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ORIGINAL ARTICLE

Assessment of tissue damage from mosquito-inspired surgical needle

Sai Teja Reddy Gidde[a], Sharad Raj Acharya[a], Shital Kandel[b], Nancy Pleshko[b] and Parsaoran Hutapea[a]

[a]Department of Mechanical Engineering, Temple University, Philadelphia, PA, USA; [b]Department of Bioengineering, Temple University, Philadelphia, PA, USA

ABSTRACT

Introduction: Many percutaneous procedures utilize surgical needles to extract tissue samples in biopsy or to apply specific cancer treatments. A design of mosquito-inspired surgical needles was proposed to improve the efficacy of these procedures by reducing the needle insertion force and the resulting tissue damage. The focus of this study is to assess tissue damage caused by the insertion of a mosquito-inspired needle into soft tissues.
Material and methods: In this work, the geometric features and the dynamic stinging (insertion) mechanism of mosquito proboscis were mimicked for the design of 3D-manufactured bio-inspired needle prototypes. A specially designed test setup was developed to measure the insertion force in bovine liver tissue. The histology assessment based on hematoxylin and eosin staining and image analysis was conducted to determine the bovine liver tissue damage.
Results: It was observed that the insertion force can be reduced by up to 39% and the bovine liver tissue damage was decreased by 27% using the mosquito-inspired needles when compared with using the standard needles.
Conclusion: The findings from this study suggested that the bioinspired needle design has great potential to advance surgical needles for more effective and less invasive percutaneous procedures.

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KEYWORDS
Surgical needle; mosquito; vibration; tissue damage; percutaneous procedure

Introduction

Surgical needles are commonly used in percutaneous procedures such as biopsy, drug delivery, blood sampling, deep brain stimulation, and brachytherapy cancer treatment [1–3]. In performing these procedures, medical professionals make a small incision into an external tissue and insert a needle to reach a specific target or internal tissue location. The efficacy of the needle insertion procedure is highly dependent on the target accuracy [4]. It has been well studied that the accuracy can be improved by reducing needle insertion force, tissue deformation, and, most importantly, tissue damage [5–9]. The tissue damage is directly influenced by the insertion force and tissue deformation [10–12]. Researchers have shown that higher needle insertion force will contribute to higher deformation, thus increasing the tissue damage causing the needle to miss the target [1,13]. It is therefore critical to reduce the insertion force to minimize tissue damage to facilitate more accurate procedures.

Critical parameters affecting tissue damage are needle shape and design [5,14] and dynamics of the insertion such as needle vibration [1,8,15] and needle rotation [16]. Bioinspired needles have recently been proposed for reducing insertion force, tissue deformation, and tissue damage. In our previous work, it was determined that the needle insertion force in a tissue phantom can be decreased by using a mosquito-inspired needle [10]. In the same study, through tests performed in a clear tissue gel phantom, it was shown that the tissue deformation decreases with the application of a mosquito-inspired needle. A similar study using a honeybee stinger-inspired needle in tissue gel phantom also showed that the decrease in the insertion force would result in a decrease in tissue deformation and tissue damage [11]. Additionally, it was also observed that introducing vibration of mosquito-inspired needles could further decrease insertion force and tissue deformation [10]. This study focuses on assessing the tissue damage caused by the dynamic insertion of mosquito-inspired needles in bovine liver tissues.

CONTACT Parsaoran Hutapea hutapea@temple.edu Department of Mechanical Engineering, Temple University, 1947 N 12th St, Philadelphia, PA 19122, USA

A mosquito inserts its proboscis through animal skin with minimal force [17]. Kong et al. found that a mosquito penetrates the tissue with a force of 18 μN [18]. The unique structure in the mosquito proboscis is taken as an inspiration in the proposed needle design to reduce the insertion force and tissue damage. A needle insertion force is defined as the combination of tissue stiffness force, cutting force, and friction force on the needle–tissue interface [19,20]. The tissue stiffness force exists from the moment the needle tip touches the tissue surface to the moment the needle tip punctures the tissue. After the puncture, the insertion force is the summation of a cutting force and a friction force. The cutting force is due to the needle tip cutting through tissue layers and the friction force is due to the sliding of the needle body surface against the tissue. Among these forces, the friction force is the largest contributor to the insertion force [21]. Therefore, in this work, it is conjectured that the mosquito-inspired geometric features on the needle reduce friction and in turn decrease the insertion force.

