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Feed Motion Resection Force Analysis of 1 mm and 2 mm Biopsy punches on tempered human Cadaver Skin use for the Acquisition of full Skin Islets for Skin Transplantation

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Abstract:

In this study we analysed the resection forces occurring when tempered human cadaver skin was punched with 1 mm and 2 mm biopsy punches as part of a project to develop a method for transplanting autologous whole skin on severely burned patients. The objective was to determine the force required to penetrate human skin with differently sized biopsy punches when resection was carried out by employing a simple forward motion without any rotation. To this end two different tests were performed with similar experimental setups. In the first test, the forces emanating from individual 1 mm and 2 mm biopsy punches were compared. For each punch size, 100 resection force curves were measured on four pieces of skin obtained from two donors. For the second test two bunch biopsy stamps were constructed, each of which contained a hundred punch biopsy cylinders. In one of the stamps the cylinders were all of the same length, while in the other they were all of a different length. It was also determined how many whole skin islets remained in the punches after the biopsy procedure, as well as the proportion of skin cylinders which could not be released from the original skin layer. The tests were performed using a Zwick material testing machine equipped with appropriate force sensors (i.e. load cells). The study revealed that the use of smaller diameter biopsy punches, as well as the use of punches with different length resection cylinders resulted in a reduction in the forces occurring upon resection. The measured resection forces were also so high that a combined forward and rotary movement of the punch proved more effective regarding the expenditure of force than a punching motion that merely involved a simple forward motion.

Introduction:

Procedures for epidermal skin transplantation provide less than optimal cosmetic and functional results and cannot ensure that all skin layers are replaced [1]. Whole (i.e. full-thickness) skin grafts that aim to produce a favourable cosmetic and functional result cannot be transplanted over large areas [2] due to the fact that there is a limit on the size of donor area which can be used. Using cultured skin it is now possible to incorporate skin appendages in transplants, such as sweat glands and hair cells, which are extremely important for ensuring a good skin quality. Despite this, there is still no laboratory-cultured whole skin substitute that can be used on a routine basis for skin operations [3,4]. Clinical observations have revealed that a whole skin biopsy removal using biopsy punches with a biopsy diameter of 1 - 2 mm results in minimal or even no scarring. Based on this observation, a new method for intraoperative whole skin transplantation was designed in which 1 - 2 mm whole skin islets recovered from biopsy punches were to be used for the purposes of an intraoperative, autologous whole skin transplantation. In a collaboration with the University of Lübeck and the Emergency Hospital Berlin, a prototype was designed for the sampling of multiple whole skin punch biopsies, each with sizes within the range of just millimetres. Using this new procedure a 5 x 5 cm sized array was designed, equipped with 1 mm or 2 mm biopsy punches, that was to be used for removing whole skin islets. After sampling, the whole skin islets can then be introduced into a substitute dermal matrix, after which the matrix can then be transplanted onto the new area scheduled for repair. In preliminary pilot experiments, compression and tension tests were carried out for the removal of biopsies from porcine skin using 3 mm punch diameters, and for the removal of biopsies from human cadaver skin using 2 mm punch diameters. This showed that although a rotary motion significantly minimised the force required to take a successful biopsy, there was still a considerable technical challenge posed by the incorporation of a simultaneous rotary mechanism, particularly when implemented in a stamp device containing numerous individual punches. Since there have been no studies on the resection forces occurring when taking a biopsy, the focus of this paper was to compare the resection forces of 1 mm and 2 mm biopsy punches when employing a simple forward motion, which alone would greatly facilitate the technical realisation of a new method for carrying out whole skin transplantations.

Materials and methods:

The forces required to penetrate the dermis and epidermis of human skin were investigated using 1 and 2 mm biopsy punches. The experiments were carried out at the Laboratory of Biomechanics at the University Hospital Schleswig-Holstein using a Zwick material testing machine and its proprietary software TextXpert II (Zwick GmbH & Co. KG, Ulm, Germany). In the first test, a 100 N force sensor (load cell type Z6FD1/10 kg Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany), and in the second test, a 2000 N force sensor (load cell type U2A, 2 kN, Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany) were used to detect the forces. For the purposes of penetrating the skin, biopsy punches supplied by the company KAI of 1 mm and 2 mm in diameter were used (standard biopsy punches, Kai Industries & Co. Ltd., PFM Medical AG, Cologne, Germany). Human cadaver skin was used as the model tissue in the experiments carried out, which was provided by the Institute of Anatomy, University of Lübeck. These were seven frozen pieces of skin which had been obtained from the thigh of a male donor aged 79. The body parts used were examined under the auspices of the "Burial Act of Schleswig-Holstein, enacted 04/02/2005, Section II, Paragraph 9 (autopsy, anatomical)".

