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Resection Force Analysis of 1 and 2 mm Punch Biospies Combined with Rotation on Temperature-Controlled Cadaver Skin use for the Acquisition of Full Skin Islets for Skin Transplantation

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Introduction

An entirely new mode of skin transplantation has been developed based on the knowledge that a) the sampling of skin punch biopsies leaves no lasting scars behind, and 2) the extracted whole skin islets contain all the appendages required to regenerate new skin. This has led to an immediate autologous, intraoperative wound treatment which eventually led to the production of a whole skin equivalent. The device designed for the purposes of autologous whole skin transplantation led to whole skin islets being acquired from the donor site using millimetre range biopsy punches before they were introduced into a collagen matrix for transplantation onto the burn wound. The purpose of this study was to carry out resection force measurements that could provide valuable information about the forces that are needed to reliably punch out a skin biopsy. Such measurements were then to be used as a technical basis for further developing a biopsy device. With whole (full-thickness) skin removal involving the use of biopsy punches, frictional forces also arise in addition to pure resection forces [1]. These resection and friction forces mathematically summate with multiple simultaneous whole skin islet removals so that in a stamping device consisting of 100 biopsy punches, the maximum force that can possibly be exerted is theoretically exceeded with simultaneous skin punching [2]. This was preceded by our own pilot pressure tests to determine the force required to penetrate porcine whole skin with a biopsy punch. The forces were registered and documented using a 100 N force sensor in a Zwick material testing machine. In a second pilot study, the experimental design was extended in that the punch was rotated 180 ° after reaching a previously set resection force (Fig. 1). The result of this was that an additional rotation of the punch meant that the force needed to cut the tissue was reduced to 25%. The maximum forces that were required to penetrate the epidermis and the dermis were between 9.4 N and 34.9 N with rotation, while without

rotation the forces were between 67 N and 101 N. In addition, the

measurements also showed us that the biopsy punch blunted over the course of several measurements due to wear and tear, meaning that the force required for resection was increased. Since the measurements were only pilot tests, the number of measurements was low (n = 6). No basis for a statistically consolidated statement existed, so that the decision was taken to repeat the tests with a higher number of measurements (n = 100). The basic properties of the porcine skin used in the pilot test (collagen content) meant it could only at best be considered as a temporary skin substitute [3]. The new measurements were conducted using tempered human cadaver skin so that results could be obtained that were much more clinically relevant.

Materials and methods

The resection force measurements were performed on four cadaver skin preparations from two donors. The four skin preparations had a size of 115 x 110 mm and were provided by the Institute of Anatomy, University of Lübeck in a frozen state. The skin pieces revealed a fat layer of 1cm. Donor 1 was an 86 year-old man whose thigh skin had been removed. Donor 2 was a 71 year old woman, and the removed skin material also came from her thigh. The cadavers of the body donors or the body parts used (in this case the skin of the thigh) were studied under observance of the "Burial Act" of the State of Schleswig-Holstein from 04/02/2005, Section II, § 9 (autopsy, anatomical). The punch design was realised with the help of a Zwick material testing machine. In order to achieve a defined pressure point at the start of the measurements, a defined initial load was set in the software TestXpert II. The force measurement started only after the initial load was measured at the sensor. The speed of the punch could be set using a speed control dial while the process was monitored on another monitor. The forces absorbed by the sensor were illustrated in a force-distance plot. In order to prevent a continuous rotation of the punch, a control for starting the rotation of the punch was integrated in addition to the speed control. After thawing, the cadaver skin was cut at the corners, placed in the skin positioning device, and the perforated plate was mounted (Fig. 2.3). After connecting a heating mat, the skin was

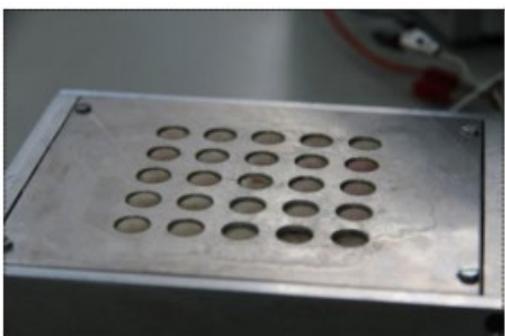Fig. 2 Thawed cadaver skin in the skin mounting device

