

**Producing a transplant from animal dermis using
sodium sulfide solution**

5 The present invention relates to a method of producing a transplant from animal dermis, in particular from porcine dermis, as well as to a transplant which can be obtained therewith.

10 Tissue transplants are used for a number of applications, for example in dental surgery to build up the jaw bone or for soft tissue regeneration to fill up bone defects in the case of a tumor, in surgical interventions to restore tendons and ligaments as in the correction of congenital defects. Furthermore, they can also be used in surgery on the spinal column, in the revision of hip prostheses or in the treatment of abdominal defects.

15 Generally, three kinds of transplants (also known as grafts) are distinguished, namely autogenous tissue grafts in which the donor and the recipient are one and the same person, with the graft usually being used at a different site to the removal site; allogeneic tissue grafts in
20 which the donor and the recipient are different individuals of one species; and xenogeneic tissue grafts in which the donor belongs to a different species, for example a pig, than the receiver, for example a human. Due to the limited availability of allogeneic and in particular autogenous tissue grafts, xenogeneic tissue grafts are of particular interest. In this respect,
25 in particular tissue implants from pigs, for example from porcine dermis, are in demand because they satisfy the demands of the human body in a physiologically better manner than tissue implants from primates, for example. In addition, there is the almost unlimited availability of porcine skin.

It is a material aspect in the production of tissue transplants that the native structure of the tissue in the finished transplant is maintained as much as possible so that it has at least largely the properties of the native tissue with respect, for example, to the inner surface, the handling capability and the elasticity. However, with the previously known processes, it is not possible to produce transplants from animal dermis and in particular not from porcine dermis while adequately maintaining the native tissue structure. As a rule, such tissue transplants are namely produced by a process in which the tissue is first treated with 1 M sodium hydroxide solution to loosen the tissue structure, to release bristles from the dermis and to depilate the dermis. However, the dermal collagen matrix is seriously damaged by this treatment step so that the native structure of the dermis is considerably impaired.

It is therefore the object of the present invention to provide a method of producing a transplant with which a tissue implant can be provided from animal dermis and in particular from porcine dermis simply, fast and inexpensively whose morphology corresponds at least largely to the native structure of the dermis.

This object is satisfied in accordance with the invention by a method of producing a transplant from animal dermis, in particular from porcine dermis, which comprises the following steps:

- a) providing animal dermis;
- b) treating the animal dermis with an aqueous solution containing sodium sulfide;
- c) treating the dermis once or several times with an aqueous saline solution;

- d) optionally, treating the dermis once or several times with an aqueous hydrogen peroxide solution; and
- e) dehydrating the dermis.

5 This solution is based on the recognition that a loosening of the tissue structure, a removal of the bristles from the dermal matrix and a depilation of the dermis can be achieved by the treatment of animal dermis with a solution containing sodium sulfide, in the same manner as by the treatment with a comparatively concentrated sodium hydroxide
10 solution, and indeed surprisingly such that unlike the treatment with a comparatively concentrated sodium hydroxide solution damage to the dermal collagen matrix of the dermis is almost completely prevented. For this reason, a sterile and cell-free xenogeneic graft can be produced simply, fast and inexpensively from an animal dermis using the method in
15 accordance with the invention, said xenogeneic graft corresponding at least largely to the native tissue structure in its morphological structure. In particular a chemical treatment of the tissue with chemical agents such as formaldehyde, glutaraldehyde or the like which cause a crosslinking of proteins contained in the collagen while losing the native tissue structure
20 can in particular be dispensed with using the method in accordance with the invention. Such a crosslinking of proteins of the tissue is disadvantageous because such a crosslinking has a negative influence on the engrafting behavior of the transplant in the recipient. In some cases, such a protein crosslinking can even have the result that the transplant
25 does not engraft in the recipient at all, but is encapsulated without previously exercising the intended function of tissue stabilization.

In order reliably to prevent the production of hydrogen sulfide in the performance of the method in accordance with the invention, it is
30 proposed in a further development of the idea of the invention to use an

aqueous alkaline solution containing sodium sulfide in method step b). In general, the alkaline solution containing sodium sulfide used in this embodiment can have any desired pH of more than 7, with good results in particular being obtained with corresponding solutions which have a pH of
5 at least 10 and preferably of at least 12.