The mosquito-inspired (also referred to as bioinspired) needle design used in this study included a labrum structure on the tip and a maxilla design on the body [22,23]. The structure of the labrum was adopted for the needle tip design of the proposed mosquito-inspired needle to lower the cutting force. Similarly, the maxilla [23] (i.e., a jagged structure of the mosquito proboscis) was mimicked for the needle body design. The bioinspired design minimized the friction force due to a less frictional interface between the needle and tissue. The reduction in friction force results in a decrease in the insertion force [1,5,14,19,22]. It was conjectured that the decrease in the insertion force of the proposed mosquito-inspired needle would also reduce the tissue damage.

The other bioinspired feature that was implemented was the vibration of the proboscis during the stinging process [24,25]. During the insertion of the proboscis into animal skin, the mosquito uses a longitudinal vibration (i.e., the back-and-forth axial motion), which is in the insertion direction at a frequency of about 5–30 Hz [17,24]. In this study, a similar vibration was applied to the mosquito-inspired needle during insertion into the bovine liver. The use of vibration during insertion into soft tissues has been studied by several other researchers [3,15,25,26]. They have all concluded that the vibration guides the needle to improve cutting performance as well as to lower the insertion force.

Studies from the literature also have shown that the bioinspired mechanism during needle insertion can improve needle placement [27–31]. But the main difference between the existing work and the work presented in this study is the combination of needle body design (geometric features) and the insertion mechanism (vibration) that influences the insertion force and tissue damage. Moreover, the assessment of tissue damage caused by the mosquito-inspired needle has not been reported. Additionally, in our previous study, it was also observed that the deformation in a clear tissue gel phantom decreased during the insertion of a vibrating mosquito-inspired needle [10].

It was therefore hypothesized that the tissue damage can be minimized if the insertion force with the use of a vibrating mosquito-inspired needle was decreased compared to a standard needle. To prove this hypothesis, a tissue damage study of mosquito-inspired surgical needle insertion was performed. A series of needle insertion tests were conducted on *ex vivo* bovine liver tissues. The tissue damage, defined as ruptured areas of the cross-section of bovine liver tissues, was evaluated by performing a histology and image analysis study.

Material and methods

Design and manufacturing of mosquito-inspired needle

The two basic design parameters considered for the design of the mosquito-inspired needle in this study were the geometrical shape of the needle and vibration. The first parameter, the needle shape, and geometry consist of two important features: maxilla shape on the needle body and labrum at the needle tip [23,24]. The microscopic features of the labrum and maxilla in a proboscis of a mosquito are shown in Figure 1(a). These geometric features were designed on the needle body to reduce the insertion force of a surgical needle. Using these two bioinspired features, 3D-printed prototypes of the bioinspired needle were manufactured. One critical consideration made in the needle design was that the radial asymmetry of mosquito proboscis was substituted by a radially symmetrical shape on the needle body. This was done because the asymmetrical structures on the needles produce an unbalanced and undesirable force [32,33]. It should be noted that a typical size of a surgical needle used in procedures such as biopsy or brachytherapy is approximately 1.5–2 mm in diameter. Due to the complexity in manufacturing the prototype of this size and lack of current manufacturing technology,

