APPARATUS AND METHOD FOR REMOVING NON-BONE MATERIAL FROM BONE

Technical Field

The present invention generally relates to an apparatus and method for cleaning bone, and in particular to an apparatus and method for cleaning bone that may be used for dental implants or used as bone grafts.

Background

A successful dental implant relies on healthy jaw bone and surface treatment of the implant. Hydroxyapatite-treated metallic implants exhibit good biocompatibility and promote bone osseointegration. Bone grafts are often needed for the case of bone loss around a dental implant.

Bone grafts can be defined as a new bone or replacement material transplanted from a donor site to a recipient site. Bone grafting refers to the surgical procedure that places the bone graft into spaces between or around fractured or defective bone to aid in the healing process.

There are several types of bone graft, which have been characterized and applied clinically, namely synthetic grafts, autografts, allografts, and xenografts. Synthetic bone grafts are made of biocompatible materials such as synthetic hydroxyapatite, tri-calcium phosphate, calcium carbonate, and bioactive glass. Autografts can be defined as grafts made of bone from the patient's own body. Allografts are non-vital, osseous tissues or bones harvested from one individual for transplantation in

another individual of the same species, whereas xenografts are made of bones or osseous tissues from another species (such as bovine or porcine bones).

Bone is comprised of organic and inorganic matrix, while the major component of the inorganic matrix is calcium phosphate in apatite form. It is well documented that a hydroxyapatite coating on the surface of an implant exhibits superior biocompatibility and has less problems with bacterial infection and immunological response than implants with no hydroxyapatite coating.

Hydroxyapatite can also be made porous, resulting in good osteoconductivity and high osteogenesis. Osteoconductivity can be defined as the ability to support the growth of bone over its surface, without creation of a fibrous intermediate layer, whereas osteogenesis refers to the formation and development of new bone by the cells contained within the bone graft.

However, synthetic hydroxyapatite is very dense, resulting in problems associated with the restoration of bone because newly forming bones do not have access to the surface of the implant, and consequently fibrous tissue encapsulation occurs. Nevertheless, the most frequently claimed problem is delamination of the coating from dental implants. Consequent release of hydroxyapatite particles requires an additional operation to prevent possible side effects.

A major challenge in dental implant is the prolonged healing period, lasting from 3 to 6 months. During this period, synthetic hydroxyapatite implants build-up interactions with cells and connect to the adjacent bone to restore normal function. Shortening the bridging

between implant and bone is highly desirable and can be achieved by improving coating methods as well as hydroxyapatite-based composites for better biocompatibility and bone osseointegration.

Disadvantages of synthetic bone grafts, such as fibrous tissue formation, coating delamination, and prolonged healing period, are one of the reasons why natural bone grafts, such as xenografts, have attracted much interest in the field of dental implants. Bones prepared from animal or xenografts have superior biocompatibility as they offer natural three-dimensional structures with original components, which are claimed to be crucial for cell differentiation and bone formation.

Differentiation is rapidly induced, by incubating pre-osteoblasts or bone marrow-derived cells with scaffolds derived from natural hydroxyapatite.

However, xenografts need to be treated prior to transplantation process. Fresh xenografts may provoke a severe immunological response from the recipient's body. Most of the bone's organic components need to be removed to prevent the risk of disease transmission. For this reason, tedious processes and toxic solvents are generally employed in preparing animal bones. The processes also have to be done carefully without altering the material's integrity and osteogenic potential.

Methods for cleaning cadaveric donor bone to produce bone grafts suitable for human transplantation are known in the art. Some of these methods involve removing bone marrow potentially containing bacteria, virus, or fungi from the donor bone by sonicating the bone in a solvent containing one or more detergents. Other disclosed

methods include the step of centrifuging a cut bone graft under conditions effective to remove bone marrow from cancellous bone spaces of the cut bone graft.

Devices for cleaning bones are known which comprise a central cleaning unit and with containers that can be connected to the later and that hold cleaning liquid.