In principle, the present invention is not restricted with respect to the quantity of the sodium sulfide contained in the aqueous solution used in method step b). The aqueous solution used in method step b) preferably
10 contains 0.01 to 10% by weight sodium sulfide, in particular preferably 0.1 to 5% by weight, particularly preferably 0.5 to 3% by weight, very particularly preferably 0.75 to 1.5% by weight, and most preferably approximately 1% by weight.

15 In order to set the above-named preferred alkaline pH, provision is made in accordance with a preferred embodiment of the present invention that an aqueous solution is used in method step b) which, in addition to sodium sulfide, contains 0.001 to 0.5 M sodium hydroxide, preferably 0.005 to 0.4 M sodium hydroxide, particularly preferably 0.01 to 0.3 M
20 sodium hydroxide, in particular preferably 0.05 to 0.2 M sodium hydroxide, very particularly preferably 0.075 to 0.125 M sodium hydroxide, and most preferably approximately 0.1 M sodium hydroxide.

Any base known to the skilled person for setting pH values can be used
25 instead of sodium hydroxide, such as, for example, any alkaline metal hydroxide or alkaline earth metal hydroxide, such as potassium hydroxide, lithium hydroxide, magnesium hydroxide, calcium hydroxide, ammonia and the like.

In general, the sodium sulfide solution should be used in a sufficiently high quantity and for a sufficiently long time in method step b) so that the dermis is effectively loosened and the bristles and hairs contained therein are almost completely removed. Good results are in particular achieved in this respect when the dermis is treated for 1 to 48 hours, preferably for 5 to 36 hours, particularly preferably for 12 to 24 hours, and very particularly preferably for 14 to 20 hours, with the solution containing aqueous sodium sulfide. In this respect, the solution containing aqueous sodium sulfide is preferably used in a 5-fold to 15-fold volume with respect to the volume of the dermis. The solution containing aqueous sodium sulfide solution is particularly preferably used in a 6-fold to 14-fold volume, in particular preferably in a 7-fold to 13-fold volume, further preferably in an 8-fold to 12-fold volume, very particularly preferably in a 9-fold to 11-fold volume, and most preferably in an approximately 10-fold volume, with respect to the volume of the dermis.

In principle, the treatment with the solution containing sodium sulfide can be carried out once or a multiple of times after one another in method step b), with the dermis being treated and/or rinsed with water between the individual treatment steps with the solution containing sodium sulfide. However, it has been found to be preferable within the framework of the present invention preferably only to carry out a single treatment of the dermis with the solution containing sodium sulfide for the previously named treatment duration in method step b).

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It is proposed in a further development of the idea of the invention to carry out the treatment with the sodium sulfide solution in accordance with method step b) in a rotating drum. A uniform and complete incubation of the dermis with the sodium sulfide is thereby achieved. For example, the rotating drum can be supported horizontally and can be rotated about its

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axis. Connectors are expediently provided at the drum via which the process solutions can be supplied into the drum and led out of the drum. In addition, connectors can also be provided at the drum to set an overpressure or underpressure as required in the drum. In addition, it is preferred that the drum is designed with double walls so that the inner space of the drum can be temperature treated via connectors provided in the hollow space. Finally, it is also preferred to configure the drum so that its rotational speed can be electronically regulated.

- 10 Any intact cells still present after method step b) are destroyed by osmotic stress by the treatment of the dermis with an aqueous saline solution in method step c). In this respect, all salts suitable for this purpose can be used such as in particular alkaline metal halides and earth alkaline metal halides. For cost reasons, in particular the alkaline metal chlorides and earth alkaline metal chlorides such as in particular sodium chloride are suitable for this purpose.

Good results are in particular obtained in this connection when the dermis is treated with an aqueous solution containing 1 to 50% by weight sodium chloride, preferably 2 to 20% by weight, particularly preferably 5 to 15% by weight, and very particularly preferably 10% by weight, in method step c). These quantities are also preferred on the use of other alkaline metal halides and earth alkaline metal halides such as potassium chloride, lithium chloride, magnesium chloride, calcium chloride and the like.