The skin supporting device consisted of a base plate measuring 120 x 148 mm with 25 mm high walls as well as a perforated plate


Fig. 1) Skin supporting device, top view of the perforated plate

A silicone heating mat (100 x 100 mm, 12 V, 10 W, rs-components GmbH, Mörfelden-Walldorf, Germany) was used to heat the skin in the experiments to a constant temperature of 30 ± 2 ° C. Before the test, skin was secured between the aluminium plate and the perforated plate to prevent it from moving when the tests were being conducted and to simulate and equally distribute the physiological stretching. For the first part of the experiment, 100 units of 1 mm and 100 units of 2 mm standard biopsy punches were used, where each consisted of a resection cylinder and a plastic overbody The experimental setup of the first part of the experiment is shown in Biopsies were taken in alternating fashion with 1 mm and 2 mm cylinders per hole so that for each piece of skin the biopsy punches were used 25 times with 1 mm and 25 times with the 2 mm punches. This procedure was carried out in the same manner with a total of four pieces of skin, so that a total measurement sample of 100 measurements with 1 mm punches, and 100 measurements with 2 mm punches was created
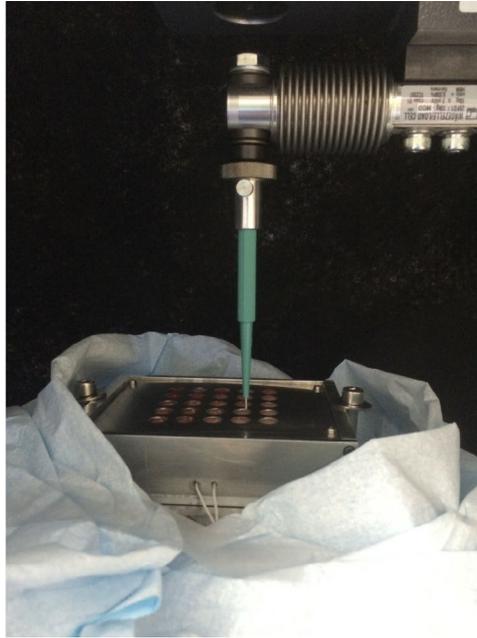

. 2) Standard biopsy punch for the first part of the experiment. Left 1 mm and right 2 mm biopsy diameter
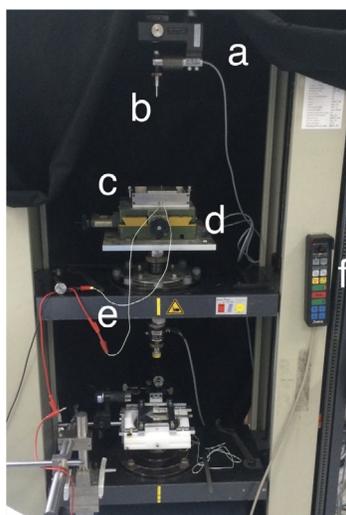
. 3) Experimental set-up the first part of the experiment, Zwick material testing machine

3a)   100 N force sensor with power connector
3b)   Thorn attachment for the punch
3c)   Skin supporting device
3d)   XY table
3e)   Power supply for the heating mat
3f)   Control module for the material testing machine

4) Experimental set-up with a clamped biopsy punch

In the first part of the experiment the resection forces were measured using a 100 Newton load cell with only the resection force being measured from a biopsy punch. In order to prevent incorrect values arising due to wear of the biopsy punches, a new biopsy punch was used for each measurement (i.e. 100 punches were used).