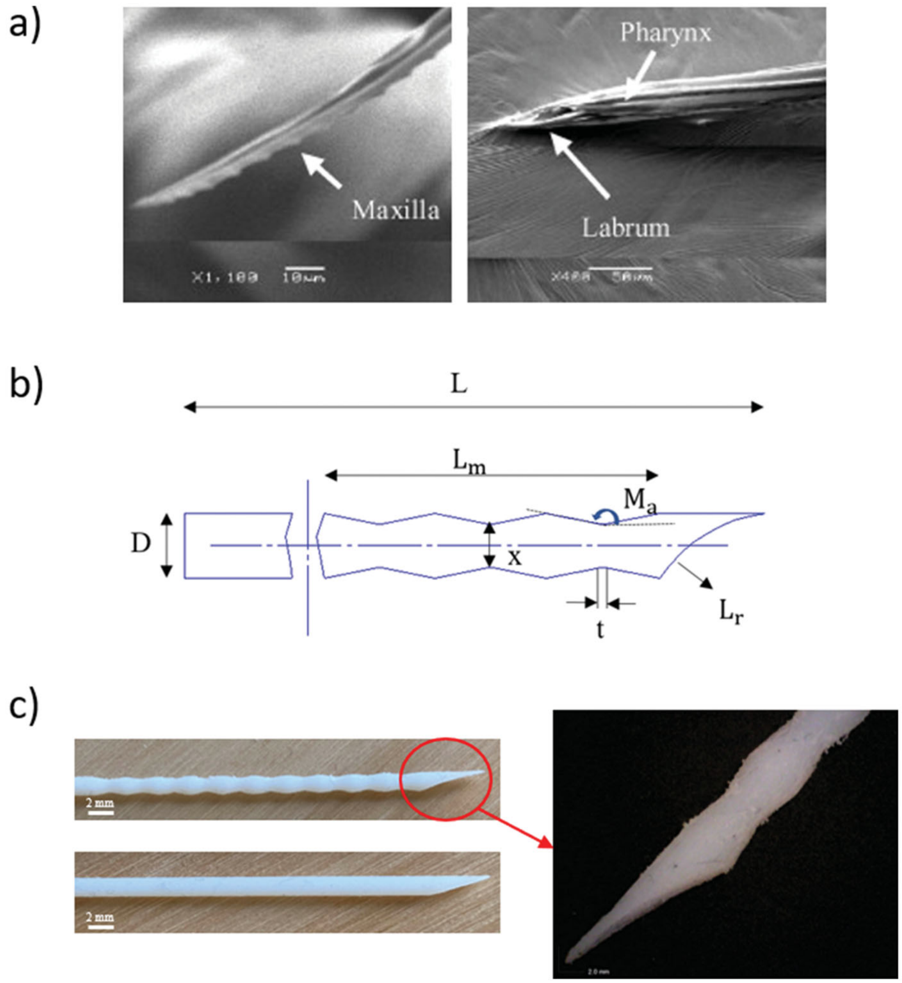

Figure 1. (a) The maxilla (jagged structure) and labrum (curved tip) in a mosquito proboscis. Adapted with permission from Elsevier (Sensors and Actuators A: Physical), Copyright (2011) [23]. (b) The schematic representation of the mosquito-inspired needle in this study with the parameters as maxilla angle (M_a) of 170°, thickness (t) of 0.2 mm, length of the needle (L) of 180 mm, length of maxilla shape on the needle body (L_m) of 65 mm, needle diameter (D) of 3 mm, inner distance (x), and radius of the labrum tip (L_r) of 35 mm and (c) the 3 D printed needles of both mosquito-inspired and standard bevel-tip designs.

the prototypes were scaled up and manufactured to D as shown in Figure 1. The exact dimensions of the maxilla are not transferred for the presented needle prototype as the needle diameter is bigger than the mosquito proboscis. But instead, the shape of the maxilla was adopted for the bigger-size needle. The aspect ratio (diameter/length) was considered for the bioinspired needle in this study. The diameter of an actual mosquito proboscis ranges from 20 to 40 μm and the length ranges from 1.5 to 2.5 mm. From the literature, we found that the maxilla of the mosquito reduces the force while steering into tissue [17].

The second parameter, the longitudinal vibration at a frequency of 200 Hz and an amplitude of 5 μm was applied during needle insertion. The final design parameters were chosen based on the set of experimental results from the study of the effect of mosquito design parameters on the insertion force. The mosquito-inspired needle design parameters and the vibration frequency follow the insertion force result of our previous study [10], which demonstrated that the maximum reduction in insertion force for a 3 mm diameter mosquito-inspired needle can be achieved at this frequency and amplitude. The bevel-tip needles have many medical applications and in general have less insertion force compared to needles with different tips such as conical, blunt, or diamond tips [5]. The mosquito-inspired needle in this study would furthermore reduce the insertion force. In addition, since the labrum-tip designed for the mosquito-inspired needle was similar to the bevel-tip shape, a fair comparison would be to use bevel-tip needles in this study. Since this study focuses on studying the influence of mosquito-inspired needles on soft tissue damage, the mosquito-inspired needle prototype was compared with the same 3 mm diameter 3D printed standard bevel-tip needle. As shown in Figure 1(b), the design parameters of the mosquito-inspired needle were maxilla angle (M_a) of 170°, thickness (t) of 0.2 mm, length of maxilla shape on the needle body (L_m) of 65 mm, and the radius of the tip (L_r) of 35 mm. The inner distance (x) depends on the change in maxilla angle. The jagged structure designed on the needle body is up to 65 mm in length and the remaining length is the same as the standard needle diameter. Also, the outer diameter is the same even with the jagged structure (see Figure 1(b)). The jagged structure on the needle body means the removal of extra material. The jagged structure is not added to the needle body; instead, it has been carved on the needle body surface. It is conjectured that the internal diameter of the hollow needle for the proposed needle will not interfere with the internal channel for performing surgical actions. The standard needle with a bevel angle of 40° was chosen and the diameter was the same as the mosquito-inspired needle, which was 3 mm. The mosquito-inspired needles and the standard needles were manufactured (a total of six needles i.e., three mosquito-inspired and three standard needles) using a high-resolution print accuracy 3D printer (Object Connex 350, Stratasys, Inc., Eden Prairie, MN, USA). The material used to manufacture the needle prototypes are polymer which consists of 30% acrylic monomer, 25% isobornyl acrylate, 15% phenol, 12% diphenyl 2, 8% titanium dioxide, 5% acrylic acid ester, and 5% composition of phosphoric acid and propylene glycol monomethyl ether acetate. The 3D-printed needles are shown in Figure 1(c).