In accordance with a further preferred embodiment of the present invention, the dermis is treated with saline solution a plurality of times in method step c), in each case interrupted by a treatment and/or a rinsing with water. The transport of cellular components from the tissue structure

by diffusion processes is promoted by this alternating bath treatment of saline solution/water due to changing osmotic relationships in the tissue and the effect of the saline bath treatment is thus improved. Good results are in this respect in particular obtained when the dermis is treated two
 5 times to ten times, preferably three times to five times, and particularly preferably four times, with an aqueous saline solution in method step c), with the dermis in each case being treated with saline solution between the treatment steps.

10 In this respect, the duration of the individual treatment steps in method step c) preferably amounts to 1 to 48 hours, with at least one of these treatment steps preferably being carried out for 5 to 36 hours, particularly preferably for 12 to 24 hours, and most preferably for 14 to 20 hours, for example for 16 hours.

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Method step c) is preferably also carried out in the rotating drum previously described as preferable for method step b).

20 In order to kill the bacteria and viruses present in the tissue and to bleach the tissue, it is proposed in a further development of the idea of the invention to carry out the optional method step d), and indeed preferably using an aqueous solution which contains 1 to 10% by weight hydrogen peroxide, preferably 2 to 5% by weight hydrogen peroxide, and particularly preferably approximately 3% by weight hydrogen peroxide.

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In this respect, the dermis is preferably treated a multiple of times with an aqueous hydrogen peroxide solution in method step d), and indeed preferably two times to five times, and particularly preferably three times, with the dermis being treated and/or rinsed with water in each case
 30 between the treatment steps with the hydrogen peroxide solution.

Good results are in particular obtained in this respect when the duration of the individual treatment steps in method step d) preferably amounts to 5 to 60 hours, particularly preferably to 10 to 36 hours, and very particularly preferably to 20 to 30 hours, preferably to 24 hours.

Method step d) is preferably also carried out in the rotating drum previously described as preferable for method step b).

To achieve a particularly effective dehydration in method step e), which allows a subsequent storage of the dermis in a frozen state without changing its structure, provision is made in accordance with a further preferred embodiment of the present invention to dehydrate the dermis in method step e) by two-time to ten-time treatment with an organic solvent, preferably by four-time to eight-time treatment, and particularly preferably by six-time treatment. In this respect, particularly good results are obtained when the organic solvent is selected from the group comprising acetone, methanol, ethanol and isopropyl alcohol.

In accordance with a particularly preferred embodiment of the present invention, acetone is used as the organic solvent in method step e) because it not only dehydrates the dermis, but rather also in particular degreases it and deactivates any viruses present.

Good results are in this respect in particular obtained with respect to the degree of dehydration when the dermis is treated several times with an organic solvent in method step e). The dermis is preferably treated with organic solvent two to ten times, preferably four to eight times, and very particularly six times. To achieve a slow water removal or a gentle dehydration at the start, it is moreover preferred to use an organic solvent

diluted with water in the first step, for example a 70% acetone solution, before then diluted organic solvents of a higher concentration are used in the following treatment steps such as a 90% acetone solution in the second treatment step and then concentrated organic solvent such as undiluted acetone.

In this respect, the duration of the individual treatment steps in method step e) preferably amounts to 1 to 48 hours, with at least one of these treatment steps preferably being carried out for 5 to 48 hours, particularly preferably for 12 to 36 hours, and most preferably for 20 to 30 hours, for example for 24 hours.

Method step e) is preferably also carried out in the rotating drum previously described as preferable for method step b).

After the end of dehydration, residual organic solvent in the dermis is preferably removed by circulating air drying at 25 to 60°C, for example at 35°C.

As presented, the present method is in particular suitable for producing a transplant from porcine dermis. However, it is also suitable for all other animal dermis such as bovine dermis.

