual polypeptides.

80. The method of claim 76, where the hyaline cartilage replacement substance comprises a pharmaceutical agent impregnated within the hyaline cartilage replacement substance.
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        Sandberg, Lawrence B
        Jimenez, Felipe

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