However, these known methods and apparatuses are usually manual and/or require a high degree of supervision. Furthermore, the cleaning processes have to be undertaken for long periods of time to ensure the bone is sufficiently clean for implantation. As a result, an unnecessarily large amount of manpower and time may have to be expended in supervision and the monitoring of the cleaning process.

In addition, most of these methods or apparatuses employ cyclical cleaning processes where fresh cleaning solvents are used for each cleaning cycle. The total amount of cleaning solvents constantly being replaced is dependant on the number of cycles being run. Consequently, upon completion of the entire cleaning process, a copious amount of cleaning solvents would have been expended. Accordingly, this is undesirable from an economical perspective. From an environmental perspective, the excessive use of such organic solvents may have an adverse impact on the environment if not disposed correctly since these cleaning solvents are usually organic in nature.

In view of the above, efficiency in the preparation of a cleaned bone graft is hence largely compromised.

There is a need to provide an apparatus and a method for removing non-bone material from bone that overcome or

at least ameliorate one or more of the disadvantages described above.

Summary

According to a first aspect, there is provided a method for removing non-bone material from bone, comprising the step ultrasonicating a bone having non-bone material thereon in a first washing solution using multiple frequencies.

According to a second aspect, there is provided- an apparatus for removing non-bone material from bone, comprising a wash chamber for containing the bone and a washing solution therein; and means for generating ultrasonic waves within said wash chamber at multiple frequency to thereby promote removal of non-bone material from bone.

Advantageously, the means for generating ultrasonic waves within said wash chamber at multiple frequencies is capable of expediting the cleaning process by the novel use of varying frequency ultrasonic waves. More advantageously, the use of the varying frequency ultrasonic waves also enables both large and small debris to be removed effectively from the bone.

In one embodiment, the wash chamber comprises an overflow pipe to configure to remove contaminants from the washing solution within said wash chamber. Advantageously, this enables oil-based contaminants such as fats (grease), to be continuously removed when the cleaning process is underway. This removes the need for any periodic stoppages of the cleaning process to actively remove the contaminants and allows the cleaning

process to continue smoothly, thus cutting down on the overall operating time.

In another embodiment, the apparatus may comprise means to automatically filling the wash chamber with a washing solution during removal of non-bone material from said bone. Advantageously, this also removes the need to constantly monitor whether the washing solution in the wash chamber has to be replaced.

The apparatus may further comprise means adapted to recycle a washing solution disposed within the washing tank. Such means adapted to recycle the washing solution may comprise a piping system comprising a network of pipes that re-circulate the washing solution after passing the washing solution through a series of filters. Advantageously, the washing solution used for each cleaning cycle can be conserved for the next cleaning cycle, reducing the need for excessive volumes of the washing solution for the entire cleaning process.

In one embodiment the wash chamber has an undulating shaped base to promote sonication during said removal of non-bone material from said bone. Advantageously, this particular shape of the wash chamber enhances the overall sonication process by promoting reflections of the sonication waves from the base of the wash chamber back toward the bone.

According to a third aspect, there is provided a bone graft cleaned by the method as disclosed herein. The bone cleaned by the method disclosed herein may be used in a dental implant and used as a bone graft.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "ultrasonic" as used herein generally refers to acoustic vibration frequencies greater than about 15 KHz. More preferably, the acoustic vibration frequencies range from about 20 KHz to about 200 KHz.

The term "non-bone material" as used herein relates generally to any material that subsist on a bone but which is chemically material that is inconsistent with bone. The non-bone material may include, but is not limited to, muscle tissue, fat tissue, ligamentous tissue, tendonous tissue, bone marrow tissue, blood cells, collagen, grease and microorganisms such as bacteria.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for

convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of an apparatus and a method for removing non-bone material from bone, will now be disclosed.

There is disclosed an apparatus for removing non-bone material from bone, comprising a wash chamber for containing the bone and a washing solution therein; and means for generating ultrasonic waves within said wash chamber at multiply frequency to thereby promote removal of non-bone material from bone.

The wash chamber of the apparatus may be impermeable to water and air as this reduces the possibility of contamination of the contents contained therein. Advantageously, the wash chamber is non-reactive to its contents and made up of an inert material. Advantageously, the wash chamber is made up of a material that is non-toxic. The wash chamber may also be sterilized at temperatures of more than about 100°C and

pressures at more than about 15 psi (~104 KPa), without an appreciable change in its desired form and properties.