Needle insertion test setup and procedure

The needle insertion test setup (schematics) as shown in Figure 2(a) includes the needle that was attached to a motorized linear slide. The base of the needle was attached to a six-axis force/torque transducer Nano 17® (ATI Industrial Automation, Apex, NC, USA), which was connected to a data acquisition system (National Instruments Corporation, Austin, TX, USA). To apply the vibration during insertion, the needle was fixed to an actuator that vibrated the needle in the longitudinal direction (Figure 2(b)). The actuator utilized in this study was a piezoelectric actuator equipped with an amplifier (Physik Instrumente, Auburn, MA, USA).

To compare the insertion force and resultant tissue damage from the mosquito-inspired needle against the standard needle, the needles are inserted into bovine liver tissues. A total of three fresh bovine livers were obtained from a local butcher shop. For each bovine liver sample, two-needle insertion experiments were performed, i.e., one with the standard needle and one with the mosquito-inspired needle, adding

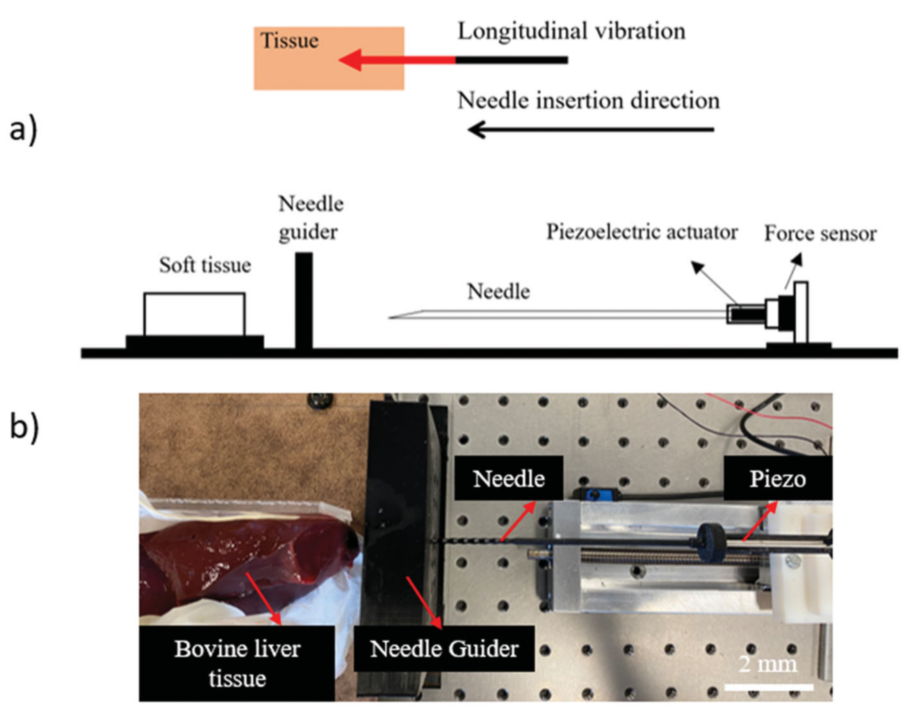

Figure 2. (a) A schematic representation of the needle insertion test setup with longitudinal vibration and (b) a top view of an insertion experiment in bovine tissue with a mosquito-inspired needle.

up to a total of six insertion experiments. The number of 3D-printed needles for each prototype was three. The number of insertion tests in each liver tissue was limited to two (one each for standard needle and mosquito-inspired needle) to avoid the intersection/crossing of the needle insertion path. This intersection inside the tissue could cause difficulty in performing histology studies. For this reason, three different bovine tissues were used, such that each set of needle prototypes (standard and mosquito-inspired) was used to perform insertion tests in one tissue, which means a total of six insertion experiments. For example, the histology process in bovine brain tissue makes it extremely difficult to examine the needle insertion location and to measure the damaged area because of the lower Young's modulus. The liver tissue was chosen in this study because it is more vulnerable to needle insertion damage compared to other tissues; this is due to its size, location, and complex mechanical behavior (in terms of Young's modulus). To prevent the nonhomogenous liver tissue from creating bias we chose to randomize the needle insertion experiments.