In accordance with a further particularly preferred embodiment of the present invention, method step a) includes the following steps, in particular in the case of porcine dermis:

- a₁) cleansing the skin of the slaughtered animal, in particular a pig, using water optionally containing a detergent, with the

water having a temperature between room temperature and a maximum of 40°C;

- a₂) removing the skin from the back area of the pig;
- a₃) removing the bristles from the skin prepared in step a₂); and
- 5 a₄) removing the subcutis and the epidermis from the dermis using a cutting machine.

It was found within the framework of the present invention that the type of preparation of the animal dermis also has an important influence on the quality of the obtained transplant in addition to the type of chemical treatment. It was in particular determined within the framework of the present invention that porcine skin produced by a conventional method in a slaughterhouse results in transplants with comparatively poor properties. This is due to the fact that, in conventional slaughterhouse processes the pigs are boiled with hot water of approximately 65°C after slaughtering to cleanse and disinfect the pigs superficially before the bristles are subsequently burnt off. The collagen contained in the pig skin is irreversibly damaged by this thermal treatment.

20 To avoid this, provision is made in accordance with the previous, particularly preferred embodiment of the present invention to cleanse the skin of the slaughtered pig gently, namely with warm water at a maximum of 40°C which optionally contains a mild detergent. In other words, in the method in accordance with the invention, no thermal treatment of the skin is carried out with a hot medium of more than 40°C.

Only the skin section from the back area of the animal, preferably a pig, is preferably used in the method in accordance with the invention because this is characterized by a tauter skin structure and a greater thickness in comparison with the skin of the belly side. The portions of the belly side

are thus removed from the complete skin and discarded in method step a₂) and only the skin of the back area having a surface of approximately 800 x 1200 mm is used.

- 5 Subsequently, in method step a₃), the bristles are preferably removed from the skin mechanically, and indeed preferably using a blade, before the skin is cut into pieces of approximately 400 x 600 mm in size, for example. The skin thickness in this stage amounts to approximately 2.5 to 3 mm. The skin prepared in this manner can be supplied directly to
10 method step a₄) or can first be stored at a temperature of a maximum of -20°C.

- Then, in method step a₄), the subcutis and the epidermis are preferably removed from the dermis using a cutting machine. For this purpose, the
15 band knife cutting machine preferably contains a continuous blade which is comparable with a band saw blade without teeth and which is drive via two wheels and is guided via additional rollers. The blade is preferably horizontally supported and is moved at a speed of a plurality of meters a second. The gap with is continuously adjustable with such a cutting
20 machine. In this respect, the dermis is supplied to the blade by two rolls and is divided into two pieces along the cross-sectional area.

- It is proposed in a further development of the idea of the invention first to remove the subcutis from the dermis in method step a₄) before the
25 epidermis is subsequently removed from the dermis. The machine is set, for example, to a gap dimension of 2 mm for the first part step of removing the subcutis from the dermis. The precut skin pieces are then introduced into the machine with the fast side facing upward, with the fat portion and connective tissue portion, including the hair roots, going beyond a
30 thickness of 2 mm being cut off and then discarded. Alternatively to this,

the gap dimension can also be set to approximately 1.5 or approximately 1.0 mm.

5 In the next part step of removing the epidermis from the dermis, the gap dimension of the cutting machine is set to 0.5 mm and the skin obtained in the previous part step is again introduced into the cutting machine to split off and discard the epidermis.

10 The dermis prepared in this manner can be supplied directly to method step b) or first be stored at a temperature of a maximum of -20°C or alternatively to this at 4°C in a 25% sodium chloride solution.

15 After carrying out the pretreatment and the chemical treatment of the dermis in accordance with method steps a) to d), finally the transplant is cut-out and/or stamped out of the dermis dehydrated in step e), optionally while using a pattern. In this respect, the transplants can be cut into rectangular, round or oval shapes. Optionally to this, the transplants can also be stamped out using a hole pattern.

20 Subsequently, the transplants are welded, preferably doubled, into flat pouches of laminated plastic film or composite film of plastic and aluminum film. The transplants are preferably dry-packed in this process. However, the transplants can also be moist-packed after a rehydration has taken place.

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The packed transplants can finally be treated with gamma radiation, for example with gamma radiation at an energy dosage of 17 to 22 kGy, for sterilization. Whereas dry transplants are preferably irradiated at a temperature of 20 to 40°C, the irradiation of moist transplants is
30 preferably carried out at a temperature of -20 to -80°C.