In one embodiment the wash chamber is made of plastic material that can be easily cleaned and which is light weight and is generally non-supportive of promoting growth of microorganisms thereon. The wash chamber may be manufactured by plastics molding, such as cold molding, compression molding, injection molding etc. The wash chamber may also be capable of withstanding temperatures of at least 100°C without an undesirable change in properties. In one embodiment, the wash tank is made of SUS 304 stainless steel 2B type with insulation heat protection.

The base of the wash chamber may have an undulating shaped surface to enhance sonication of the contents contained therein. In one embodiment, the undulating shaped surface of the base is a "V"-shaped base. In another embodiment, the undulating shaped surface of the base is a "W-shaped base", that is an irregular surface consisting of a pair of depressions disposed adjacent to each other. Advantageously, the undulating shaped base of the wash chamber is configured to allow enhanced transmission of the ultrasonic waves from a ultrasonic wave generator that may be located below said wash chamber. The wash chamber may also be equipped with heat protection insulation and a temperature sensor.

In one embodiment, the apparatus also comprises an overflow pipe configured to remove contaminants from the washing solution with said wash chamber. The overflow pipe is configured to allow low-density contaminants such as grease and oil to be eliminated from the washing

solution contained within the wash chamber by the continuous overflowing of said washing solution. Typically, the contaminants are of a lower density than the washing solution such that these contaminants tend to float at the top of the washing solution. These floating contaminants can then be removed from the washing solution by being transmitted from the wash chamber via an over-flow pipe located at an upper part of the wash chamber. The wash chamber may also be comprised of an overflow sensor for maintaining a sufficient level of washing solution to enable continuous drainage of the contaminants by the overflow pipe. The apparatus may comprise a pump configured to pump the washing solution containing contaminants from the overflow pipe during removal of non-bone material from the bone.

The apparatus may also comprise a heater configured to heat the wash solution during removal of non-bone material from said bone. The heater may be any heating means that is capable of transmitting heat towards the contents of the wash chamber such that the temperature of the contents can be raised to the desired temperature. Exemplary heaters may include an open flame, electric heater, microwave heater or the like. The heater is present within said wash chamber or physically coupled to it. Any heater that is capable of generating inductive heat within the wash chamber may also be used. In one embodiment, the heater is an inductive heater installed within the wash chamber.

The apparatus may also comprise means to automatically fill the wash chamber with a washing solution during removal of non-bone material from said

bone. This can ensure constant feeding of the wash chamber with a predetermined volume of fresh washing solution to maintain constant overflowing of the washing solution from the wash chamber and ensure that fresh washing solution containing substantially no or a limited amount of non-bone material that has been released from the bone during washing. In one embodiment, the means to automatically fill the wash chamber with a washing solution is coupled to the wash chamber via a piping system. An inline heater may be further coupled to the piping system to heat the washing solution to the desired temperature during transfer of the washing solution to the wash chamber via the piping system.

In one embodiment, a pre-heat tank for heating up the fresh washing solution to a desired temperature before being supplied to the wash chamber is further provided. Advantageously, this reduces the amount of time taken to heat up the washing solution when the washing solution is resident in the wash chamber. The pre-heat tank may also be comprised of a flow meter and a temperature sensor to monitor the temperature and volume of the washing solution that is fed into the wash chamber. The pre-heat tank may be connected to the wash chamber by a piping system that allows the washing solution from the pre-heat tank to be fed to the wash chamber. The pre-heat tank itself may also comprise a means to automatically fill the pre-heat tank with washing solution that is to be heated up. An inline heater may be further coupled to the piping system to heat the washing solution to the desired temperature

during transfer of the washing solution to the pre-heat tank via the piping system.

In one embodiment, the apparatus further comprises means adapted to recycle a washing solution disposed within the washing tank. The means adapted to recycle the washing solution may be a piping system coupled to a series of filters. The filters may be comprised of filter papers having pore sizes of from about 0.2 microns to about 50 microns.