A paired *t*-test was performed for tissue damage due to two types of needles on each liver under the assumption that the distribution is Gaussian. But increasing the number of liver samples as well as labor-intensive histology samples was not feasible for this study. This study also found that even with the three samples for each group, the conclusion can be made based on the study by De Winter [34] for using *t*-test with extremely small sample sizes ($N \leq 5$). Also, previous studies with fewer sample numbers have been able to conclude hypothesis testing on insertion force and its effect on the tissue damage [7,12,14]. It showed that paired *t*-test was feasible even with the sample size of three if the within pair co-relation was high. Based on the insertion depth and the puncture hole, the site of needle insertion was located [1] in bovine liver tissue. Then, the histology study was conducted to determine the influence of the proposed mosquito-inspired needle design on tissue damage.

Histology study

Sample preparation

The soft tissue samples that were studied for insertion force experiments were cut and trimmed to investigate the tissue damage by histology method. With two histology experiments on each of the three liver tissues, one to assess damage due to standard needle and the other to assess damage due to mosquito-inspired needle, a total of six histology experiments were conducted. The histology assessment in this study followed all the standard protocols for evaluating the tissue damage [7,35]. Locating the needle insertion path after needle insertion and extraction is challenging. It was located based on the insertion direction and the insertion depth. Then a section of the insertion path with a cross-section of 40 mm × 40 mm and depth of 4 mm is cut halfway along with the insertion depth and extracted. The final tissue sample for the histology was taken at 30 mm insertion depth from the surface (half of the insertion depth) for all experiments. During the sectioning of the bovine liver samples, they were handled with a guider, which helps to cut and trim gently. They were placed in the cryomolds to begin the histology preparation. This process was repeated for the remaining bovine liver tissue samples.

Before adding a formalin solution (10%), 4-mm-thickness samples were immersed three times in Dulbecco's phosphate-buffered saline (DPBS) solution to wash the tissue samples before dissociation, which is a process of eliminating water content before sectioning them. This DPBS solution prevented cells in the tissue samples from rupturing due to osmosis. The soft tissue samples were then immersed in formalin solution for 24 h and then in DPBS solution. In the next step, the optimal cutting temperature (OCT) compound was added to the cryomold with the tissue samples and stored in a −80 °C freezer. The OCT compound was used to embed the tissue samples and freeze them before sectioning. These frozen samples were sectioned using a cryostat (Leica CM3050 S, Leica Biosystems, Lincolnshire, IL, USA) as shown in Figure 3 and the thickness used to section the samples was 0.5 µm and they were placed smoothly on the micro slides.

Staining and imaging

To perform the histological evaluation of the bovine liver tissue samples, hematoxylin (1%) and eosin Y (1%) solutions and xylene were prepared and added in tissue-tek dishes. To obtain the hematoxylin and eosin (H&E) stain, graded ethanol (70%, 80%, 90%, and 100%) was made to obtain the desired concentration by diluting 100% ethanol with deionized water. The tissues were then processed for H&E staining. After finishing the staining process, the sample slides were left to air dry for 24 h before observing the slides for the damaged area using a microscope. To measure tissue damage on the stained slides, a microscope (Olympus CX53 Inverted Microscope) with an

Figure 3. The cryostat machine for sectioning the bovine liver tissue samples with a thickness of 0.5 µm.

Olympus color camera DP22 was used. A 4× magnification was applied during the imaging of the slides. The snapshot images captured from the microscope were evaluated using ImageJ [36] to measure the cross-sectional area of the tissue damage due to the mosquito-inspired needle. The area of the damage is estimated by setting the calibration scale measurement (2×) in the ImageJ and the threshold black area is selected and adjusted to see the desired levels of darker areas in the image. Next, the threshold regions are measured by setting the size range and the display results option will show the area of the selected region in the image. This process was repeated for the other captured images and the average area was presented in the results.

