BIOMATERIAL AND PREPARATION METHOD THEREOF

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ABSTRACT
A biomaterial prepared from a process comprising dehydrating the fish scales until the fish scales containing less than about 50% of water, and grinding the dehydrated fish scales into ground particles each having an average size of less than about 10,000 µm in diameter, wherein the ground particles contain a mixture of sponge-like matrix and powder is provided. The invention also provides a biomaterial prepared from fish scales by a process comprising subjecting the fish scales to a heat treatment at a temperature of less than about 200°C.
FIG. 1 A

Flowchart:
- Scales
  - Clean
    - Dehydrated
      - Ground
        - Matrix
        - Powder
  - soaked
    - Extrusion
      - Flaky
Cleaning the fish scales

Dehydrating the fish scales until the fish scales contain less than 50% of water

Grinding the fish scales into ground particles each having a size of less than 10000 \( \mu \text{m} \) in diameter

Separating matrix and powder from the ground particles

**FIG. 1 B**
Cleaning the fish scales

Dehydrating the fish scales until the fish scales contain less than 50% of water

Grinding the fish scales into ground particles each having a size of less than 10000 μm in diameter

Separating matrix and powder from the ground particles

Extrusion at a temperature less than 200°C

FIG. 1 C
BIOMATERIAL AND PREPARATION METHOD THEREOF

[0001] This application is a divisional application of pending of U.S. patent application Ser. No. 11/882,328, filed Jul. 31, 2007 (of which the entire disclosure of the pending, prior application is hereby incorporated by reference).

BACKGROUND OF THE INVENTION

[0002] The present invention relates to a biomaterial and preparation method thereof, and particularly to a biomaterial prepared from fish scales for use in tissue repair and implantation.

[0003] Tissue engineering that involves the incorporation of a biomaterial with biologics and/or pharmaceutics and upon implantation in a patient will stimulate angiogenesis, tissue integration, and/or tissue remodeling. The biomaterial is a synthetic and biocompatible material that is used to construct artificial organs, rehabilitation devices, or prostheses and replace natural body tissues.

[0004] For over decades, collagen fiber, hydroxyapatite (HAP) and tri-calcium phosphate (TCP) are some biomaterials with great biocompatibility and safety to be used in human tissue implant. However, these biomaterials have disadvantages such as low mechanical strength, risk of chemical residue in cross linking, terrestrial animal transmitted disease.

[0005] Therefore, it is desirable to develop a biomaterial having a high mechanical strength, low possibility of contracting with the terrestrial contagious disease and is applicable to tissue repairs or implants.

BRIEF SUMMARY OF THE INVENTION

[0006] It is an aspect of the invention to provide a biomaterial prepared from fish scales by a process which includes dehydrating the fish scales until the fish scales containing less than about 50% of water, and grinding the dehydrated fish scales into ground particles having an average size of less than about 10,000 in diameter, wherein each of the ground particles contain a mixture of sponge like matrix and powder. In an embodiment of the invention, the fish scales contain less than about 25% of water, and the ground particles have an average size of less than about 5,000 μm in diameter.

[0007] It is another aspect of the invention to provide a biomaterial prepared from fish scales by a process, which comprises subjecting the fish scales to a heat treatment at a temperature of less than about 200°C.

[0008] It is a further aspect of the invention to provide a method for preparing a biomaterial. The method comprises dehydrating the fish scales until the fish scales containing less than about 50% of water, and grinding the dehydrated fish scales into ground particles having an average size of less than about 10,000 μm in diameter, wherein the ground particles contain a mixture of sponge like matrix and powder. In an embodiment of the invention, the fish scales contain less than about 25% of water, and the ground particles have an average size of less than about 5,000 μm in diameter.

[0009] It is one other aspect of the invention to provide a method for preparing a biomaterial comprising subjecting the fish scales to a heat treatment at a temperature of less than about 200°C.