The apparatus may also comprise at least one nozzle configured to spray a fluid towards the bone during removal of non-bone material from the bone. At least one of the nozzles may be configured to spray a fluid towards the bone during removal of non-bone material from said bone. At least one of the nozzles may also be configured to spray the fluid to thereby promote a centrifugal fluid flow path for the washing solution within the washing chamber. In another embodiment, at least one of the nozzles may also be configured to spray the fluid to thereby agitate the washing solution so as to create flow circulation of the washing solution within the wash chamber. Advantageously, the flow circulation of the washing solution within the wash chamber provides a physical element that aids in the removal of non-bone material from the bone. At least one of the nozzles may also be angled at from about 70 degrees to about 85 degrees from the horizontal plane of the base of the wash chamber. In one embodiment, at least one of the nozzles is angled at about 80 degrees from the horizontal axis of the wash chamber. In one embodiment, there are at least three sets of nozzles. The fluid being sprayed by the

nozzles may be selected from at least one of water, air and air bubbles.

The means for generating multi-frequency ultrasonic waves may be a multi-frequency ultrasonic generator that is able to vary the frequency of ultrasonic waves based on predetermined settings. In one embodiment, the means for generating multi-frequency ultrasonic waves is capable of generating multi-frequency ultrasonic waves at frequency ranges for from about 20KHz to 50KHz, from about 60KHz to about 90KHz, from about 100 KHz to about 150KHz and from about 160KHz to about 200KHz. The means for generating multi-frequency ultrasonic waves may also be a 900W ultrasonic generator. A mixing tank may also be present and coupled to the wash chamber. The mixing tank may be capable of mixing different volumes of liquids to make up the washing solution. In one embodiment, the mixing tank is capable of mixing at least two different solutions together to form the resulting washing solution. In one embodiment, the mixing tank mixes a predetermined volume of pure alcohol to a predetermined volume of water to form the second washing solution as disclosed herein. The mixing tank may further comprise a fluid level indicator and a flow meter. Preferably, the mixing tank is in fluid communication with an alcohol storage source and a water source. The alcohol from the alcohol source may be then transferred to the mixing tank with the aid of a pump such as a diaphragm pump. The final composition of washing solution in the mixing tank may then be transferred to the wash chamber via the piping system of the wash chamber disclosed above.

In one embodiment, the apparatus further comprises a control means. The control means may be capable of coordinating the functions of the different components coupled to the apparatus and also capable of ensuring that the entire cleaning process of the bone is automated. For example, the control means may be able to receive feedback from the temperature sensor of the wash chamber and modulate the temperature of the washing solution in the wash chamber via the heating means. The control means may also ensure the automatic refilling of the wash chamber and pre-heat tank with washing solution when required. Preferably, the control means can also automatically control the volume of the different solutions to be mixed in the mixing tank based on+ predetermined instructions.

The control means may incorporate a processor capable of interrogating a memory having predetermined instructors for automatically controlling the entire cleaning process of the bone. The control means may be linked to a user interface, such as a keyboard, and a graphical user interface such as an LCD display for allowing an operator to interact with the control means.

In one embodiment, the control means comprises a memory having a computer algorithm thereon for storing said predetermined instructions. Advantageously, the LCD display linked to the control means is touch sensitive and is capable of relaying user's instructions to the other components of the control unit.

The control means may be able to store the cleaning history of the apparatus. The control means may be able

to store and process the cleaning parameters (i.e duration) inputed by a user.

Advantageously, the control means has a learning ability to allow it to call on prior knowledge or memory to apply instantaneous settings. This learning ability is preferably encoded by software. The prior knowledge, or stored history, is based on past events, including dispensing rates and dispensing periods, and is stored in a temporary memory over a period of time.

There is also disclosed a method of removing non-bone material from bone, comprising the step of ultrasonicating a bone having non-bone material thereon in a first washing solution using multiple frequencies. The method may further comprise the step of boiling a bone having non-bone material thereon in a second washing solution. The method may also further comprise the step of removing said non-bone material obtained during said ultra-sonicating step, from the second washing solution. This removal may be done by passing the second washing solution through a series of filters. In one embodiment, the ultra-sonicating step and removal step are repeated until no turbidity is observed. In another embodiment, the method also comprises the step of supplying constantly the first washing solution during the boiling step. The supplying step may also be carried out at predetermined intervals.