Fig. 3 Mounting of the perforated plate on the cadaver skin

kept at a constant temperature of 29-30° C over 1-2 hours. Skin temperature was measured every 15 minutes using a thermometer. The 2 mm biopsy punch was adapted to the material testing machine and and the test series was initiated with the following parameters (Figs. 4 and 5):
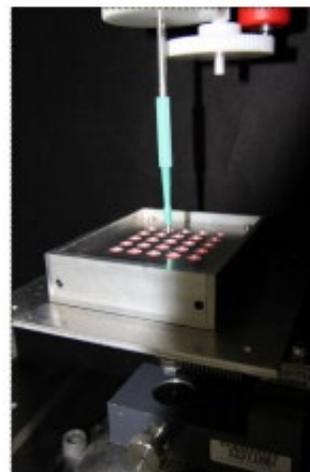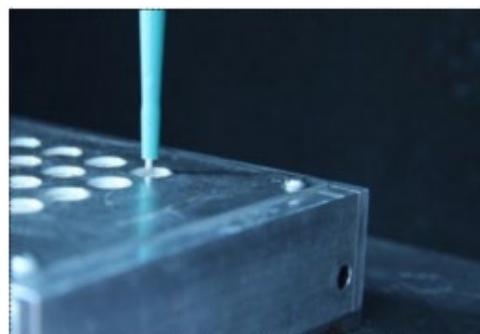

Fig. 4. 2mm biopsy bunch in the material testing machine

Fig. 5 Perforated plate in the material testing machine

- •   Rotation of the punch (600 ° / min) by 180 °
- •   Total penetration depth 6 mm
- •   A forward speed of 12 mm/min

The measurements were repeated in the same manner on the remaining 24 measurement points. By measuring four cadaver skin preparations at 25 measurement points on each preparation, a total of 100 measurements could be recorded. In order for the measurement curves to be assigned to the measurement locations, the skin preparations were divided up into row and column positions. The measuring point on the top left was designated as 1.1 while the next measuring position (Fig. 6) on the right was designated as 1.2 and so on.

Results

Force measurement and penetration depth: In Figure 7, the force profile is shown in relation to the penetration depth. The force measurement ends once the punch has protruded 6 mm into the skin (f). The rising part of the curve b describes the compression of the skin by the biopsy punch. At the point at which the force
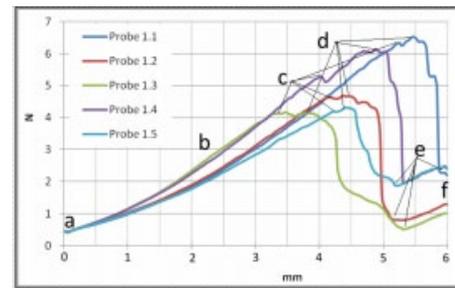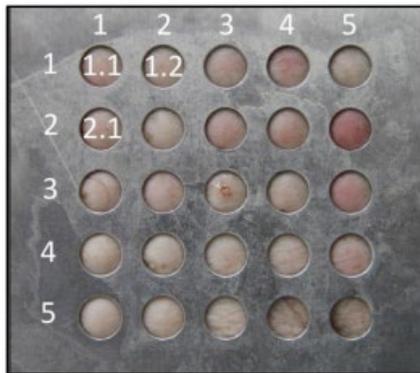
Skin piece	Donor 1 Sample 1	Donor 1 Sample 2	Donor 2 Sample 1	Donor 2 Sample 2
Mean maximum force (N)	6.8	4.9	5.2	6.4
σ (N)	1.8	1.4	0.9	1.9
Mean penetration depth (mm)	4.6	4.4	4.5	4.6
σ (mm)	0.5	0.6	0.7	0.6

begins to drop for the first time (c), the blade severs the first fibre bundle. The further rise in the curve describes the ongoing compression of the skin. This process continues until all the fibre bundles and as such the entire skin is cut (d). The force achieved at this point is hereinafter referred to as the maximum force. If the skin is cut, the force decreases. The blade in this area has now penetrated into the subcutaneous tissue under the skin. The slopes of the 5 curves are different. In addition to the maximum force that must be applied to the skin to cut it (sample 1.1: 6.5 N; sample 1.3: a little over 4 N), the associated penetration depths (sample 1.1: 5.5 mm; sample 1.3: 3.8 mm) are also different. One reason for the variations in the curves might be the inhomogeneity of the skin. Table 1 shows an overview of the mean maximum forces, the mean penetration depths and their standard deviations. The results of the measurements in Table 1 show a reduction in force with combined advancement and rotation of the punch, unlike the earlier tests which involved advancement without rotation. Unlike the penetration depth, the maximum force varied from skin sample to skin sample. In seven measurements from the 100, the skin was not severed by the biopsy punch. An overview of the measurements is provided in figs. 8-11.
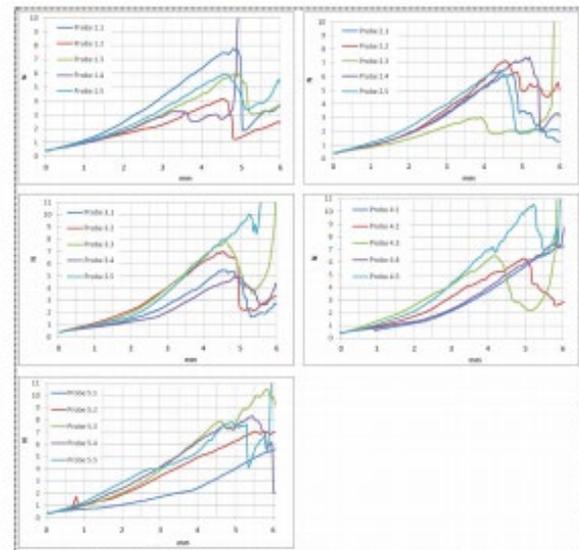
Required maximum forces: Figure 12 shows the maximum forces, depending on the measurement position on skin sample 2 of the second donor. The diagram is aligned in such a way that measuring position 5.5 is located on the front right, as applied in figure 6. Columns of the same colour lie in the same row (row 1: blue, row 2: red, etc.).

