DEHYDRATED SUBMUCOSAL TISSUE BIOACTIVE POWDER FOR PROMOTING CELL GROWTH IN A HOST AND METHOD OF PREPARING THE SAME

TECHNICAL FIELD

[0001] The present disclosure relates to extracellular matrix bioactive compositions and their use in repairing damaged or diseased tissues. More particularly, the present disclosure relates to a dehydrated submucosal tissue bioactive powder obtained from a pig urinary bladder submucosa tissue, or from any suitable submucosa tissues extracted from a warm-blooded vertebrate, for promoting cell growth (endogenous tissue growth), and further to a method of preparing the same.

BACKGROUND

[0002] In biology, the extracellular matrix (ECM) is a collection of extracellular molecules secreted by support cells that provides structural and biochemical support to the surrounding cells. Because multicellularity evolved independently in different multicellular lineages, the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM. ECM has been found to cause regrowth and healing of tissue.

[0003] In terms of injury repair and tissue engineering, the ECM serves two main purposes. First, it prevents the immune system from triggering from the injury and responding with inflammation and scar tissue. Second, it facilitates the surrounding cells to repair the tissue instead of forming scar tissue. For medical applications, the ECM required is usually extracted from pig bladders, an easily accessible and relatively unused source. It is currently being used regularly to treat ulcers by closing the hole in the tissue that lines the stomach, but further research is currently being done by many universities as well as the U.S. Government for wounded soldier applications. In 2007, testing was being carried out on a military base in Texas. Scientists were using a powdered form of the pig bladder ECM (pig bladder submucosal tissue) on veterans whose body parts were damaged in the war.

[0004] Common events to this remodeling process include: widespread and very rapid neovascularization, proliferation of granulation mesenchymal cells, biodegradation/resorption of implanted submucosal tissue material, and lack of immune rejection.

[0001] Submucosal tissues harvested from warm-blooded vertebrates appears to be a collagenous matrix that has shown great promise for inducing the repair of damaged or diseased tissues, and for inducing the proliferation and differentiation of cell populations. Submucosal tissue consists primarily of extracellular matrix material prepared by mechanically removing selected portions of the mucosa and the external muscle layers and then by lysing resident cells with hypotonic washes.

[0002] Numerous studies have shown that submucosal tissue is capable of inducing host tissue proliferation, remodeling and regeneration of tissue structures following implantation in a number of in vivo microenvironments including lower urinary tract, body wall, tendon, ligament, bone, cardiovascular tissues and the central nervous system. Upon implantation, cellular infiltration and a rapid neovascularization are observed and the submucosa extracellular matrix material is remodeled into host replacement tissue with site-specific structural and functional properties.

[0003] Submucosal tissue can be obtained from various sources, including intestinal tissue harvested from animals raised for meat production, including, for example, pigs, cattle and sheep or other warm-blooded vertebrates. Direct interactions between extracellular matrix components and cells are known to mediate processes which are fundamental to migration, proliferation and differentiation during development. However, the role of the extracellular matrix in wound healing and tissue regeneration has been less well studied.

[0004] It is well known in the art to process a submucosal tissue of a warm-blooded vertebrate into a fine powder, prior it is lyophilized (freeze-dried) for its conservation. Such powder can then be mixed with collagenase to form an ECM derived hydrogel (self-healing hydrogels). Though hydrogels do not yet have direct clinical relevance, they have shown promise as a method of assisting in organ regeneration.

[0005] Even if the powder described above provides interesting remodeling and rejuvenation features, it will remain hard to be commercialized as it needs to be stored in a frozen hydrated state until its usage (the fine powder is lyophilized). It brings many challenges in designing suitable preservation processes for such material and in integrating it into a supply chain (material needs to remain at about -18°C, and thus, has no shelf life at room temperature).

[0006] There is therefore a need for a method for preparing a submucosal tissue bioactive powder and further for a submucosal tissue bioactive powder that would be able to be stored at room temperature for a certain amount of time, while providing same host-remodelling responses.

SUMMARY

[0007] It is an object of the present disclosure to provide a method for preparing a dehydrated submucosal tissue bioactive powder useful for promoting cell growth that overcomes or mitigates one or more disadvantages of known methods of preparation, or at least provides a useful alternative.

[0008] It is a further object of the present disclosure to provide a dehydrated submucosal tissue bioactive powder useful for promoting cell growth that overcomes or mitigates one or more disadvantages of known submucosal tissue bioactive compositions, or at least provides a useful alternative.

[0009] According to an embodiment, there is provided a method for preparing a dehydrated submucosal tissue bioactive powder useful for promoting cell growth comprising the steps of: providing a submucosal tissue of a warm-blooded vertebrate in a saline solution; dehydrating the submucosal tissue to obtain a dehydrated submucosal tissue; and pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder.

