

Fabrication of Microneedle Molds and Polymer Based Biodegradable Microneedle Patches: A Novel Method

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ABSTRACT

Objective: Microneedles are a micro sized needle-like structure which has the ability to pierce the skin in a non-invasive and painless way. The present research work aims to design microneedle molds in a novel way and fabricate and characterize biodegradable polymer based micro-needle patch utilizing polymer casting.

Methods: Fabrication of polymer patch involved two steps, one is to fabricate microneedle array mold and the other is to prepare biodegradable polymeric microneedle patch using the molds. Molds are prepared by manually piercing the mixture of resin and hydrate (emseal) using needles having micro tips and patches are prepared using polymer solution. Characterization of microneedle patch was done using scanning electron microscope and skin piercing ability was understood from histological studies of the rat skin.

Results: The micro-needles on the patch were found to be uniform in size and shape, with concentric circular features, The size of the microneedle tip was found to be between 20-50 μm and base around 200 μm and the shape was found to be conical with sharp tip. The micro-needles showed good penetration in to the skin which was observed by the histological studies performed using rat skin.

Conclusion: The present study demonstrates that the microneedle molds can be prepared using resins and microneedles can be developed using polymer casting method. The developed microneedles showed comparable structural features with those reported in the literature. These microneedles possessed good mechanical strength and can pierce the rat skin.

Keywords: Microneedle molds, Micro needle, Micro fabrication, polyvinyl alcohol, skin penetration.

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INTRODUCTION

Micro needle [MN] is a micron-scaled needle-like structure which measures maximum up to a length of 1 mm with thickness in microns. MN pierce the skin in a non-invasive and painless way to deliver drugs beneath the epidermis. MNs enable delivery of hydrophilic drugs as well as lipophilic drugs and macro molecular therapeutics through micro channels that were physically made by the MN while disrupting the stratum corneum (SC). MNs do not trigger the pain receptors and do not enter the blood vessels which enables painless delivery without damage to the blood vessels.¹⁻⁴ MN penetration into skin generates temporary micropores in the skin which aid the delivery of those drugs that do not diffuse passively through upper layer of the skin.⁵

The first generation MNs were made from silicon, metals or organic polymers and were designed to create the micropore in the skin into which the drug or vaccine is diffused.⁶ The use of microneedles eliminates the sharps waste, that are required to administer drugs or vaccines.⁷⁻⁹ Dissolvable MNs have also been reported and are known to be made from many biocompatible materials such as sugars,¹⁰ and polymers. Most popular polymers that are used in the fabrication of microneedles are poly lactic acid, polyglycolic acid, poly lactic-*co*-glycolic acid (PLGA), poly (vinyl) alcohol, poly- vinylpyrrolidone (PVP) and carboxymethyl cellulose (CMC).¹¹

Different types of MNs act differently in delivering drugs and other therapeutic moieties. Solid MNs are used as skin pretreatment, after inserting and removing these MNs to form micropores on the surface of the skin, a drug formulation is applied to the skin for diffusion of drug. MNs can be sometimes coated with a drug in which case after insertion of MNs into the skin, the coated drug dissolved into the

skin, after which the MNs can be removed. In some cases, MNs are made completely from water soluble or biodegradable polymer that can entrap the drug within the matrix. This type of MNs completely dissolve or degrade in the skin to release the entrapped drug. Using MNs sites other than skin such as eye can be targeted.¹²

The first patent on MNs based drug delivery has been filed in US in the year 1971. At that time inventors Gerstel and Place called the microneedles as 'puncturing projections'.¹³ However, the first successful attempts on MNs emerged in the 1990s which demonstrated the use of silicon MNs to successfully facilitate the delivery of calcein, across human skin.¹⁴ Revolution in the microelectronics industry lead to the advent of micro fabrication technology tools enabled the evolution of the manufacturing facilities necessary to produce micro-conduits in silicon MNs. MNs have been shown to be effective in the delivery of many therapeutic molecules across biological membranes including skin, mucosal tissue and sclera. In a report it was suggested that MN arrays can not only transport small molecules but can be used to transport larger molecules and even microparticles.^{15,16}

Since more than 150 years, hypodermic needles are delivering drugs to patients. The hollow needle was invented in 1844, and the first injection was administered later on.¹⁷ During the early days syringes and needles were one piece and are made up of metal; the drug used to be injected via a leather plunger.¹⁸ These syringes were meant to be reused and are very difficult to sterilize. Presently, the syringe and hypodermic needle have evolved into a two-part disposable device. In current clinical setting, the syringe is typically made of plastic, and the needle is made up of medical-grade stainless steel.