Statistical analysis

The analysis was performed between the two samples (due to standard and mosquito-inspired needles) that were used to determine the significance. Statistical analyses between the measured tissue damage area data were done with a 95% confidence interval. The significance of this reduction in tissue damage area was calculated using a paired *t*-test. The within pair correlation coefficient for our study was measured to be 0.98. Moreover, the statistical power of this study was found to be significant. It was concluded that the *t*-test could be applied for extremely small sample size if the effect size was expected to be big. The effect size measured as Cohen's *d* for our sample size was 5.92 (which is high).

Results

Effect of mosquito-inspired needle on insertion force

The insertion force data presented was the average of three insertion tests for each needle prototype. Due to the nonhomogeneous properties of bovine liver tissue [8,37], the force curve was not continuous throughout the insertion depth as shown in Figure 4. However, the insertion tests with mosquito-inspired needles showed a significant reduction in the insertion force compared to the standard needles. The forces in both standard and bioinspired needles are showing vibrating patterns because of the heterogeneous and anisometric nature of the liver tissue. The needle insertion in liver tissue was done from the left lobe to the right lobe in all experiments to maintain identical conditions for comparison between the two types of needles. The insertion location was exposed based on the insertion depth and the bovine tissue was sectioned immediately to locate the path and implemented the histological method. A similar reduction of insertion force due to the design of the needle geometry was seen in the literature [7]. Upon the observation of the insertion force data in this study, it is evident that without any geometrical design and vibration (which is for standard needles) the force will be higher compared to mosquito-inspired needles, where the insertion force was lower.

The maximum insertion force recorded for the full depth of insertion (which is 60 mm) for the mosquito-inspired needle is 4.97 ± 0.19 N whereas that for the standard needle is 8.26 ± 0.17 N. The average

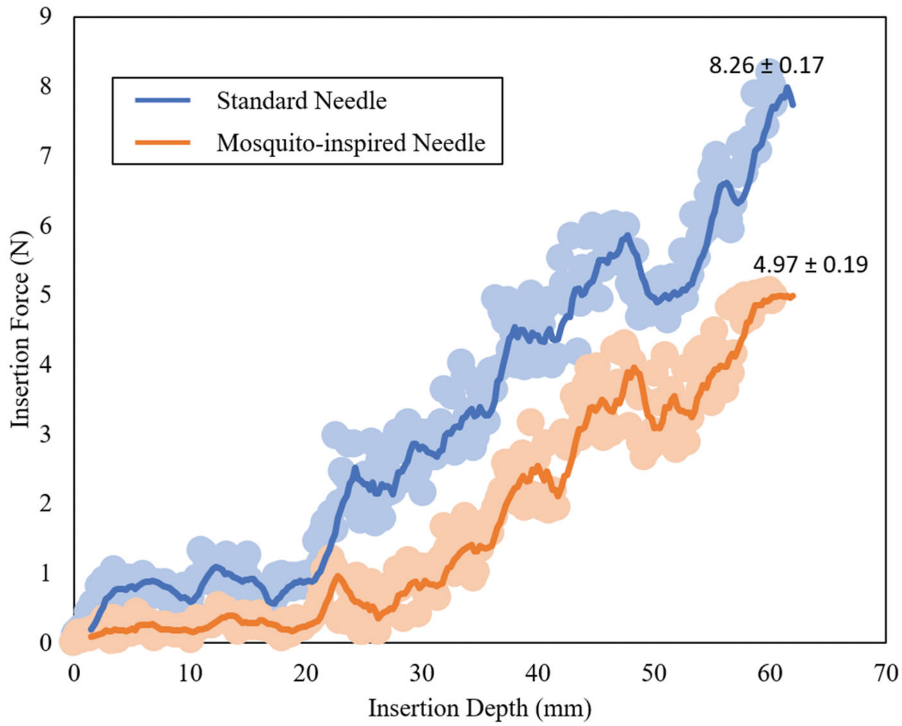

Figure 4. The average insertion force plot for the standard needle and the mosquito-inspired needle (a vibration of 200 Hz, and an amplitude of 5 µm). Insertion was performed in the bovine liver for 0–60 mm insertion depth with 1 mm/s insertion velocity (standard deviation (SD) range values for the standard needle: $0.05 \leq SD \leq 0.17$ and mosquito-inspired needle: $0.07 \leq SD \leq 0.19$).

insertion force was reduced by 39% using a mosquito-inspired needle compared to a standard needle. It was observed that the insertion force decreased with mosquito-inspired needles compared to standard needles as shown in Figure 4. This reduction was conjectured due to the geometrical shape of the needle and the applied vibration during insertion [8,10]. The total velocity (insertion speed and vibration) was also the reason for the reduction in insertion force [38].