[0010] According to another embodiment, there is provided the method defined above, wherein the submucosal tissue is selected from the group consisting of: a urinary bladder submucosa tissue, an intestinal submucosa tissue, a stomach submucosa tissue, and a uterine submucosa tissue.

[0011] According to a further embodiment, there is provided the method defined above, wherein the warm-blooded vertebrate is selected from the group consisting of: a pig, a veal and a sheep.

[0012] According to yet another embodiment, there is provided the method defined above, wherein the method further comprises the step of sterilizing the submucosal tissue using a sterilizing agent prior the step of dehydrating the submucosal tissue.

[0013] According to another embodiment, there is provided the method defined above, wherein the sterilizing agent comprises peroxide.

[0014] According to a further embodiment, there is provided the method defined above, wherein the step of pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder comprises at least one of: tearing, cutting, grinding and shearing the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder.

[0015] According to yet another embodiment, there is provided the method defined above, wherein the submucosal tissue is a urinary bladder submucosa tissue and wherein the method further comprises the step of: delaminating the urinary bladder submucosa tissue from abluminal muscle cell layers and at least the luminal portion of the mucosal layer of a segment of urinary bladder of the warm-blooded vertebrate.

[0016] According to another embodiment, there is provided a dehydrated submucosal tissue bioactive powder useful for promoting cell growth obtained using the method defined above.

[0017] According to a further embodiment, there is provided a submucosal tissue bioactive composition for promoting cell growth, the submucosal tissue bioactive composition comprising: the dehydrated submucosal tissue bioactive powder defined above; and a pharmaceutically acceptable carrier.

[0018] According to yet another embodiment, there is provided the submucosal tissue bioactive composition defined above, wherein the pharmaceutically acceptable carrier is honey.

[0019] According to another embodiment, there is provided the submucosal tissue bioactive composition defined above, wherein the pharmaceutically acceptable carrier is one of: a moisturizing cream, a moisturizing ointment and a moisturizing gel.

[0020] According to a further embodiment, there is provided an encapsulation of the dehydrated submucosal tissue bioactive powder defined above, which exhibits a capacity to induce remodeling in a host.

DETAILED DESCRIPTION

[0021] According to an embodiment, there is provided a method for preparing a dehydrated submucosal tissue bioactive powder, that is useful for promoting cell growth.

[0022] The method includes a step of providing a submucosal tissue of a warm-blooded vertebrate in a saline solution and a step of dehydrating the submucosal tissue to obtain a dehydrated submucosal tissue. The method further includes the step of pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder. The step of pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder may include, without limitation, tearing, cutting, grinding, shearing, and the like the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder. It is important, however, that such step does not create an important amount of heat, so the ability of the dehydrated submucosal tissue to induce host-remodelling responses is not affected.

[0023] The submucosal tissue may be, without limitation, a urinary bladder submucosa tissue, an intestinal submucosa tissue, a stomach submucosa tissue, a uterine submucosa tissue or any other suitable submucosa tissue. On the other hand, the warm-blooded vertebrate defined above may be, without limitation, a pig, a veal, a sheep or any other suitable warm-blooded vertebrate.

[0024] Prior the step of dehydrating the submucosal tissue defined above, the method may further include the step of sterilizing the submucosal tissue using a sterilizing agent. The sterilizing agent may include peroxide, but a person skilled in the art to which the above described method pertains would understand that any other suitable sterilizing agent may be employed.

[0025] According to another embodiment, there is provided the method defined above, where the submucosal tissue is a urinary bladder submucosa tissue. The method would therefore further include the step of delaminating the urinary bladder submucosa tissue from abluminal muscle cell layers and at least the luminal portion of the mucosal layer of a segment of urinary bladder of the warm-blooded vertebrate, a pig for instance.

[0026] According to a further embodiment, there is provided a dehydrated submucosal tissue bioactive powder useful for promoting cell growth obtained using the method defined above.

[0027] According to yet another embodiment, there is provided a submucosal tissue bioactive composition for promoting cell growth. The submucosal tissue bioactive composition includes the dehydrated submucosal tissue bioactive powder defined above and a pharmaceutically acceptable carrier, where the pharmaceutically acceptable carrier may be honey.

[0028] According to another embodiment, the pharmaceutically acceptable carrier may be, without limitation, a moisturizing cream, a moisturizing ointment, a moisturizing gel, and the like.

[0029] According to a another embodiment, there is provided an encapsulation of the dehydrated submucosal tissue bioactive powder defined above, which exhibits a capacity to induce remodeling in a host.