In one embodiment, the multiple frequencies utilized comprises of four different frequency ranges. These four frequency ranges may include frequency ranges of from about 20KHz to 50KHz, from about 60KHz to about 90KHz,

from about 100 KHz to about 150KHz and from about 160KHz to about 200Hz.

The washing solution disclosed herein may include water, alcohol of varying compositions or any other solution that is suitable to enhance or aid in the removal of non-bone material from the bone. In one embodiment, the first washing solution as disclosed herein is water. In another embodiment, the second washing solution is an alcoholic solution having from about 60% to about 90% alcohol composition by volume. Preferably, the alcohol composition by volume is 70%. Exemplary alcohol includes methanol, ethanol, propanol, butanol, pentanol, hexanol and the like. In one embodiment, the second washing solution is 70% ethanol.

Preferably the bone biomaterials prepared by the method disclosed herein may be used in a dental implant and used as bone grafts.

Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 shows a configuration of the various components of an apparatus for removing non-bone material from bone in accordance with one embodiment disclosed herein.

Fig. 2 is a perspective view of the apparatus for removing non-bone material from bone as illustrated in Fig. 1.

Detailed Description

Referring now to Fig. 1 there is shown the configuration of various components of an apparatus 100 for removing non-bone material from bone in accordance with one embodiment disclosed herein. The sources of intact bones mainly include, but are not limited to, femur or femur heads. Other parts of bones could be processed as well. Prior to cutting, bones are cleaned by tap water and the residual meats are removed manually as much as possible. Bones to be cleaned are cut into approximately 2 x 2 x 2 cm^3 bone pieces. The bone pieces are then placed within a removable basket 17 within a wash tank 10.

There are two main steps for removing non-bone material from the bones i.e. cleaning the bones, namely heating the bones with a first washing solution such as boiling water and ultrasonicating the bones in a second washing solution such as ethanol. Both of the steps take place in the wash tank 10.

The boiling step removes slurry that has formed during boiling, abrades residual meat, and denatures organic matrix that is integrally formed with inorganic matrix of the bones. Hot water needed for boiling is provided by pre-heat tank 20, which is a preheated tank for water storage with heat protection insulation 22. The pre-heat tank 20 is equipped with a built in heater 21 for heating up the water to approximately 85 to 90 °C

before the cleaning process starts. It is also equipped with a temperature sensor 23, a flow meter and means for automatically filling the pre-heat tank such as a built-in automatic refill system. The automatic refill system is configured to by a control system (not shown) to open a valve 24 once the water level is below a preset threshold, as indicated by a level indicator tubing 25 and let the water flow in.

The pre-heated tank 20 is connected to the wash tank 10 by a predetermined piping system 54. Since, the water supplied to the wash tank 10 is already pre-heated in the pre-heat tank 20, the cleaning process can start immediately after the bone pieces are placed in the removable basket 17.

The wash tank 10 is equipped with heat protection insulation 56, a heating means in the form of a ceramic heater 11, a temperature sensor 18 and an automatic refill system. A 5 KW ceramic heater for a 45-liter tank is employed for keeping the water in the wash tank 10 boiling. The automatic refill system serves the purpose of maintaining sufficient water level needed for overflow process. Water for auto refill purposes is directly supplied from a water inlet 50, and is the same as the one used to supply water to the pre-heat tank 20. The pre-heat tank 20 is in fluid communication with the wash tank 10 by piping conduit 51. An inline heater 52 is attached to the piping conduit 51 in order to maintain the temperature of the water supply. The pre-heat tank 20 is further equipped with a flow meter 55 for monitoring, measuring or recording the flow rate of water.

There are three sets of nozzles 12 installed with three different angles in the wash tank 10. Air bubbles generated from these nozzles 12 are intended to promote removal slurry attached on the bones more efficiently, as well as to remove any dirt stuck on the surface of the wash tank 10. One of the three sets of nozzles 12 is placed at spray angle of 80° aiming at the bone samples.