Effect of mosquito-inspired needle on tissue damage

The cross-sectional areas of the tissue damage in the bovine liver caused by both the needles are shown in Figure 5. The histology results showed that the damaged cross-sectional area due to the mosquito-inspired needle was less than that due to the standard needle (Figure 5(a)). The average of the three quantified cross-sectional areas of the damage on the tissue with mosquito-inspired needles (Figure 5(b)) ($n = 3$) was $4.71 \pm 0.45\,mm^2$ and that with the standard needles ($n = 3$) was $6.42 \pm 0.72\,mm^2$ (see Table 1). Furthermore, it was also confirmed that the damaged perimeter, which is the estimated border length of the tissue damaged region, was reduced in mosquito-inspired needle insertions. The perimeter due to the mosquito-inspired needle was 10.91 ± 0.31 mm and the standard needle was 19.04 ± 0.44 mm.

The images captured from the microscope showed clear tissue damage and its boundaries around the cross-section of the needle insertion site. The white region in the captured images represents the area of the damage, where the needle created an insertion path in the tissue. Because of higher insertion force (which depends on friction force), the damaged area enlarges when using the standard needles compared to mosquito needles as shown in Figure 5. The tissue damage area caused by the mosquito-inspired needle is smaller compared to the damage caused by the standard needles. It was found that the area of tissue damage was reduced by 27% using the mosquito-inspired needle. The p value (0.01) for tissue damage area using the two-tail test was less than the alpha (0.05). Therefore, it could be confirmed with a 95% confidence interval that the insertion of the mosquito-inspired needle in the soft tissue significantly reduced the tissue damage compared to the standard needle.

Discussion

The needle insertion experiment and the histology study on bovine liver tissue were performed to

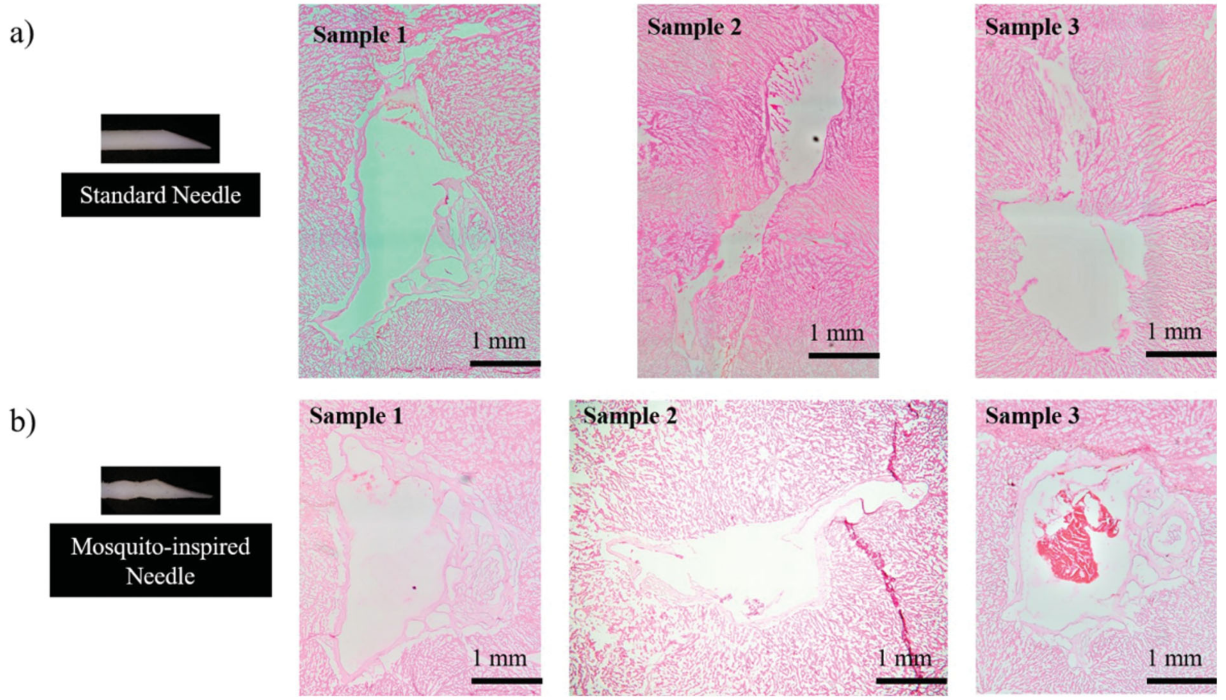

Figure 5. Magnified (4×) view of the tissue damage showing the cross-section of the hole at needle insertion points in three different bovine liver tissue samples caused by (a) standard needle and (b) mosquito-inspired needle with the vibration of 200 Hz and an amplitude of 5 μm (insertion velocity of 1 mm/s is the same for both needles).