Transdermal delivery offers many advantages over conventional drug delivery. However, this has limitations due to the inability of majority of drugs to cross the skin at the desired rates because of the presence of a relatively impermeable thick outer stratum corneum layer.¹⁹ MNs are one of advanced drug delivery systems which by mechanically penetrating the skin can inject the drug just under the stratum corneum from where it is rapidly absorbed by the capillary bed into the bloodstream.²⁰⁻²¹

The performance of the MN array enhancing drug delivery is based on 3 main factors. First, the geometry (length, radius, shape) and the number of needles (MNs density) of the MN patch are important as they directly affect the drug passage and the insertion behavior. Second, the force applied on the patch determines its insertion behavior and, hence, its performance. The force needed has to be sufficient to pierce the upper layer of the skin, stratum corneum which governs the overall viscoelasticity of the skin and is also the main barrier in the transdermal drug delivery. Third, the molecular weight of the drug molecule, the larger molecules are mostly benefited from the MN delivery. Smaller and optimum lipophilic molecules can cross the stratum corneum easily to enter the skin.²²

There are many methods reported in the literature for the fabrication of MNs that are dissolvable. The first step in the manufacture of the MNs is to prepare MN molds. These molds are made typically from UV lithography. However, our aim is to prepare molds from resin that can be solidify with time without using any high end technology. Then the MNs can be fabricated by pouring the polymeric material into the molds.

MATERIALS AND METHODS

Materials

Poly vinyl alcohol (hot water soluble, MW 30,000-50,000) has been purchased from Nihal traders private limited, Hyderabad. Resin and hydrate (emseal) were purchased from local market. All other chemicals were of analytical grade supplied by Nihal traders private limited, Hyderabad.

Preparation of mold

Microneedle molds are prepared by using mixture of resin and hydrate material (emseal) and a micron sized sewing needle tip. The resin and hydrate material taken in a 1:1 ratio and mixed well properly by hand mixing. The mixed preparation was placed into a micro centrifuge tube of 1.5ml quantity and the upper surface area of tube had been evenly distributed. It was then pierced with micron sized needle tip to get array mold. After preparation of mold arrays, they were dried for 20 minutes at room temperature for formation of hard solid molds (Fig 1).

Fabrication of microneedle patch

The polymer solution used to fabricate the micro needle patch is polyvinyl alcohol (PVA). 20% w/v polymer solution was prepared by heating on amagnetic stirrer at 80°C for 20 minutes. Prepared polymer gel solution was transferred into micro-molds and immediately centrifuged at 1500 rpm for 10 minutes for even and uniform distribution and to remove the air spaces. After completion of the process the micro centrifuge tube was removed from the centrifuge and dried for 2 days and then kept in freezer for 30 minutes at 4 °C for easy separation of microneedle patch from the micro molds (Fig 1).

Characterization of polymeric microneedle arrays

Scanning Electron Microscopy (SEM) Analysis of MN Arrays

The scanning electron microscope (Carl Zeiss Model EVO 18) was used to understand the surface morphology and size and shape of the MNs. The magnification, tilt degree, spots, width and other imaging characteristics were reported on the SEM images.

Histology study

Male Sprague Dawley rat skin was collected from freshly euthanized animals used for other experimentations. The skin was shaved and pierced with the microneedle patch. The patch was applied for 1 minute by applying pressure with the hands. The skin was immersed in 10% neutral buffered formalin solution after piercing with the microneedles. These tissues were fixed for at least one day before dehydration. Tissues were dehydrated using a series of ethanol, ethanol and his to clear mixture (50:50) and his to clear. Then, the tissues were paraffin embedded, and thin (5 μ m) sections were cut using rotary microtome (Leica RM2235) and mounted onto slides. The slides were rehydrated and stained using haematoxylin and eosin (H&E).

RESULTS

The present study demonstrates that microneedle molds can be prepared out of resin and the patch can be prepared out of biodegradable polymers. The prepared microneedle molds in the micro centrifuge tubes were shown in figure 2. It is observable that the holes are uniformly distributed throughout the area. The depth of the micromolds was uniform in all the micro wells that can be understood after observing the final MNs. Sharp tips on the

microneedles were achieved in our study which can be perceived by touch (Fig 3).