In the areas around measurement points 1.3, 1.4, 1.5, 2.5 and 3.5 there are particularly noteworthy readings (i.e. 8.8 N, 10.9 N, 9.8 N, 15 N, and 15 N, respectively). The maximum force at these locations, in contrast to measurement points 5.1 - 5.5 (5.2 N, 4.5 N, 4.7 N, 5.3 N and 4.75 N, respectively), is almost twice as high. At measuring points 2.5 and 3.5 the measurement was stopped to protect the force sensor. In this area of the skin sample the tissue seems to have been more elastic under certain circumstances than
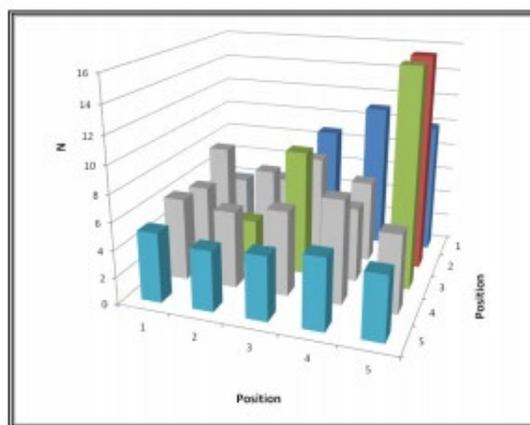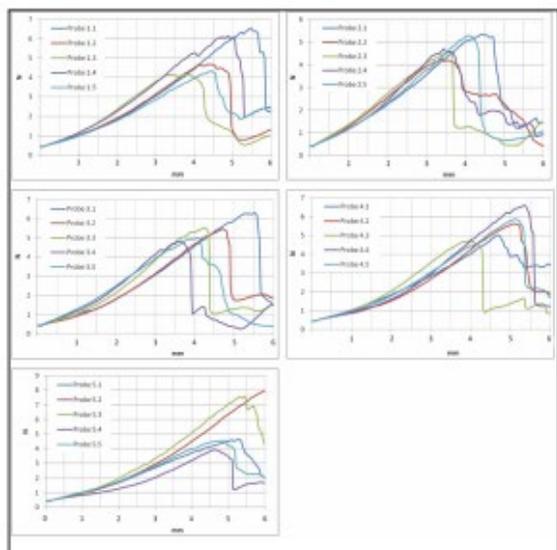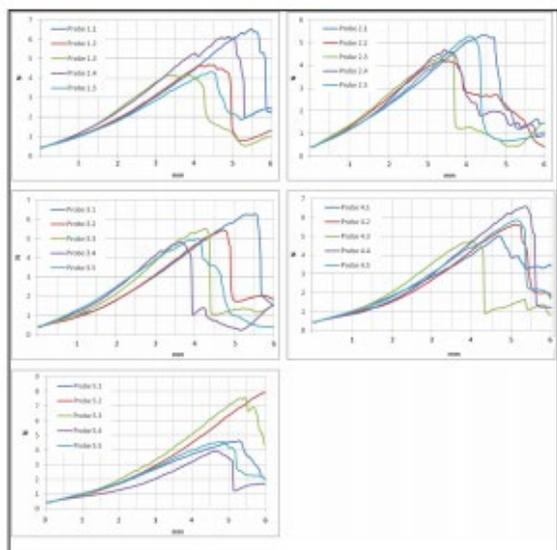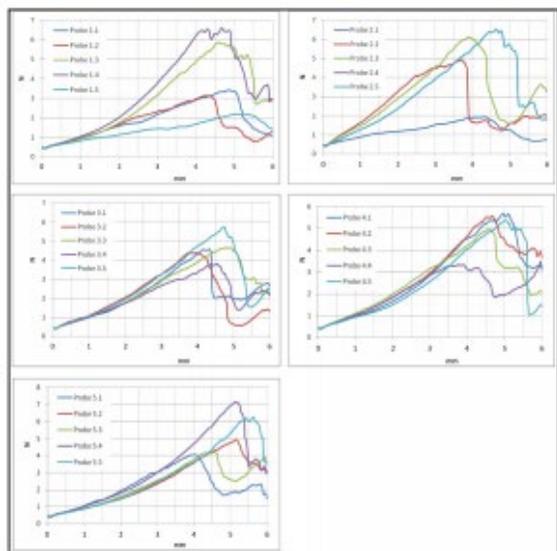
it was in other areas of the sample (5.1 -5.5 or 3.2). Here it was not possible for the punch to sever all the fibres, and in some