[0030] Therefore, the dehydrated submucosal tissue bioactive powder defined above may be provided from a urinary bladder submucosa of a pig delaminated from adjacent bladder tissue layers. The dehydrated submucosal tissue bioactive powder thus includes the bladder submucosa delaminated from abluminal muscle cell layers and at least the luminal portion of the mucosal layer of a segment of the pig's urinary bladder. Typically the delamination technique described above would provide a pig urinary bladder submucosa tissue.

[0031] Indeed, dehydrated submucosal tissue bioactive powder is typically prepared from bladder tissue harvested from animals raised for meat production, including, for example, pigs, veal, cattle and sheep or other warm-blooded vertebrates. Indeed, there is an inexpensive commercial source of urinary bladder tissue for use in preparation of the dehydrated submucosal tissue bioactive powder.

[0032] In the preparation of the dehydrated submucosal tissue bioactive powder, a segment of urinary bladder tissue is first subjected to abrasion using a longitudinal wiping motion to remove both the outer layers (particularly the abluminal smooth muscle layers) and the luminal portions of the tunica mucosa layers (the epithelial layers). The resulting urinary bladder submucosa tissue has a thickness of about 80

micrometers, and consists primarily (greater than 98%) of a cellular, eosinophilic staining extracellular matrix material. Occasional blood vessels and spindle cells consistent with fibrocytes are scattered randomly throughout the tissue. Typically, the urinary bladder submucosa tissue is rinsed with a saline solution (such as mixed in a 0.9% sea salt saline solution for about a minute) and rapidly dehydrated, so the dehydrated urinary bladder submucosal tissue is not required to be stored in a frozen hydrated state until its usage or its pulverization. Urinary bladder submucosa tissue may be dehydrated using, for example, a conventional dehydrator, for about 8 to 12 hours at about 35°C. Dehydrated urinary bladder submucosa tissue may indeed be stored at room temperature for a certain amount of time.

[0033] Dehydrated urinary bladder submucosa tissue is then put into powder, as it may be comminuted/pulverized by, without limitation, tearing actions, cutting actions, grinding actions, shearing actions and the like.

[0034] Prior to its dehydration, the above mentioned urinary bladder submucosa tissue may be sterilized using conventional sterilization techniques. A sterilization technique which does not significantly weaken the mechanical strength and biotropic properties of the urinary bladder submucosa tissue is preferably used. For instance, it is believed that some techniques may cause loss of strength in the urinary bladder submucosa tissue. Because one of the most attractive features of such dehydrated powdered urinary bladder submucosa tissue is its ability to induce host-remodelling responses, it is desirable not to use a sterilization approach which will detract from that property. Preferred sterilization techniques may include, without limitation and as mentioned above, exposing the urinary bladder submucosa tissue to a peroxide solution (such as exposed to 2 oz. of peroxide combined with 2L of 0.9% sea salt saline solution). Urinary bladder submucosa tissue, after its sterilization, may further be rinsed with (or exposed to) water.

[0035] The present disclosure also contemplates the use of a dehydrated submucosal tissue bioactive powder (corresponding to a solid substantially anhydrous particulate composition), the use of a submucosal tissue bioactive composition (dehydrated submucosal tissue bioactive powder combined with its pharmaceutically acceptable carrier), and further, the use of an encapsulation of the dehydrated submucosal tissue bioactive powder.

[0036] Such compositions (powder; powder and acceptable carrier; and encapsulation of the powder) lead to a wide variety of applications relating to the repair or replacement of damaged tissues, including, for example the repair of vascular and connective tissues. Connective tissues for the purposes of the present disclosure include, without limitation, bone, cartilage, muscle, tendons, ligaments, and fibrous tissue, including the dermal layer of skin.

[0037] In accordance with the present disclosure, such compositions may be used advantageously to induce the formation of endogenous tissue at a desired site in a warm blooded vertebrate, a human for instance (the host). They can be administered to the vertebrate host in an amount effective (when in the powder form or in the form of an encapsulation) to induce endogenous tissue growth at a site in the host in need due to the presence of damaged or diseased tissue. The effective amount may be, for example, a 500mg encapsulation or 1g of powder. Indeed, such compositions can be administered to the host in either solid (encapsulation of the powder or membrane/sheet of powder) or powdered form, by surgical implantation, or by injection. For example, powder may be introduced into chirurgical molds, such as to form replacing tissues, such as, without limitations, an esophagus tissue or any other tissue. The submucosal tissue bioactive composition (dehydrated submucosal tissue bioactive powder + pharmaceutically acceptable carrier) may also be applied directly on the host's dermal layer of skin, as a moisturizing cream, a moisturizing ointment or a moisturizing gel, such as to treat, for example, burns, cuts, bites, scratches, ulcers, varicose veins, cysts, skin diseases, skin inflammations, and the like. Moisturizing creams, moisturizing ointments or moisturizing gels may further be used for periodically skin rejuvenation. The powder may further be manufactured to provide substantially flexible sheets or membranes comprising the dehydrated submucosal tissue bioactive powder, and further, put into interaction with the host's specific sites of interaction.