Scanning Electron Microscopy (SEM) Analysis of MN Arrays

To characterize the micro needles, the patches were examined using SEM. The PVA micro needle patch had a rugged texture and all the needles are uniform in size and shape, indicating the orderly self-assembly and the presence of sharp tips (Fig.4). We found that the needles had some concentric circular features on the surface (Fig. 4). The patch show microneedles with height less than 1 mm and thickness at the base around 200 μ m and at the tip around 20 μ m (Fig. 5).

Histology study

To test the capability of these micro needles to penetrate into rat skin, we applied the PVA polymer micro-needles onto rat skin. The images in Fig. 6 show that the PVA micro needles can penetrate the skin and can make transient micropores. The results also show that the PVA micro needles result in less apparent tissue trauma.

DISCUSSION

Microneedlemolds are prepared using UV lithography in literature reports most of the times. In our studies we have prepared these molds using a novel method applying very cheap materials. We have selected a resin (emseal) that can solidify after treatment with a hydrate. Before the resin can solidify completely we have made micron sized apertures using the microtip of a needle. The manual microwell formation is easy and requires minor skills. Master micro array templates can be fabricated by resin and hydrate mixture (such as a emseal), filled in a micro centrifuge tube (1.5ml quantity) for microinjection molding. This method is very simple and requires no costly equipment. The microwells can be seen in

Figure 2. These wells are well arranged and the micro centrifuge which is used in the study is highly useful as it can be centrifuged during the casting process of microneedles from the PVA solution. The micromold process can be easily scaled up to produce large number of molds. Initially we have tried using various sugars like maltose, sucrose and polymers like PVP for the micro fabrication of microneedles, however the integrity and the ease of manufacture was only observed with PVA. The conditions used during manufacturing are mild and the process is simple, inexpensive and easily scalable.

Figure 2 shows a view of the top of a emsealmicromold, into which the polymeric materials flow after centrifugation. In figure 6 the distance between the microholes, indicated as the MN inter base spacing, was determined to be less than a mm. The MN base diameter was determined to be similar in the SEM images and histology sections. Both measurements were consistent with the physical dimensions of the actual polymeric MNs. Although, the needles of each polymeric MNs were essentially the exact replicas of the master needles, the differences may have resulted from shape transitions during solidification. Digital photographs of MN sections from PVA microneedle arrays fabricated from the emsealmicromolds were illustrated in figures 3, 4&5. It is revealed that cylindrical needles were rigid; the bases were regular, intact and smooth shaped.

The work performed in this study has significance to both micro fabrication and drug delivery. While these fabrication tools have been used before in other contexts, this study presents their application to polymer microneedles. Additionally, cone shaped microneedles were fabricated using a novel resin based technique, which is a new method to the field of micro fabrication. The molding approach described here is also of

potential significance. The use of emseal molds to replicate microstructures and filling these molds is easy but MNs surface texture is rough which is a persistent problem. In this study a centrifugation method is applied to fill molds with polymeric solution. This modified injection molding technique could find additional applications in a variety of micro molding scenarios.

Another character of microneedles is skin penetration, it is studied by histology. We applied the PVA polymer micro-needles onto rat skin. We inserted the microneedles into the rat skin and removed them within minutes. The skin has been stained with haematoxyllin and eosin and the photographs were captured under the microscope. These results show that the PVA micro needles can penetrate rat skin. The results are in accordance with the previous literature reports where the authors have prepared the dissolvable microneedles using other methods than the one reported in the present study and polymer casting.⁵ Altogether, these results show that biodegradable polymeric microneedles can be fabricated using manufacturable micro fabrication techniques, have sufficient mechanical strength to insert into skin with a wide safety margin.

CONCLUSION

This study demonstrates that micromolds can be made using cheaper materials without the usage of UV lithography and micro needles can be made out of biodegradable polymers. Sharp tips were achieved by adapting micro fabrication techniques to produce cylindrical micro needles. Micro fabricated master structures were replicated using emseal molds and a novel centrifugation method to fill the molds with biodegradable polymer melts has been described. This fabrication method is

expected to be suitable for rapid scale up for inexpensive, mass production.

Characterization of polymeric microneedles has included testing of microneedle size, shape and also the skin penetration. The following microneedle dimensions were identified; *Microneedle length*: below 1000 μm ; *microneedle width*: 200 μm ; *microneedle tip*: 20 μm ; and the *number of microneedles in the array*: 16.