For the second part of the experiment two biopsy stamps were constructed which contained either 100 x 1 mm or 100 x 2 mm cylinders without their plastic overbodies

In these tests a 2000 N load cell was used to measure the resection forces occurring when 100 punches simultaneously penetrated the skin. It was assumed that with different punch lengths in a stamp there would be a difference in resection force compared to the stamp with equally long punches. Figure 7 shows the stamp with identically long punches and Figure 8 shows the stamp with different punch lengths. The experimental setup of the second part of the experiment is shown in Figure 9. Here, the resection forces of 100 x 1 mm punches and 100 x 2 mm punches were examined which simultaneously penetrated the skin. The stamp measurements for each skin piece were performed on three areas and on two pieces of skin per donor, meaning that a total of six measurements were made per stamp (and indeed per donor).

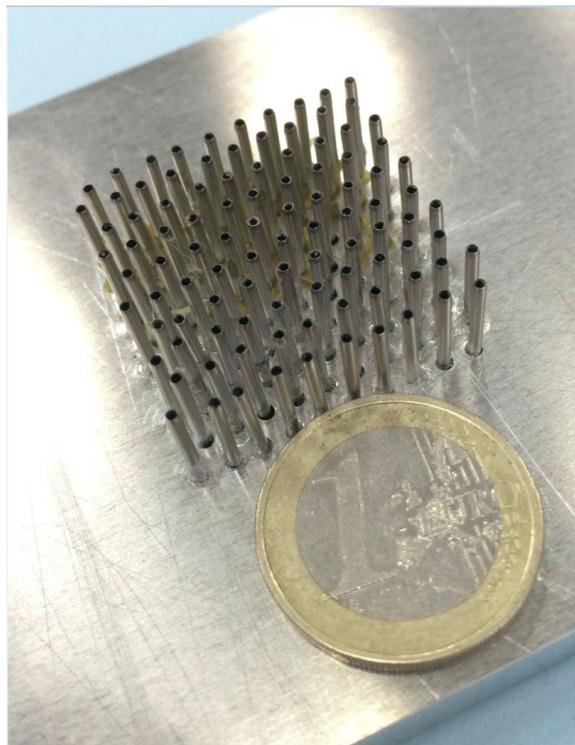Figure 6) Biopsy punch without plastic body before incorporation into the stamp

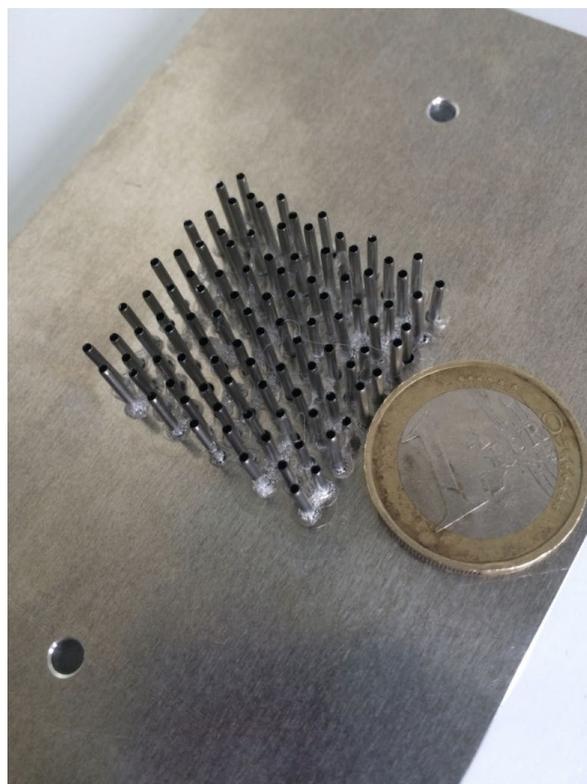Fig. 7) Stamp with 100 punches of equal length
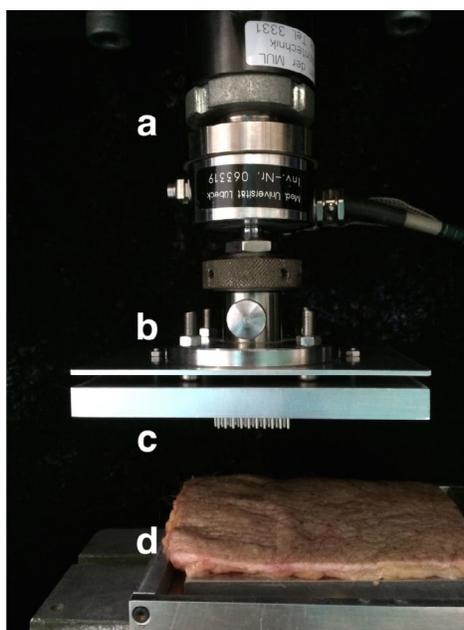
Fig. 8) Stamp with 100 punches of different length