[0010] It is yet another aspect of the invention to provide a use of the biomaterial prepared by the process described above for repairing tissues.

[0011] It is yet another aspect of the invention to provide a use of the biomaterial prepared by the process described above for tissue implantation.

DETAILED DESCRIPTION OF THE INVENTION

[0022] Reference will now be made in detail to the present embodiments of the invention, examples of which are illustrated in the accompanying drawings.

[0023] Referring to FIG. 1A, the present invention relates to a biomaterial prepared from fish scales 10. The biomaterial is prepared by a process which comprises dehydrating the fish scales 10 (S12) until the fish scales 10 contain less than about 50% of water. The dehydrated fish scales 10 are then ground (S13) into a ground product having a particle size of less than about 10,000 μm. In an embodiment of the invention, the fish scales 10 contain less than about 25% of water, and the ground particles have an average size of less than about 5,000 μm in diameter.

[0024] In accordance with some examples of the invention, the fish scales 10 may be freshly provided in a chilled or frozen manner. In a specific example of the invention, the fish scales 10 each having an average size less than about 20 cm in diameter may be selected for preparing the biomaterial. Prior to dehydration of the fish scales, the fish scales 10 may be cleaned by washing (S11) in a steam with other cleaning agents including but not limited to surfactant, detergent, warm water and polar solvent such as ethanol at about 60°C. However, the present invention is not limited to any particular cleaning step. For example, the fish scales are clean enough to pass Limulus Amebocyte Lyse (LAL) test which is an assay
for detection and quantitation of bacterial endotoxin. Preferably, the cleaned fish scales would have a LAL test score less than about 100 EU/ml.

[0025] According to one example of the invention, the fish scales 10 may be dehydrated (S12) (but not limited) after they are cleaned (S11) as described above. The fish scales may be dehydrated by air drying, oven, freeze drying or any other conventional dehydration methods available so far. Also, the fish scale may be dehydrated by soaking the fish scales in the ethanol or other polar organic solvent. The fish scales are dehydrated until their water content is less than about 50%, preferably less than about 25%. According to another example of the invention, the dehydrated fish scales may be ground into particles each having an average size of less than about 10,000 μm, preferably about 5000 μm, in diameter. The ground particles may contain a mixture of sponge like matrix 14 and powder 15. For example, the ground particles may be further filtered using an optimal sieve to isolate the matrix and powder. The filtering step may be carried out with an aid of a vibrating means to enhance the sieving effect. As a result, the particles filtered out from the sieve provide the biomaterial in a powder form, whereas the filtrate that remains from the filtering step makes up the biomaterial in a matrix form. Also, the device adopted for grinding the fish scales are not limited to using any particular grinder, grinding machine or any equipment used to reduce the particle size of the fish scales, as long as each of the ground particles does not exceed an average size of less than about 10,000 μm, preferably less than about 5000 μm, in diameter.

[0026] These products may be further processed, for example, by extrusion (S16), filtering, fully or partially drying and sterilizing to yield sterilized biomaterials. In a preferred embodiment, these steps may be performed with or without heating. These products may be used in combination with a variety of connective tissue repair compositions, or in combination with other active or inactive ingredients.

[0027] In one other example, the dehydrated fish scales may be subjected to a heat treatment, such as an extrusion process performed at a temperature of less than about 200°C, preferably, about from 110°C to 200°C, to produce the biomaterial in a flaky form. As one specific example, the dehydrated fish scales may be subjected to the extrusion with or without one or more cross linking ingredients. However, the present invention is not limited to the above method for producing the biomaterial in the flaky form. For example, the fish scales may also be subjected to an extrusion process performed at the temperature of less than about 200°C after the cleaning step (S11) to produce the biomaterial in flaky form 17. An additional step (S18) of soaking the fish scales in water is selectively performed before the extrusion process (S16), but after the cleaning step (S11). Moreover, in yet another example of the invention, the biomaterials in the matrix 14 or powder form 15 may be further subjected to an extrusion process (S16) performed at a temperature of less than about 200°C to convert into the biomaterial in the flaky form 17. However, the heat treatment in the present invention is not limited to the extrusion described above. One skilled in the art may also adopt other heat treatments such as thermal extrusion of any type, thermal pressing and molding steps to produce the flaky biomaterial.