In conclusion, this study presents a micro needle fabrication method based on micro molding a master structure generated by resin and hydrate mixture (such as emseal) with PVA micro needles. These microneedle patches were found to pierce the skin with small application force; it showed good penetration and also found the micro needle diameters within the desired dimensions. Further it is concluded that the microneedle patch prepared can be used to prepare drug loaded microneedle patch for pain less drug delivery systems and should be evaluated for other official parameters.

Declaration of interests

The authors report no declarations of interest.

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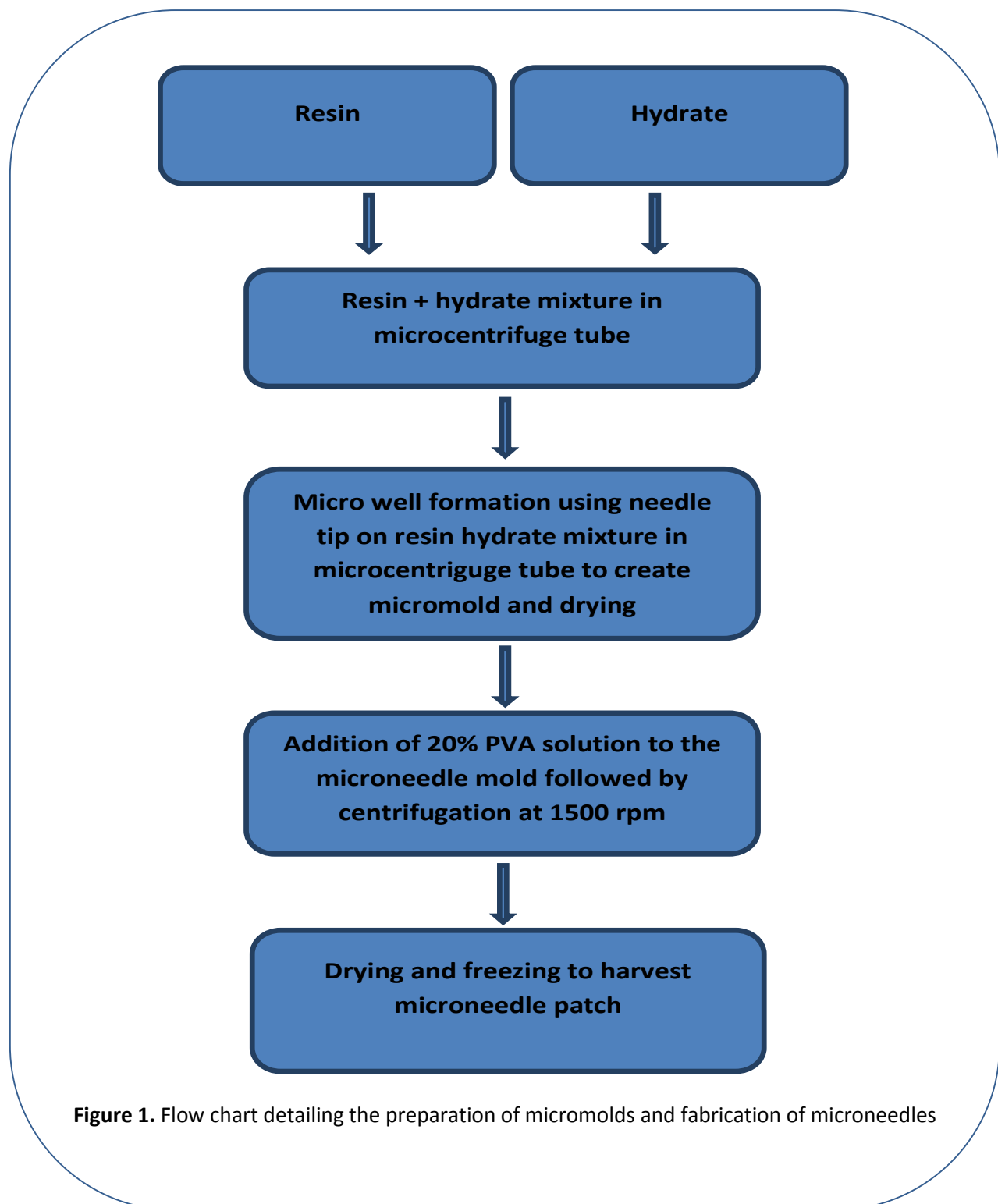