Fig. 9) Experimental setup for the second part of the experiment

9a)   2000 N force sensor with power supply
9b)   Aluminium base plate with adapter
9c)   Punching stamp with 100 punches
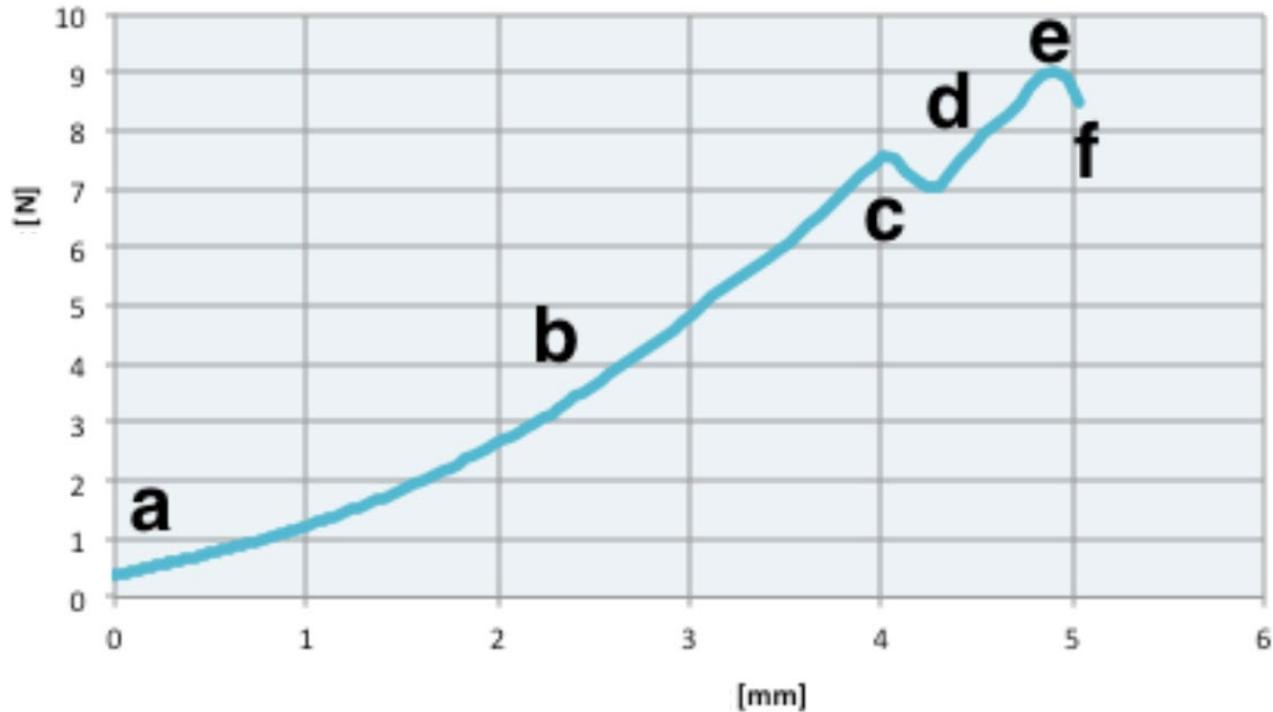9d)   Skin layer construction with human skin

Fig. 10) Resection curve for a biopsy punch
10 a) Start of measurement
10 b) Gradient
10 c) Resection the first fibre bundle
10 d) Gradient
10 e) Maximum force (or force required for rupture)
10 f) End of measurement

Results:

a typical resection force curve for a 1 mm biopsy punch as it penetrates the skin. The diagram reveals how the force changes in relation to the depth of penetration. In a total 8 of the 100 measurements with 1 mm punches and 17 of the 100 measurements with 2 mm punches, there was no cut or penetration of the skin when applying the combined forward and rotary motion (Tab. 1). From the maximum forces the arithmetic mean was calculated for the individual pieces of skin, with the measured values being depicted in Although all pieces of skin originated from the same donor, the resection forces measured with the four skin pieces used in the first part of the experiment varied substantially. In addition, forces measured within a single piece of skin also varied greatly. the averaged and rounded-up resection forces of the 1 mm and 2 mm biopsy punches with the four different areas of skin. Both lower and higher resection forces are distributed over the pieces of skin, and no general organised pattern could be ascertained. The resection forces materialising with the 2 mm punches were approximately twice as high as those for the 1 mm punches