[0028] The biomaterial of the invention contains tissue repair factors and may be manufactured into a tissue repair material for repairing a variety of tissue damages and tissue defect sites. For example, the biomaterial of the invention may be prepared for injection or insertion at, into, onto or near bone defect sites, cartilage repair sites, dental alveolar repair site or other soft tissue defect sites. In other examples, the biomaterial may be made as a coating material coated on surgical grafts or implants to be implanted at, into, onto or near bone defect sites, cartilage repair sites or other tissue defect sites. Accordingly, the invention is also applicable to connective tissue surgical implant with the tissue repair material derived from fish scales, whereby the surgical implant is implanted at a connective tissue defect site.

[0029] Summarizing from the above, the invention relates to a biomaterial in powder and/or matrix and/or flaky form prepared from the fish scales for use in a variety of tissue repairs and implantations. These fish scale derived products, also referred to herein as the biomaterial in a powder form, matrix form or flaky form may contain tissue repair factors and may be further processed to produce a variety of formulations and consistencies.

[0030] The invention will now be described in further detail with reference to the following specific, non-limiting examples.

**Example 1**

**Matrix Form**

[0031] Referring to FIG. 1A and FIG. 1B, the process for preparing the biomaterial in a matrix form from fish scales begins with step (S20), where the fish scales are cleaned with a steam or vapor, for example, until the clean fish scales have a LAL test score of less than about 200 EU/ml. The process then proceeds to step (S21). In step (S21), the fish scales are dehydrated until the fish scales contain less than 25% of water. Next, in step (S22), the dehydrated fish scales are ground into particles each having an average size of less than 5000 μm in diameter. The ground particles contain a mixture of sponge like matrix and powder. The process proceeds to step (S23). In step (S23), a filtering step is carried out using an optimal sieve for separating the matrix from the mixture. Accordingly, the filtrate from the filtering step makes up the matrix. The matrix contains fibrous tissue that is composed of HAP, TCP and collagen. The size of the fibrous is less than 2.5 mm in diameter.

**Example 2**

**Powder Form**

[0032] Referring to FIG. 1A and FIG. 1B again, the process for preparing the biomaterial in a powder form is similar to that for the biomaterial in matrix form except that the biomaterial in powder form include the particles filtered out and left on the sieves after the filtering step is carried out. Therefore, the powder form differs from the matrix form in that the powder form has a definite or mechanical structure. And the powder size is less than 5000 μm in diameter.

**Example 3**

**Flaky Form**

[0033] There are a number of ways for preparing the biomaterial in flaky form from the fish scales according to the process flow illustrated in FIG. 1A and FIG. 1C. Referring to FIG. 1A and FIG. 1C, a process for preparing the biomaterial in a flaky form from fish scales begins with step (S30), where the fish scales are cleaned with a steam or vapor, for example,
until the clean fish scales have a LAL test score of less than about 200 Eu/ml. The process then proceeds to step (S31). In step (S31), the fish scales are dehydrated until the fish scales contain less than 25% of water. Next, in step (S32), the dehydrated fish scales are ground into particles each having an average size of less than 5000 µm in diameter. The ground particles contain a mixture of sponge-like matrix and powder. The process proceeds to step (S33). In step (S33), a filtering step is carried out using an optimal sieve for separating the matrix and powder from the mixture. The process proceeds to step (S34) which includes subjecting the matrix and powder to extrusion.

[0034] Alternatively, the process for preparing the biomaterial in flaky form may proceed directly to step (S34) after the cleaning step to simplify the overall process. Also, the process may include the cleaning and dehydration steps before performing the extrusion step on the cleaned and dehydrated fish scales as shown in FIG. 1C.

