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Development of an intraoperative procedure for producing an autologous whole skin equivalent - prototype design and pilot resection force and tensile strength measurements

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Introduction

Existing epidermal transplantation procedures still represent the state of the art for chronic wound care and burns surgery, but despite this they do not provide a true replacement for skin that is complete in all its layers [1]. Dermal skin layers are indispensable for allowing the body to displace itself with respect to its external environment, for being able to carry vital skin appendages such as hair, sweat and sebaceous glands, and nerve receptors for detecting pressure, vibrations and temperature sensations, and as such for assuring the quality of the skin being grafted: „Epidermis is Life, Dermis is Quality of Life" [2]. Due to restrictions on the maximal permitted sizes of donor tissue, whole skin grafts cannot be transplanted over large areas, i.e. only 2-3% of the body's whole surface area can be covered using autologous whole skin [3]. Skin culture methods based on lab cultured skin cells are not yet developed to the point that a whole skin substitute cultured within a laboratory can be provided [4]. Cultured substitute skin which contains all its appendages such as sebaceous glands and hair cells remains an enigma [5]. Considering that the sampling of punch biopsies leaves no lasting scars behind, and that with whole skin islets one can also obtain skin appendages, a new transplant procedure was developed that resulted in an immediate autologous, intraoperative wound treatment which eventually led to the production of a whole skin equivalent. The goal of this undertaking was to develop a construct design, the prototypes of which should allow the removal of 3 mm diameter autologous full skin islets and their introduction into a commercially available collagen matrix. The collagen matrix with the implemented full skin islets were then to be transplanted on the burn wound [6]. In addition to the methodological design development, the study also included two experimental tests on the porcine skin for investigating its mechanical properties.

Materials and methods:

1.) Prototype design: the main objectives of the design stage, such as the identification of meaningful functional structures, so that the search for a suitable technical solution was carried out using a Pahl and Beitz morphological box [5]. The morphological box is a two-dimensional organizational chart in which possible solutions constitute the columns, and problems to be addressed constitute the rows. The aim of this tool from morphological analysis is to arrive at an agreed solution through a process of eliminating those solutions that can be deemed impractical, illogical or otherwise unfeasible. Based on the final solution, construction drawings are then created using CAD programmes to create the prototype design [6].

2.) In addition two tests on the mechanical properties of skin were carried out. Both experiments were carried out in the Laboratory of Biomechanics and Experimental Orthopaedics at the Luebeck Campus of the University Hospital of Schleswig-Holstein. The resection force test as well as the test for determining the tensile strength of the subcutaneous tissue were performed using a material testing machine (Zwick, Ulm, Germany) and its corresponding software TestXpert II [7]. The measuring unit was a U2A load cell (Hottinger Baldwin Messtechnik, Darmstadt, Germany) [8]. For punching the skin a biopsy punch of the manufacturer KAI (Seki, Japan) was used which had a nominal diameter of 3 mm [9]. The skin of the German Edelschwein breed was used as the skin model.

2.1) Resection force test. The test was designed to assess whether the effort invested in developing a mechanism which allows rotation during advancement was justified [10]. The test included six measurements with and six without rotation during advancement with an initial load of 6N and a forward speed of 6 mm/min. The fresh porcine skin was warmed to a temperature of

29 ° C at the start of the test [11]. In the first part of the test the biopsy punch was braced directly within the socket of the material testing machine and could not as a result be rotated. During the second part of the test the biopsy punch was guided with a brass pin. Above the punch this was limited in the axial plane through the use of a metal spacer. After the initial load of 6 N was reached, the punch was mechanically rotated through 180 ° (fig. 1).

2.2) Tensile strength test: the subcutaneous adipose tissue is not always completely severed with every punching procedure,

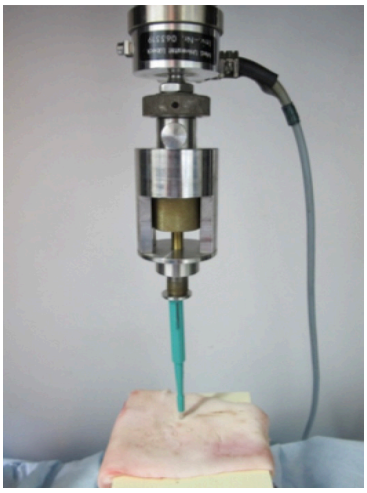

Fig. 1. Resection force test using the Zwick material testing machine.

meaning that the whole skin islet can remain in the cutaneous covering or be pulled away from the punch blade when retracting the biopsy punch from the skin [12]. In an attempt to determine the force with which the whole skin islet is held by the subcutaneous tissue, the tensile resistance of the subcutaneous adipose tissue was determined experimentally in another test. A probe was used as a tension adaptor. Twelve measurements were made with the settings initial load 0.025 N and forward speed 6 mm / min (fig. 2).