cases the punch drove right through to the aluminium plate upon which the skin was mounted. In addition, the higher force at measuring position 3.3 (9.3 N) was also unusual. Skin sample 1 of donor 2 showed a maximum of 15 N (Fig. 13) albeit at only one location
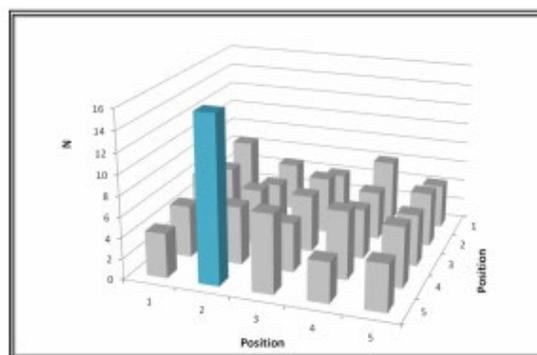
(position 5.2, turquoise). Unlike skin piece 2, the surrounding measurements showed no increase in maximum force apart from position 5.3. No explanation was forthcoming for this fact, and it should normally be assumed that the skin thickness and as such the maximum force of closely spaced positions should be similar. The skin samples of the first donor showed no abnormalities with regard to the maximum forces (Fig. 14, 15).
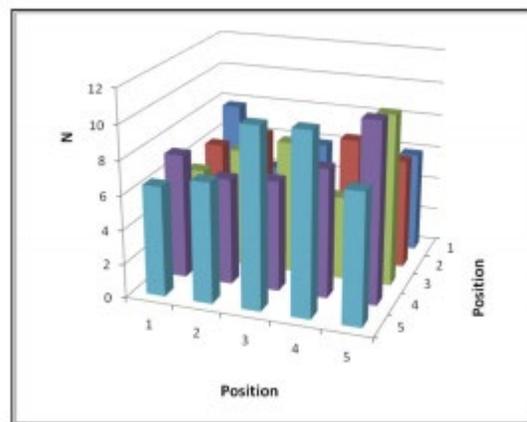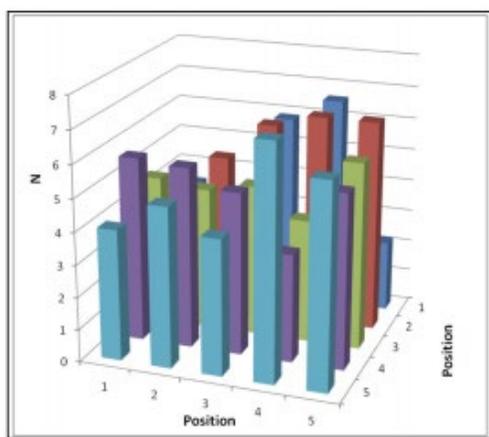
Discussion