	No penetration of the skin, 1 mm biopsy punch, 100 measurements	No penetration of the skin, 2 mm biopsy punch, 100 measurements
Skin piece 1	5 of 100	12 of 100
Skin piece 2	0 of 100	2 of 100
Skin piece 3	2 of 100	1 of 100
Skin piece 4	1 of 100	2 of 100

Tab.1) Number of punches that did not penetrate the skin

Mean resection force	1 mm biopsy punch	2 mm biopsy punch
Skin piece 1	13 N	23 N
Skin piece 2	11 N	19 N
Skin piece 3	12 N	19 N
Skin piece 4	9 N	15 N

Tab. 2) Mean maximal forces per skin piece and punch

4.69 N	13.58 N	6.14 N	13.14 N	10.95 N
5.03 N	7.63 N	5.97 N	7.17 N	n.n.
8.80 N	5.59 N	8.22 N	11.94 N	10.85 N
7.84 N	6.53 N	8.91 N	8.87 N	8.11 N
9.67 N	6.99 N	6.29 N	7.21 N	11.36 N

Tab. 3) Measured resection force of the 1 mm punches on skin piece 4

7 N	13 N	14 N	10 N	15 N
10 N	8 N	11 N	11 N	13 N
11 N	11 N	14 N	12 N	14 N
15 N	8 N	9 N	8 N	10 N
11 N	9 N	9 N	11 N	12 N

Tab. 4) Mean measured resection forces for the 1 mm punches (averaged) with the four skin pieces

14 N	16 N	15 N	18 N	20 N
19 N	17 N	19 N	19 N	16 N
14 N	14 N	16 N	17 N	15 N
21 N	25 N	20 N	19 N	14 N
20 N	17 N	21 N	19 N	16 N

Tab. 5) Mean measured resection forces for the 2 mm punches (averaged) with the four skin pieces

The mean resection forces for 1 mm and 2 mm punches could be determined for all measuring points on the four pieces of skin. With the 1 mm biopsy punch a mean resection force of 11 N arose from all 100 punches with a standard deviation of 2 N. With the 2 mm biopsy punch on the other hand, the mean resection force from all 100 punches was 18 N with a standard deviation of 3 N. In addition to the tests for determining resection forces, it was also important to determine how many whole skin islets remained after resection and retraction of the punches from the skin, i.e. those that had not been completely removed from the skin. With the 2 mm punches all whole skin islets were retracted from the skin by the biopsy punches. With the 1 mm punches, 17 of the 100 did not cut through the skin at all, and of the remaining 83 that were cut, only 47 of these skin cylinders were successfully extracted (56.62%).

In the second part of the experiment it was assumed that the mean measured resection force for the 1 mm punch from the first part of the experiment would be 100 times higher when using the specially constructed 100 punch biopsy stamp.

Of the in all 12 attempts at measurement (three measurements on four pieces of skin), resection of the skin only occurred in 7 of these cases. Table 6 shows the mean resection forces measured in the second part of the experiment for the two stamps having the same and different punch lengths. In the first part of the experiment employing single punches, a mean resection force of 11 N was determined for the 1 mm punch. By employing a 100 punch stamp, as used in the second part of the experiment, a resection force of 1,100 N was expected (i.e. 100 x 11 N = 1100 N). With the stamp equipped with the same punches lengths, the mean resection force measured was surprisingly low at 639 N, i.e. only 58% of the expected 1100 N. When using the stamp with different punch lengths, an even lower mean maximum force of 478 Newton was measured, i.e. 44% of the calculated maximum force from the individual measurements.