Results:

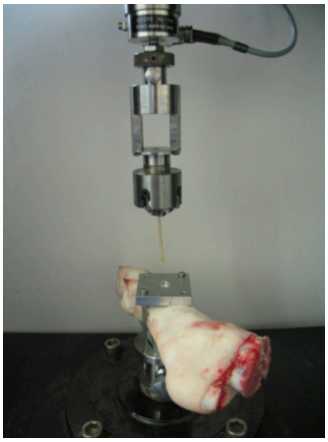

Fig. 2. Tensile strength test using the Zwick material testing machinemachine

The punch adapter as the centrepiece of the prototype consists of a tube sheath and features two opposing guidance pins on its side. Each of the guidance pins has a threaded cavity running right through it. Grub screws are screwed into these threads. The grub screws secure the biopsy punch into the punch adapter (fig. 3).

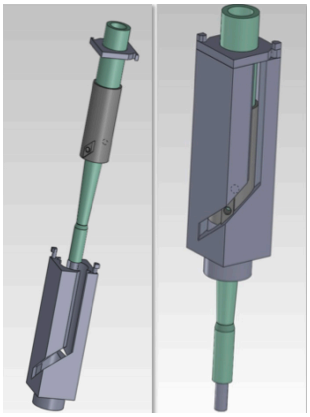

Fig. 3. Punch adaptermachine

The prototype design was designed so that the skin islets could be extracted despite the presence of remaining subcutaneous tissue connections using either a vacuum suction nozzle or a vacuum wound drainage system. Only one adapter is needed which connects the full skin biopsy punch to the vacuum system selected. Figures 4 and 5 show the biopsy stamp.

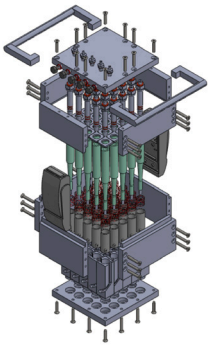

Fig. 4. Close-up of the biopsy stamp

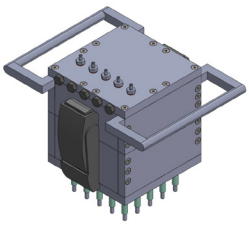

Fig. 5. Biopsy stamp

The implementation station is used to transfer the whole skin islets from the punches to the collagen matrix. The guidance pins in

the spring blocker are screwed in at the lower side. The spring blocker is there to restrict the travel of the springs. This prevents the implementation pins from penetrating too far and the whole skin islets from becoming displaced by the collagen matrix (fig. 6). Figure 7 shows the entire construction of the prototype design.

Resection force test: the measurements obtained showed that

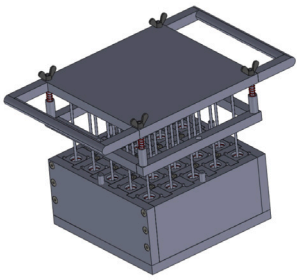

Fig. 6. Implementation stamp

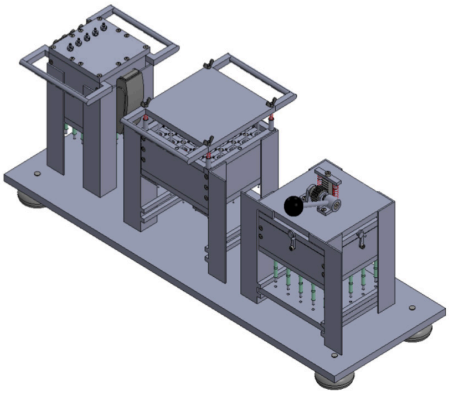

Fig. 7. Overall view of the prototypes

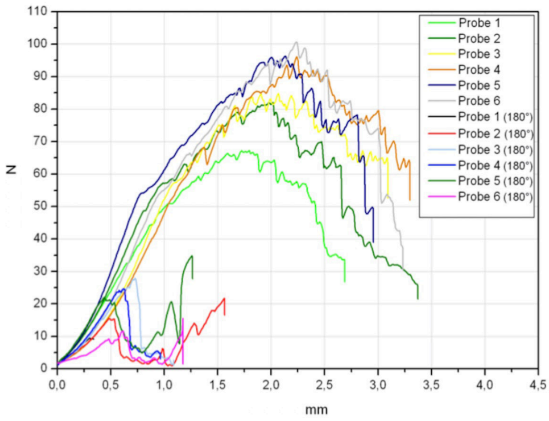

Fig. 8. Graphical representation of the resection force test

when a rotation of 180 ° was combined with the cutting motion, penetration of the skin layers was significantly easier. This was due to the dynamic friction which arose from the cutting action between the contact surfaces moving relative to one another [13]. The results of individual measurements and their standard deviations can be found in table 1, while the curves resulting from the measurements are displayed graphically in Figure 8.