In order to allow a better understanding of the mechanical behaviour of skin, a number of other attempts have been made historically to take measurements and investigate the mechanical properties of human skin. As long ago as 1933 Jochims studied the elasticity and resistance of human skin [4]. Using a skin resistance gauge he determined the retraction force of the skin. He found that the retraction force only reflected the elastic properties of the skin. The total force required, however, reflected both the elastic and inelastic (viscous) properties of the skin. In 1947 Stroebel recognised a relationship between tissue changes and the age of the skin. Skin aging already begins in mid-life and is associated with changes occurring in collagen fibres located within the dermis [5]. Accordingly, the number of collagen fibres starts to decrease from the age of 30. In addition, there is also an association between these collagen fibre changes and a later remodelling of the skin. In his studies in 1949, Wenzel, using a tensile strength test, found that the elasticity of the human skin decreases with increasing age, while the modulus of elasticity increases [6]. If skin in the earlier years of life still expands by up to 91% of its initial length, this value for individuals over 42 years falls to under 65%. In 1965, Zink confirmed the assumption that the mechanical properties of the skin are primarily determined by collagen [7]. With a specially designed construction he examined the tensile strength and elasticity of human skin in 29 human cadavers (aged 18 to 75 years). In that study, the independence of the two parameters on the expansion rate was confirmed. Fazekas in 1967 examined the tension of the skin in 121 cadavers (aged between 0.5 and 88 years). He used a device he referred to as a machine, upon which 10 cm long, 1 cm wide strips of skin were stretched and studied. Fazekas came to the conclusion that the tensile strength of human skin differs depending on gender, body region and age [8]. As such, the tensile strength of female skin was less than that of male skin. Irrespective of gender, it decreases with ageing. Fazekas considered the cause for the decrease to be the fact that the number of collagen fibres reaches its maximum by the age of 20. From this point on only the diameter of the individual fibres increases, although this has no effect on the tensile strength of the skin. Furthermore, he found that the tensile strength was at its strongest on the back and at its weakest in the extremities. In 1970, Holzmann et al. examined thigh skin samples from 52 patients to investigate their elasticity, modulus of elasticity, resistance to tearing and tensile strength. After separation from the subcutaneous tissue, the 5 mm wide strips were stretched within the clamps of an Instron device. Again, the result was that the skin reaches biological maturity at an early age (15 - 20 years) at which point it then starts to age [9]. Recent studies support the idea that collagen plays a role in the aging of the skin [10-12]. In addition to the work on the mechanical properties of the skin, investigations were also carried out in the past on the forces which were required to penetrate the skin and other materials with differently shaped objects. In 1999, O 'Callaghan et al. carried out measurements on human cadaver tissue within the scope of a forensic investigation. They were trying to determine the effort which was expended to carry out a knife attack using individual layers of skin and muscle tissue [13]. O 'Callaghan et al. concluded that the force required for penetration of adipose tissue was lower than that required for tissue consisting of skin, fat and muscle (2 N in contrast to 49.5 N). Skin required a greater effort to penetrate than fat (12 N). A similar experiment was carried out by Shergold and Fleck in 2005. They examined the force curves upon penetration by sharp-tipped and flat-bottomed cylindrical punches into silicone rubber blocks. They found that both a reduced diameter and a sharp-tipped geometry of the cylinder resulted in a reduction of the required forces [14]. In conclusion it can be stated from the literature that because of the different experimental setups and differing parameters (in vivo vs. in vitro studies, different ages of the skin etc.), it is difficult to compare individual results and provide a general statement on skin properties. The measurements, however, all consistently led to the realization that human skin is a viscoelastic material with a non-linear stress-strain relationship which from the age of 20 changes markedly in its characteristics [15-17]. A non-linear stress-strain relationship exists when the curve resulting from the deformation of the material cannot be surpassed by a curve generated from a simple elastic recovery. Viscoelasticity is deemed to exist when a material obeys both Hooke's and Newton's laws. Hooke's law, which describes elastic properties, is defined by a linear relationship between strain and stress. A constant loading of the material leads to an increasing deformation and an increased tension in the tissue. If a purely elastic material is stretched it will return to its initial state when the tensioning process is stopped. If a material is liquid or partly made of liquid it will have viscous properties. With these materials, Newton's law of viscosity takes effect, which is defined by a formula. With viscoelastic materials, both Hooke's and Newton's law describe the mechanical behaviour of the skin. The knowledge of the properties of viscoelastic materials is important when handling skin tissues, especially within the subject of skin transplantation. In order to achieve a cosmetically favourable transplant outcome, it should not be possible to deform the graft by mechanical stresses after surgery, since otherwise the re-

integration process would be disrupted and scarring might result.

Conclusions:

An experimental setup could be presented which allowed the implementation of an in vitro resection force measurement on temperature-controlled human cadaver skin using a biopsy punch. The design allowed the forward movement of the biopsy punch to be combined with a rotation. One hundred force measurements were carried out, of which 93 resulted in resection of the skin. Whether the parameters set were optimal for allowing a minimal effort for resection could not be shown here. The parameters need to be varied in future studies so that an optimized minimal resection force can be achieved. Another noteworthy finding was the observation that different, even closely spaced sampling points, revealed different required forces for resection in the biopsy. The results from these measurements can be used for the further development of a method for whole (full-thickness) skin transplantation employing punched whole skin islets.

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