A further subject of the present invention is a transplant which can be obtained using the method in accordance with the invention.

- 5 For example, the transplant in accordance with the invention can have a rectangular shape having a size of, for example, 50 x 100 mm to 350 x 350 mm, a round shape having a diameter between 30 and 100 mm or an oval shape having a size of, for example, 100 x 155 mm to 250 x 350 mm.
- 10 Furthermore, the present invention relates to the use of the previously described transplant in hernia surgery, for example for treating incisional hernias or for treating a burst abdomen.

- In the treatment of hernias, the transplant in accordance with the
- 15 invention is sewed to the surrounding intact tissue and first serves as a defect closure. As part of the subsequent tissue modeling, the transplant serves as a guide rail for the patient's own cells which populate the collagen structure of the transplant. The defect is closed by newly formed connective tissue by the conversion of the transplant, i.e. by the reduction
- 20 in the transplant collagen and the parallel build-up of the patient's own tissue.

The present invention will be explained in more detail in the following with reference to an embodiment not restricting the invention.

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Example

- A freshly slaughtered pig was superficially cleansed using hot water of 40°C which contained 0.1 to 1% by weight sodium dodecyl sulfate (SDS)
- 30 before it was skinned. The portions of the belly side were removed from

the complete skin and discarded and only the skin of the back area having a surface of approximately 800 x 1200 mm was further processed.

The bristles were subsequently mechanically removed from the pig skin using a blade before the skin was cut into pieces of approximately 400 x 600 mm in size. The skin was then introduced into a cutting machine set to a gap dimension of 2 mm with the fat side facing upward to cut off the fat portions and connective portions, including the hair roots, going beyond a thickness of 2 mm. In this respect, the cutting machine contained a horizontally supported continuous blade driven via two wheels and guided via additional rollers.

Subsequently to this, the gap dimension of the cutting machine was set to 0.5 mm and the skin was again conducted through the cutting machine to split off the epidermis. A dermis was thus obtained having a thickness of 1.5 mm.

The dermis was then treated in a rotating drum in accordance with the following treatment scheme:

Step	Action	Time (h)	Purpose
1	Rinse with water (3x)		
2	Store in water	0.5	
3	Store in water	0.5	
4	Treat with aqueous 1% sodium sulfide, 0.1 M NaOH solution	16	Hair removal
5	Rinse with water (3x)		Diffusion, Osmosis
6	Store in water	1	

7	Store in 10% NaCl	3	
8	Store in water	1	
9	Store in 10% NaCl	16	
10	Rinse with water (3x)		Diffusion, Osmosis
11	Store in water	1	
12	Store in 10% NaCl	3	
13	Store in water	1	
14	Store in 10% NaCl	16	
15	Rinse with water (3x)		
16	Store in 3% hydrogen peroxide	24	Deactivation of bacteria and viruses, Bleaching of pigments
17	Store in 3% hydrogen peroxide	24	
18	Store in 3% hydrogen peroxide	24	
19	Rinse with water (3x)		Wash out H ₂ O ₂
20	Store in 70% acetone	2	
21	Store in 90% acetone	2	Slow water removal
22	Store in 100% acetone	2	Water removal/Degreasing
23	Store in 100% acetone	16	Water removal/Degreasing
24	Store in 100% acetone	24	Water removal/Degreasing
25	Store in 100% acetone	24	Virus deactivation
26	Drying at 35°C	24	Acetone removal

After the chemical treatment, oval transplants having a size of 160 x 250 mm respectively were finally stamped out of the dermis and dry-welded pair-wise in a laminated plastic film and irradiated at 35°C with gamma
5 radiation at an energy dosage of 20 kGy.

The transplants thus produced had a morphology almost identical to the native structure.