A substantial amount of slurry and fat may be released from the bones during the boiling process. The slurry is mainly from the inner bone marrow of the bones. Due to its density, released slurry and fat will float to the surface of the water. An overflow system is employed to continuously remove all grease from the wash tank 10, which comprises an overflow sensor 19 installed to work together with the automatic refill system of the wash tank 10, such that a sufficient water level for overflow process is maintained. An overflow pipe 53 is connected from the top surface of the wash tank 10 and extended downwardly adjacent and parallel to the periphery of the wash tank 10, as illustrated in FIG. 1. The overflow pipe 53 terminates at an open end adjacent to the bottom surface of the wash tank 10 for allowing slurry and fat from the wash tank 10 to drain away.

Instead of floating like slurry and fat, residual meat rubbed down from the bones in the boiling process is settled down on a V-shaped base of the wash tank 10 and removed by pumping air through air compressor. One of the three sets of nozzles 12 which is installed at spray angle of 80° aiming at the bone samples, are meant to create bubbles, which will result in water movement around the bone pieces. As the result, a circulation

effect is generated without compromising other functions of the wash tank 10. The nozzles draw air via a blower 13 equipped with air filters 14. In another embodiment, the blower can be replaced by a pneumatic pump system.

The use of nozzles also offers several other advantages as compared to conventional design using a fan or shaker. There are some problems attributed to the use of a fan or shaker, such as absorption of some ultrasonic power due to installation of the fan right above the ultrasonic generator or transducer. The overflow function also can be easily obstructed by a spinning motion at the bottom of the wash tank 10 due to the use of fan or shaker. Moreover, the fan only generates movement and not circulation of water, such that the debris and dirt is only moved around without settling down at the w-shaped bottom of the wash tank 10.

Every thirty minutes, new fresh boiling water is supplied from the pre-heated tank 20 to the wash tank 10 for another boiling cycle. No more slurry was observed after several hours of treatment. At this stage, most of residual meat has been abraded for further removal.

The second cycle of cleaning is emphasized on removing cells and organic matrix from the bones by ultra-sonication. A multi-frequency ultrasonic generator 15 is fixed outside the wash tank 10. An ultrasonic vibrator 16 is connected to the multi-frequency ultrasonic wave generator 15 and located inside the wash tank 10 for receiving and transmitting ultrasonic waves. The multi-frequency ultrasonic generator 15 is an advanced digital generator, which offers the ability to select multiple frequencies for a single process tank

although single frequency could be used in certain disclosed embodiments.

The ultra-sonication procedure is performed using 70% ethanol, which not only serves as a solvent, but also as an anti-microorganism agent. The alcohol mixture used to clean the bone pieces is supplied from mixing tank 30. The mixing tank 30 is also equipped with a level indicator tubing 34 and read with the flow meter. A diaphragm pump 31 is used to transfer pure alcohol from an alcohol storage source 32 to the mixing tank 30. The prime mover of a pump in the form of a diaphragm pump 31 is air supplied from compressed air inlet 33. The amount of pure alcohol from the storage source influx to the mixing tank 30 is subjected to a sensor and thus adjustable by a control means. Another sensor is installed at the mixing tank 30 to measure the amount of other solution, such as water provided by the pre-heat tank 20. Thus, the ratio of the solvent mixture can be controlled according to specific needs. It should be noted that other solvent mixtures comprising of at least two different solvents, such as bleach agents (sodium hypochloride, hydrogen peroxide) can also be used. More sensors can also be added if more than two different solvents are used for the cleaning process.

The ultrasonic frequency is the factor controlling the size of the cavitations, which is correlated with different properties of the particles to be removed. There are two different frequencies used in the present invention. Low frequency is used in the first step of ultra-sonication process, such that a large exposed surface with interconnected pore structures and tightened

organic matrix can be cleaned or loosened initially. Low frequency of 40 KHz is effective to remove tough and thick debris by powerful impact wave, although there is a possibility to damage the sample. In other words, the use of 40 KHz frequency enforces cleaning of bone surfaces and loosening up any leftover residual meat such that it can be easily removed in the next step. The optimal time is modulated and kept within 4 to 6 hours.