Table 1. Comparison of the bovine tissue damage area caused by standard and mosquito-inspired needles (n is the number of needles, where one kind of needle prototype is used in a tissue sample).

	Bovine liver tissue damage area (mm^2)			
Needle prototype	Sample 1	Sample 2	Sample 3	Mean tissue damage area (mm^2)
Standard needle ($n = 3$)	7.11	5.67	6.47	6.42 ± 0.72
Mosquito-inspired needle ($n = 3$)	5.18	4.29	4.67	4.71 ± 0.45

investigate the performance of the proposed mosquito-inspired needle. The evaluation of a cross-sectional area of the tissue damage was also conducted immediately following insertion experiments so that the tissue did not shrink [34]. It should also be noted that it was challenging to identify the insertion path on the bovine liver tissue [1,8]. As shown in Table 1, it was demonstrated that the tissue insertion with the standard needles resulted in a larger damage area compared with the insertion with the mosquito-inspired needles. The comparison of the measured damage area in bovine liver tissue suggests that the needle insertion force required to penetrate tissue was less for the mosquito-inspired needles. From our literature review, it was found that there were only a few studies that did histology to measure the cross-sectional area of the damage caused due to the needle insertion [7,12]. The histology study based on our previous group on how to measure the cross-sectional area of the damage and to compare the tissue damage caused due to the two types of needles [7].

For histology study, it was understood that the tissue elasticity after the insertion and the formalin fixation could influence the damaged area [39]. Also, the sectioning of the frozen samples would have some minor mechanical effects on the damaged area of soft tissue samples [40]. However, since tissue samples for both types of needle insertion tests were equally affected, the comparison of the damaged area could be made. The frozen tissue samples could lose their mechanical properties if they are kept for longer periods in the freezer [41], which was the reason the tissue samples were immediately sectioned after freezing for a limited time (24 h.) and to follow the next steps in staining and imaging. Besides, the prototypes of standard and mosquito-inspired needles have their similarities and differences. Moreover, the total diameter is the same for both needles except the diameter for the maxilla length (which is up to 65 mm) is the only difference. The friction between the needle and the tissue is reduced by designing a jagged structure on the needle body as shown in Figure 1 because this reduces the frictional contact between tissue and needle body. This reduction in friction led to the overall reduction in the insertion force [8,20]. The material used for manufacturing both the needle prototypes was the same [10]. The differences between the two needle prototypes were the needle geometry and the vibration (utilized for mosquito-inspired needles), which resulted in less tissue damage area.

In summary, the histology results correlated positively with the proposed hypothesis that reducing needle insertion force leads to a decrease in the tissue damage area. The use of mosquito-inspired needles reduced the insertion force by 39% compared to the standard needles. The histology study of the bovine liver tissue damage showed that the mosquito-inspired needle could significantly decrease the tissue damage by 27%. Based on the histology study, the reduction in tissue damage matched with the reduction in insertion force, implying that the proposed mosquito-inspired design is acceptable. To transfer these design concepts to the clinical use needle dimensions, the main design parameters such as maxilla angle, thickness, and vibration will be evaluated experimentally. The other challenge in true scale size is the manufacturing of a clinically used stainless-steel needle. The manufacturing challenge needs to be addressed by three-dimensional metal printing in our future study, specifically with mosquito-inspired design on a needle body. Also, manufacturing the needles will be studied and its limitation of developing the complex geometry on stainless steel needles by collaborating with the needle manufacturing companies. For future work, technologies such as digital image correlation [11,42] and deformation sensing method [43] at insertion location can be utilized. Moreover, a study using true-scale such as 16-gauge and 18-gauge needles could be performed in different biological tissues. This would furthermore ensure the feasibility of the proposed mosquito-inspired needle in reducing tissue damage for more effective and less invasive percutaneous procedures.

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Declaration of interest

All authors declare that they have no conflict of interest.

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ORCID

Sai Teja Reddy Gidde http://orcid.org/0000-0003-3153-3902
Sharad Raj Acharya http://orcid.org/0000-0001-7615-2041
Shital Kandel http://orcid.org/0000-0003-0771-0112
Nancy Pleshko http://orcid.org/0000-0001-8656-3936
Parsaoran Hutapea http://orcid.org/0000-0001-6917-1252

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