Claims:

1. A method for producing a transplant from animal dermis, in particular from porcine dermis, comprising the following steps:
 - a) providing animal dermis;
 - b) treating the animal dermis with an aqueous solution containing sodium sulfide;
 - c) treating the dermis once or several times with an aqueous saline solution;
 - d) optionally, treating the dermis once or several times with an aqueous hydrogen peroxide solution; and
 - e) dehydrating the dermis.
2. A method in accordance with claim 1, characterized in that an aqueous alkaline solution containing sodium sulfide is used in step b).
3. A method in accordance with claim 1 or claim 2, characterized in that an aqueous solution is used in step b) which contains 0.01 to 10% by weight sodium sulfide, preferably 0.1 to 5% by weight, particularly preferably 0.5 to 3% by weight, very particularly preferably 0.75 to 1.5% by weight, and most preferably 1% by weight.
4. A method in accordance with at least one of the preceding claims, characterized in that

an aqueous solution is used in step b) which, in addition to sodium sulfide, contains 0.001 to 0.5 M sodium hydroxide, preferably 0.005 to 0.4 M sodium hydroxide, particularly preferably 0.01 to 0.3 M sodium hydroxide, in particular preferably 0.05 to 0.2 M sodium hydroxide, very particularly preferably 0.075 to 0.125 M sodium hydroxide, and most preferably approximately 0.1 M sodium hydroxide.

5. A method in accordance with at least one of the preceding claims, characterized in that the dermis is treated in step b) for 1 to 48 hours, preferably for 5 to 36 hours, particularly preferably for 12 to 24 hours, and very particularly preferably for 14 to 20 hours with a volume of an aqueous solution containing sodium sulfide relative to the dermis of 5-fold to 15-fold, preferably a volume of 6-fold to 14-fold, in particular preferably a volume of 7-fold to 13-fold, further preferably a volume of 8-fold to 12-fold, and very particularly preferably a volume of 9-fold to 11-fold.

6. A method in accordance with at least one of the preceding claims, characterized in that the dermis is treated in step c) with an aqueous solution containing 1 to 50% by weight salt, preferably 2 to 20% by weight, particularly preferably 5 to 15% by weight, and very particularly preferably 10% by weight, with the salt preferably being an alkaline metal halide or an earth alkaline metal halide, particularly preferably an alkaline metal chloride or an earth alkaline metal chloride such as potassium chloride, lithium chloride, magnesium chloride or calcium chloride.

7. A method in accordance with at least one of the preceding claims, characterized in that
the dermis is treated two to ten times, preferably three to five times
and particularly preferably four times with an aqueous saline
5 solution in step c), with the dermis being treated and/or rinsed with
water in each case between the treatment steps with saline solution.
8. A method in accordance with at least one of the preceding claims, characterized in that
10 the dermis is treated with an aqueous solution in step d) which
contains 1 to 10% by weight hydrogen peroxide, preferably 2 to 5%
by weight hydrogen peroxide, and particularly preferably 3% by
weight hydrogen peroxide.
- 15 9. A method in accordance with at least one of the preceding claims, characterized in that
the dermis is treated two to five times, and preferably three times,
with an aqueous hydrogen peroxide solution in step d), with the
dermis being treated and/or rinsed with water in each case between
20 the treatment steps with the hydrogen peroxide solution.
10. A method in accordance with at least one of the preceding claims, characterized in that
the dermis is dehydrated in step e) by two-time to ten-time,
25 preferably by four-time to eight-time, and particularly preferably by
six-time treatment with an organic solvent selected from the group
comprising acetone, methanol, ethanol and isopropyl alcohol.

11. A method in accordance with at least one of the preceding claims, characterized in that porcine dermis is provided in step a).
- 5 12. A method in accordance with claim 11, characterized in that the provision of the dermis in step a) comprises the following steps:
- 10 a₁) cleansing the skin of a slaughtered pig using water optionally containing detergent, with the water having a temperature between room temperature and a maximum of 40°C;
- a₂) removing the skin from the back area of the pig;
- a₃) removing the bristles from the skin prepared in step a₂); and
- 15 a₄) removing the subcutis and the epidermis from the dermis using a cutting machine.
13. A transplant which can be obtained in accordance with a method in accordance with at least one of the preceding claims.
- 20 14. Use of a transplant in accordance with claim 13 in hernia surgery, for treating incisional hernias or for treating a burst abdomen.