	Same punch lengths	Different punch lengths
Mean maximum force	639 N	487 N
Standard deviation	83 N	576 N

Tab. 6) Mean maximum forces and standard deviations for both stamps

Discussion:

Due to the three dimensional structure of skin, its biomechanics are extremely complex and follow a non-linear stress-strain relationship that is non-homogeneous, incompressible, anisotropic (not having the same properties in all directions), and subject to a certain prestretching and stretching force [5,6]. To protect against external mechanical influences, the skin is also viscoelastic [7]. These properties can be attributed to the complex network of collagen, elastin fibres and proteoglycans that are localised in the dermis [8]. When the skin is pierced, Langer observed that the prick wound assumes an oval shape, since the skin pulls apart along what he termed "fissure" lines. Once the skin is placed under tension, the collagen-containing fibres tighten parallel to these lines [9]. In 1933 Jochims conducted experiments to determine the mechanical resistance of skin in children. By creating a fold of skin through the pushing together of two plates placed on the skin, he measured the total force and the retraction force. The results of his measurements were that the retraction force only reflected the elastic resistances occurring, whereas the total force reflected both the elastic and inelastic (viscous) resistances [10]. Although the elastin and collagen fibres are linearly elastic, the stress-strain curve of the skin for one-sided traction is non-linear, since the structure of the skin is not homogeneous [11]. In 1965 Zink investigated the stretching of human skin using cadaver skin strips with a constant strain rates from a non-stretched state all the way to rupturing of the skin. More than 2,000 tests revealed a highly characteristic stress-strain relationship. Zink divided this into four phases. In the first phase, the unstretched collagen fibres are first unpleated, with the force required for this being small. During the second phase, more collagen fibres become unpleated, during which the stretching obeys Hooke's law. This stretching creates an increase in force. In the third phase, all fibre bundles are stretched and a few start to tear. In the fourth phase, a further stretching of the tissue results in a complete rupture of the collagen fibres and the tissue then starts to separate [12]. Shergold and Fleck as well as Bischoff et al. used silicone rubber as a substitute model in some

of their experiments, as this shows similar physical properties to human skin [13,14]. However, due to its heterogeneous structure and its complexity, the design of the skin is far more complex than that of silicone rubber, and even results from animal experiments can only be extrapolated to human skin with major reservations. In our own preliminary porcine skin experiments, higher resection forces were found than was the case for human cadaver skin. Brett et al. confirmed that the force to penetrate human skin is lower than is the case with porcine skin [15]. However, despite the use of tempered human cadaver skin, the results of this work need to be looked on with caution since after thawing of the skin an outflow of liquid was observed. Wenzel et al. found that the elasticity of the skin was related to the water content, so that the elasticity might have actually increased in the experiments described there [16]. Although the skin pieces originated from the same donor, the resection forces between the four pieces of skin varied as an indication that the skin material was of a highly heterogeneous nature.

Through a combined forward and rotational movement of the biopsy punch, a mean resection force of just 5.6 N was measured for the 2 mm biopsy punch in an earlier study. A forward rotational motion of the punch therefore results in a substantial reduction in the forces required for resection. Given that a simple forward motion showed an average resection force of 18 N, the additional rotation resulted in a reduction of the force required for resection to just 31%. Due to the fact that the resection forces were lower with simple forward movement and when using a 1 mm (compared to 2mm) biopsy punch, a combined forward-rotational movement with a 1-mm-punch would lead to a further reduction of force during penetration of the skin.

Conclusion:

The aim of the work was to investigate resection forces when carrying out 1 mm and 2 mm biopsies from tempered human cadaver skin. In the first part of the experiment, a mean resection force of 11 N was measured for the 1 mm biopsy punch, while 18 N was measured for the 2 mm punch with forward movement alone.

As expected, the measured resection forces when using the 2 mm punch were on average higher than was the case with the 1 mm punch. However, the resection forces tended to vary substantially even within skin pieces from the same donor. Based on the results of the first part of the experiment, 2 stamps consisting of 100 punches with a diameter of 1 mm were constructed for the second part of the experiment. For this purpose, two types of stamps were made, where one featured identical punch lengths and the other had punches of different length. An average resection force of 639 N was determined for the stamp with equally long punches, whilst this figure was just 487 N for the stamp fitted with different punch lengths. In summary it can be concluded that very high resection forces were required to penetrate human skin with a experimental set-up involving forward punch motion alone. The authors consider a combined forward-rotational movement to be advantageous over a feed motion alone regarding the forces required to penetrate human skin with biopsy punches.

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