As such an average force of 87.9 ± 5.1 N had to be applied per biopsy punch during punching alone in order to penetrate the epidermis and the dermis. When punching was combined with rotation (by 180 °) an average force of only 22.35 ± 3.69 N was needed per biopsy punch. This meant that there was a difference of 65.55 N between the two modalities. Compared to a pure punching motion alone, the combination of punching and rotation resulted in a “power saving” of 75%.

Tensile strength test: The maximum forces and linear strains arising from the measurements are shown in table 2 with the corresponding standard deviations. Sample 8 can be considered as a failed attempt, since after punching the biopsy remained in the punch. The curves resulting from the measurements are displayed

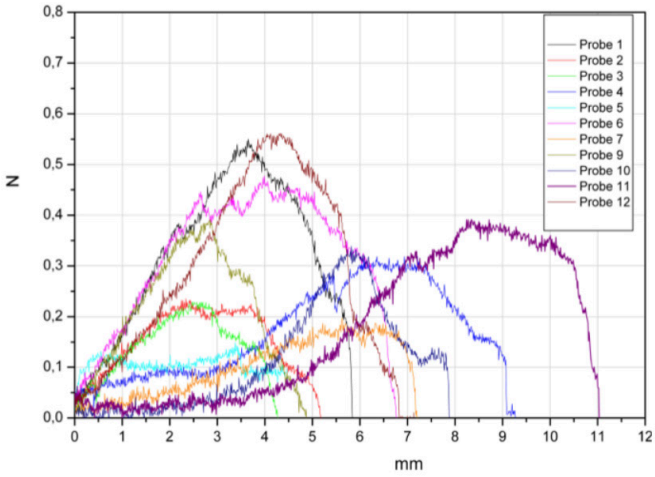

Fig. 9. Graphical representation of the tensile strength test

graphically in Figure 9.

A required negative pressure with a maximum value of 0.56 N was calculated to ensure that when removing the whole skin islets they would be held within the biopsy punch, they would not slip out, and they would not remain within the cutaneous covering. From this it could be calculated that the required negative differential pressure had to be set at - 0.79 bar (i.e. -594.2 mmHg).

Discussion

The greatest technical challenge with the proposed prototypes was to ensure that the whole skin islets would be taken up by the biopsy punch despite the presence of bridges of connective tissue which might prevent this. Both the high-vacuum wound drainage system we favoured and the vacuum suction nozzle system are based on

	without rotation	with rotation (180°)
Probe/ Sample	FDmax [N]	FDmax [N]
1	67,2 ± 9,257	9,4 ± 5,791
2	82,1 ± 2,594	21,7 ± 0,291
3	84,8 ± 1,386	28,0 ± 2,527
4	96,1 ± 3,667	24,6 ± 1,006
5	96,2 ± 3,712	34,9 ± 5,613
6	101,0 ± 5,858	15,5 ± 3,063

Tab.1 Resection force test with standard deviations

Probe/ Sample	maximum force [N]	linear strain [mm]
1	0,55 ± 0,069	3,65 ± 0,157
2	0,24 ± 0,024	2,34 ± 0,55 2
3	0,23 ± 0,027	2,76 ± 0,425
4	0,32 ± 0,000	6,93 ± 0,832
5	0,15 ± 0,051	3,38 ± 0,238
6	0,48 ± 0,048	3,99 ± 0,054
7	0,19 ± 0,039	5,62 ± 0,437
8	-	-
9	0,39 ± 0,021	2,86 ± 0,395
10	0,34 ± 0,006	5,82 ± 0,497
11	0,39 ± 0,021	8,35 ± 1,260
12	0,56 ± 0,072	4,35 ± 0,054

Tab.2 Tensile strengths and standard deviations of the 12 tensile strength samples

the same principle [14]. With both variants a negative pressure is built up which creates a suction effect and pulls the biopsy while it is being taken up into the punch. The vacuum suction nozzle functions on the basis of the Venturi principle [15]. The high-vacuum wound drainage system on the other hand is pre-evacuated to a preset, negative pressure. A balancing of pressure between the negative pressure in the bottle and the positive pressure in the system can be brought about by releasing the pipe clamp. This also results in a suction effect that holds the biopsy within the punch. An important benefit of the high-vacuum wound drainage system is that it is disposable and need not be sterilized. The disadvantage compared to the vacuum suction nozzle is that the system needs to be absolutely airtight so that the negative pressure is maintained. Unlike the case with the suction nozzle, no constant suction is produced [16].