[0038] Therefore, such compositions (powder; powder and acceptable carrier; and encapsulation of the powder), in use, undergo biological remodelling. It serves as a rapidly vascularized matrix for support and growth of new endogenous connective tissue. The dehydrated submucosal tissue bioactive powder material has also been found to be remodelled (resorbed and replaced with autogenous differentiated tissue) to assume the characterizing features of the tissue(s) with which it is associated at the site of interaction.

[0039] It is further to be noted that even if the encapsulation of the dehydrated submucosal tissue bioactive powder (or the powder itself), when orally administered to the host, is not associated to a specific site of interaction (as is when applying the powder, the membrane or the powder composition directly on the host's outer skin), such encapsulation or powder may further exhibit a capacity to induce remodeling in a host. Indeed, for example, such encapsulation or powder may be administered to a host, overnight, or at any other time, so that the host, having its body at rest, may benefit from reparation and/or rejuvenation. Stimulation of stem cells overnight may increase sleep quality of the host, may increase overall energy of the host at wakeup time and during the day (better mood, better respiration, positive energy, better endurance, decreased recuperation time in-between efforts, etc.), may decrease overall or specific host pain, and the like. Additionally, when orally administered to the host, such encapsulation or powder may further help healing from many conditions, diseases, sicknesses, or illnesses (asthma, arthritis, joint pain, lumbar pain, diverticulitis, headache, migraine, skin inflammation, external/internal bleedings, intestinal bleedings, Crohn's disease, fibromyalgia, erythromelalgia, etc.)

[0040] While preferred embodiments have been described above and illustrated in the accompanying drawings, it will be evident to those skilled in the art that modifications may be made therein without departing from the essence of this disclosure. Such modifications are considered as possible variants comprised in the scope of the disclosure.

CLAIMS

1. A method for preparing a dehydrated submucosal tissue bioactive powder useful for promoting cell growth comprising the steps of:

- providing a submucosal tissue of a warm-blooded vertebrate in a saline solution;

- dehydrating the submucosal tissue to obtain a dehydrated submucosal tissue; and

- pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder.

2. The method of claim 1, wherein the submucosal tissue is selected from the group consisting of: a urinary bladder submucosa tissue, an intestinal submucosa tissue, a stomach submucosa tissue, and a uterine submucosa tissue.

3. The method of claim 1, wherein the warm-blooded vertebrate is selected from the group consisting of: a pig, a veal and a sheep.

4. The method of claim 1, wherein the method further comprises the step of sterilizing the submucosal tissue using a sterilizing agent prior the step of dehydrating the submucosal tissue.

5. The method of claim 4, wherein the sterilizing agent comprises peroxide.

6. The method of claim 1, wherein the step of pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder comprises at least one of: tearing, cutting, grinding and shearing the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder.

7. The method of claim 2, wherein the submucosal tissue is a urinary bladder submucosa tissue and wherein the method further comprises the step of:

- delaminating the urinary bladder submucosa tissue from abluminal muscle cell layers and at least the luminal portion of the mucosal layer of a segment of urinary bladder of the warm-blooded vertebrate.

8. A dehydrated submucosal tissue bioactive powder useful for promoting cell growth obtained using the method of any one of claims 1 to 7.

9. A submucosal tissue bioactive composition for promoting cell growth, the submucosal tissue bioactive composition comprising:

- the dehydrated submucosal tissue bioactive powder of claim 8; and

- a pharmaceutically acceptable carrier.

10. The submucosal tissue bioactive composition of claim 9, wherein the pharmaceutically acceptable carrier is honey.

11. The submucosal tissue bioactive composition of claim 9, wherein the pharmaceutically acceptable carrier is one of: a moisturizing cream, a moisturizing ointment and a moisturizing gel.

12. An encapsulation of the dehydrated submucosal tissue bioactive powder of claim 8, which exhibits a capacity to induce remodeling in a host.

ABSTRACT OF THE DISCLOSURE

The present disclosure relates to extracellular matrix bioactive compositions and their use in repairing damaged or diseased tissues. More particularly, the present description relates to a dehydrated submucosal tissue bioactive powder obtained from a pig urinary bladder submucosa tissue, or from any other suitable submucosa tissues extracted from a warm-blooded vertebrate, for promoting cell growth (endogenous tissue growth), and further to a method of preparing the same. The method for preparing the dehydrated submucosal tissue bioactive powder is described as comprising the steps of: providing a submucosal tissue of a warm-blooded vertebrate in a saline solution, dehydrating the submucosal tissue to obtain a dehydrated submucosal tissue, and pulverising the dehydrated submucosal tissue to obtain the dehydrated submucosal tissue bioactive powder.