Next, high frequency ultrasonic treatment will generate small size cavitations, such that micro-pores or any other complicated structures lying inside the bones can be cleaned thoroughly. At the same time, organic matrix lying within inorganic matrix of bone could be partially removed or loosened. High frequency around 100 to 200 KHz is chosen to effectively clean small debris and cells attached on interconnected pores of the bones. Applying appropriate and interactive frequencies enables maximal cleaning effect without damaging any bone structures. The ultrasonic cleaning is also combined with interval water jettings through nozzles 12 to speed up the cleaning process.

The value of output power is another factor controlling the cavitations density or number of cavitations. A unique 900 W ultrasonic generator with multiple frequencies of 40, 72, 104, and 170 KHz is used. In principle, 15 to 20 W of output power is required for 1-liter volume in ultra-sonication.

Following the ultrasonic treatment with two different frequencies using 70% ethanol, used solvents are recycled through a pipe equipped with multi layer filter cartridge 57. Different layers of filtration

papers have different pore size ranging from 50 μm to 0.2 μm, for removing different size of debris, cells, bacteria and virus accordingly. The filter cartridge 57 is activated only when the ultrasonic cleaning process is ongoing. Normally, the cartridge 57 with 50 μm filter is activated initially when low frequency ultrasonic wave is applied. It is followed by activation of the cartridge 57 with 10 μm, 1 μm, or 0.2 μm filter individually for obtaining the final result of bacteria- and virus-free biomaterials. The filter cartridge 57 is equipped with pressure gauges, which provide a reading as an indicator of when to change a filter to prevent damage.

The chosen solvent is effective on solubilizing greases, inactivating or killing bacteria, virus, and cells captured in the filters during recycling process. Pressurized flow is used to redirect recycled solvents to the wash tank 10 so that they can be reused in the subsequent cleaning process.

Used solvents containing grease and dirty particles are recycled, by pumping to the first layer of filter having 50 μm pore size. The recycled solvents are then reused for the second interval of ultra-sonication. The reused solvents are then further recycled to the subsequent layer of filter having smaller and smaller pore size, such as 10 μm, 1 μm, or even 0.2 μm. Filtering is undertaken to ensure removal of viruses in the final run of cleaning process. The ultra-sonication step is a one-hour interval process and is repeated for 4-6 times, until no turbidity is observed.

The solvent recycle process disclosed herein offers the advantages of cost reduction and prevention of

environmental contamination. Bone samples are successfully cleaned using environmentally friendly process without involving any toxic solvents. As a result, the amount of waste generated at the end of the process is significantly reduced. The waste is finally collected in a waste tank 40, for disposal.

After ultra-sonication, the cleaned bone pieces are dried using air blow from the nozzles 12. The bone pieces are subjected to a further drying process in an 80°C oven for one hour. The final products of the processed bone pieces are then routinely checked for cell activity contamination using in vitro cell culture system for a period of several months.

Referring now to Fig 2, there is provided a perspective view of the apparatus 100 disclosed herein. The overall process for cleaning bone is automated and existed as a whole compact apparatus, as illustrated in FIG. 2. It is controlled by a touch screen control panel 60 and is further equipped with a user interface display 61, which is able to indicate cleaning stage and cycle times. The user interface allows the operator to set different cleaning cycle and timing. A total of 10 programs with multiple steps can be programmed. The control panel 60 also includes a reset key and settings for frequencies, timing, and steps.

While the present invention has been described with reference to particular embodiments, it will be understood that the embodiments are illustrative and that the invention scope is not so limited. Alternative embodiments of the present invention will become apparent to those having ordinary skills in the art to which the

present invention pertains. Such alternate embodiments are considered to be encompassed within the spirit and the scope of the present invention. Accordingly, the scope of the present invention is described by the appended claims and is supported by the foregoing description.

Applications

It will be appreciated that the apparatus for removing non-bone material from bone disclosed herein is a simple yet efficient and useful system for the preparation of a bone graft for implantation and coating.