Here a solution involving a compressed air supply within the operating theatre can be considered. The difference here is that the pressure is generated not by a pump, but is instead fed from a central gas supply system. The pressure emanating from the supply system can then be adjusted downward with a pressure regulator to the required operating pressure and fed to the punch. The disadvantage is that a centralized compressed air system needs to be present [17]. The easiest method for guiding the biopsy from the punch involves the use of a stamping device, as confirmed in the Pahl and Beitz morphological box. In this process a rod with a diameter of 1.8 mm is guided through the 3 mm biopsy punch. When the rod is inserted through the biopsy punch, it presses against the epidermal side of the whole skin islet and pushes it out of the punch.

Substantial work on the transplantation of whole skin islets has been carried out by the research group of Rox Anderson, whose work on the technical implementation of fractionated skin grafts is very advanced and has already been patented [18]. Their approach is to place a matrix over the removal site so that the whole skin islets from the whole skin are directly extracted into the matrix as a kind of “dermal photocopy”. Anderson et al. also used specially patented biopsy needles which did not require any rotation [19].

Our own attempts to patent the procedure presented here proved unsuccessful since the degree of inventive ingenuity was deemed to be insufficient. There is already a plethora of publications that describe the punching of whole skin islets and their further use, especially within the contexts of hair transplantation and vitiligo [20, 21]. Whole skin punching has also been described in a number of patents filed by the food industry [22]. There are also numerous patents for various pieces of equipment designed to transplant autologous whole skin islets [23].

The results of our research group have been made available to the scientific community so that our prototype can be further studied and technically implemented. One focus of the prototype design up until now has been to create a robust mechanical design that can easily be sterilized without much technical equipment, and that consists largely of already approved sterile single use medical devices (punches and vacuum drainage system). As regards the tests on porcine skin, the results from the punch-cut experiment are a matter of some discussion. Meyer et al proclaimed that porcine skin differs from human skin too much to provide any clinically meaningful results. In contrast to the other skin layers, the dermis of pig skin due to its extensive fat deposition, relative thickness, massive compactness and reinforcement with powerful collagen fibre bundles bears no acceptable resemblance to the human dermis [24]. Based on this observation and the lessons learnt with regard to the experimental set-up, the experiment should not just be repeated with higher punch numbers, but should also be repeated using tempered fresh human cadaver skin rather than porcine skin. The skin tensioning device should be set in such a way that any additional mechanical tension arising in the subcutaneous tissue does not affect the measurement results. The tensioning device should also be mounted on an artificial table that can be adjusted in three dimensions according to the position of the subcutaneous tissue and subdermal tissue layers. The possibility to align the skin tensioning device in three axes ensures an accurate positioning within the sensor axis of the material testing machine. A cross laser can be used to facilitate the positioning of the adjustable table with its mounted skin tensioning device. Before the start of the tensile strength test the thickness of the subcutaneous tissue must be measured with each sample so that the relationships between the force applied and the subcutaneous adipose tissue can be calculated. The curves for the samples in the tensile strength tests differed from one another. A comparable increase in the linear elasticity range of curves 1, 2, 3, 6, 9, 10, 11 and 12 could, however, be seen. The eleven curves shown in the graphic revealed no pronounced yield points. The stretching of the subcutaneous tissue that will lead to rupture lies between 3.8 and 10.6 mm. With increased stretching an increase in force can be seen which increases up to a maximum value. The maximum value of this force lies between 0.15 and 0.56 N. After reaching the maximum value, a force reduction can be seen which can be attributed to a constriction of the subcutaneous tissue. At the ends of the curves there are rapid, almost vertical drop-offs in force down to 0 N. The beginning of the rapid descent marks the point where the subcutaneous tissue is constricted so strongly that rupture occurs. The different curves suggest that the subcutaneous tissue, despite the small biopsy areas, shows strong variations in its structure [25].

Conclusions

In this paper the first design problems associated with the development of a procedure for autologous whole skin transplantation could be conceptualised and constructive solutions for developing a prototype could be implemented. Furthermore, the removal, expansion and implementation of the autologous whole skin islets could all be developed within a single structural unit. The measurements resulting from the resection force test showed that when a rotation of 180 ° was combined with the cutting motion of the punch, penetration of the skin layers was significantly easier. The tensile strength test showed that with 3 mm wide whole skin islets the required force and underpressure at 0.8 bar (594 mmHg) are very high, as far as the results from porcine skin can be compared to those for human autologous whole skin. As such, other research groups are invited to carry out follow-up

experiments so that the proposed procedure for autologous whole skin transplantation can be developed further.

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