[0035] Accordingly, the dehydrated fish scales, cleaned fish scales without dehydration, or the biomaterial derived from the fish scales in matrix or powder form are subjected to the extrusion step to produce the biomaterial in flaky form. The intact fish scales or different types of biomaterials derived from fish scales, previously cold pressed with a pressure of more than 100 kg in 2.5 cm², preferably more than 1 kg in 2.5 cm², are subjected to hot pressing performed at a temperature of less than about 200° C. in a desired mold. The cross-linking of the biomaterials can be achieved physically by heating or chemically by adding with a cross linker at an optimal concentration before extrusion is performed. The cross linker is reactive with the amine group or other reactive group in the biomaterials.

[0036] FIG. 2A is an SEM picture of 3T3 (fibroblast cell 22) culture with the biomaterial 20 described in present invention for 5 days. FIG. 2B is an SEM picture of osteoblastoma 24 culture with the biomaterial 20 described in the present invention for 5 days. These pictures show that cells (fibroblast 22 or osteoblastoma 24) can grow well on the biomaterial 20 described in the present invention.

[0037] FIG. 3A is a confocal picture of 3T3 (fibroblast cell) culture with this invention for 5 days. FIG. 3B is a confocal picture of osteoblastoma culture with this invention for 5 days. These pictures show that cells (fibroblast or osteoblastoma) can grow well on this invention.

[0038] FIG. 4 is a H&E stain picture of osteoblastoma cell 24 culture with the biomaterial 20 described in the present invention for 5 days. These pictures show that osteoblastoma cell 24 can grow well on the biomaterial 20 described in the present invention.

[0039] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[0040] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the appended claims.

1. A method for preparing a tissue repair biomaterial from fish scales, comprising the step of:
   - thorough cleaning of a fish scale, enough to pass Limulus Amebocyte Lysate (LAL) test;
   - grinding said fish scale into a plurality of ground particles, wherein the ground particles contain a mixture of sponge made of fibrous tissue and powder.

2. The method according to claim 1, further comprising subjecting the sponge made of fibrous tissue and powder to an extrusion performed at a temperature of less than about 200° C. for forming a flaky form.

3. The method according to claim 1, further comprising a step of dehydrating the fish scale before grinding the fish scale, but after cleaning the fish scale.

4. The method according to claim 3, wherein the step of dehydrating the fish scale comprises dehydrating the fish scale until the fish scale containing less than about 50% of water.

5. The method according to claim 1, further comprising a step of separating the sponge made of fibrous tissue and powder from the ground particles.

6. The method according to claim 1, wherein the step of grinding comprises grinding the fish scale into a plurality of ground particles until the ground particles having an average size of less than about 10,000 µm in diameter.

7. The method according to claim 1, wherein the step of grinding comprises grinding a fish scale into a plurality of ground particles, wherein the fish scale has an average diameter of less than 20 cm.

8. A method for preparing a tissue repair biomaterial from fish scales, comprising the step of:
   - thorough cleaning of a fish scale, enough to pass Limulus Amebocyte Lysate (LAL) test;
   - subjecting the fish scale to an extrusion process for forming a flaky form.

9. The method according to claim 8, wherein the extrusion is performed at a temperature of less than about 200° C.

10. The method according to claim 8, further comprising a step of dehydrating the fish scale after cleaning the fish scale, but before subjecting the fish scale to the extrusion process.

11. The method according to claim 10, wherein the step of dehydrating the fish scale comprises dehydrating the fish scale until the fish scale containing less than about 50% of water.

12. The method according to claim 8, further comprising a step of soaking the fish scale in water before subjecting the fish scale to the extrusion process.

13. The method according to claim 8, wherein the step of subjecting the fish scale to the extrusion process comprises subjecting the fish scale to the extrusion process for forming a flaky form, wherein the fish scale has an average diameter of less than 20 cm.

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