The presently disclosed apparatus is capable of automatically controlling the cleaning process. Advantageously, a high degree of supervision throughout the cleaning process would not be required. Furthermore, by the use of ultrasonic waves with multiple frequencies, the presently disclosed apparatus and method ensures that effective cleaning of the bone within a short period of time. Consequently, a large amount of manpower and time would be conserved for the overall cleaning process.

Furthermore, the use of a wash tank with an undulating base such as a V-shaped or W-shaped base enhances sonication of the contents of the wash tank. Advantageously, this aids in the removing non-bone material for the bone during the sonication step.

In addition, throughout the sonication process, recycling of the washing solution in the form of alcohol is ongoing. Accordingly, this removes the need to constantly replace the washing tank with fresh alcohol solution throughout the sonication process. This is

advantageous from an economical perspective. From an environmental perspective, the reduction of use of such organic solvents would mean that there would be a subsequent reduction in organic waste (which is potentially hazardous environmentally) that would have to be disposed.

The presence of an overflow pipe configured to remove contaminants from the washing solution within said wash tank also advantageously enables contaminants such as grease, to be continuously removed when the cleaning process is underway. More advantageously, this allows the cleaning process to process smoothly and effectively and removes the need for constant stoppages to actively remove the contaminants, thus cutting down on the overall operating time. While reasonable efforts have been employed to describe equivalent embodiments of the present invention, it will be apparent to the person skilled in the art after reading the foregoing disclosure, that various other modifications and adaptations of the invention may be made therein without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A method of removing non-bone material from bone, comprising the step of ultra-sonicating a bone having non-bone material thereon in a first washing solution using multiple frequencies.

2. The method as claimed in claim 1 comprising the step of boiling a bone having non-bone material thereon in a second washing solution.

3. The method as claimed in claim 1 comprising the step of removing said non-bone material obtained during said ultra-sonicating step, from said second washing solution.

4. The method as claimed in claim 3, wherein said ultra-sonicating step and removal step are repeated until no turbidity is observed.

5. The method as claimed in claim 2, wherein said second washing solution is a solvent having from 50% to 70% alcohol content.

6. The method as claimed in claim 1, wherein said multiple frequencies comprises of four different frequency ranges.

7. The method as claimed in claim 6, wherein said four different frequency ranges are 20 KHz to 50 KHz, 60 KHz to 90 KHz, 100 KHz to 150 KHz and 160 KHz to 200 KHz.

8. The method as claimed in claim 2 comprising the step of supplying constantly the first washing solution during the boiling step.

9. An apparatus for removing non-bone material from bone, comprising:

a wash chamber for containing the bone and a washing solution therein; and

means for generating ultrasonic waves within said wash chamber at multiple frequency to thereby promote removal of non-bone material form said bone.

10. The apparatus as claimed in claim 9, wherein said wash chamber comprises an overflow pipe configured to remove contaminants from the washing solution within said wash chamber.

11. The apparatus as claimed in claim 10, comprising a pump configured to pump said washing solution containing contaminants from said overflow pipe during removal of non-bone material form said bone.

12. The apparatus as claimed in claim 9, comprising a heater configured to heat the wash solution during removal of non-bone material form said bone.

13. The apparatus as claimed in claim 10, comprising means to automatically fill the wash chamber with a washing solution during removal of non-bone material form said bone.

14. The apparatus as claimed in claim 9, further comprising means adapted to recycle a washing solution disposed within the washing chamber.

15. The apparatus as claimed in claim 9, further comprising at least one nozzle configured to spray a fluid towards said bone during removal of non-bone material form said bone.

16. The apparatus as claimed in claim 15, wherein said at least one nozzle is configured to spray the fluid to thereby promote a centrifugal fluid flow path for said washing solution.

17. The apparatus as claimed in claim 9, wherein said wash chamber has an undulating-shaped base to promote sonication during said removal of non-bone material from said bone.

18. A bone graft obtained from the method as claimed in claim 1.

19. The bone graft as claimed in claim 18 for use in a dental implant.