Figure 1. Flow chart detailing the preparation of micromolds and fabrication of microneedles



Figure 2. Representative picture of micro needle molds prepared using a novel fabrication technique in the microcentrifuge tube



Figure 3. Micro needle array patches with sharp tips and uniform assembly

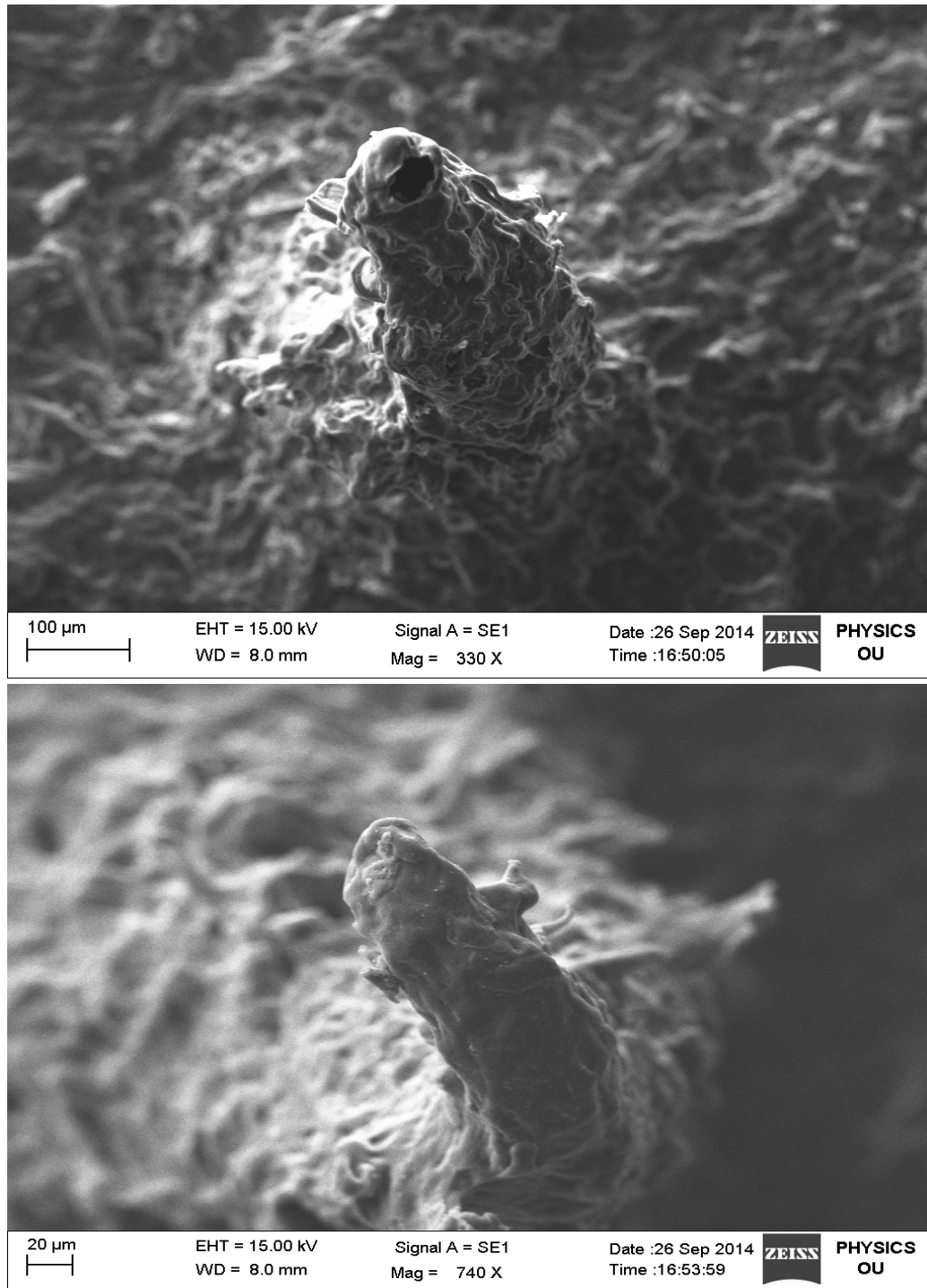


Figure 4. SEM photographs of parts from MN arrays. Show single microneedle on a patch tip size around 20 μ m

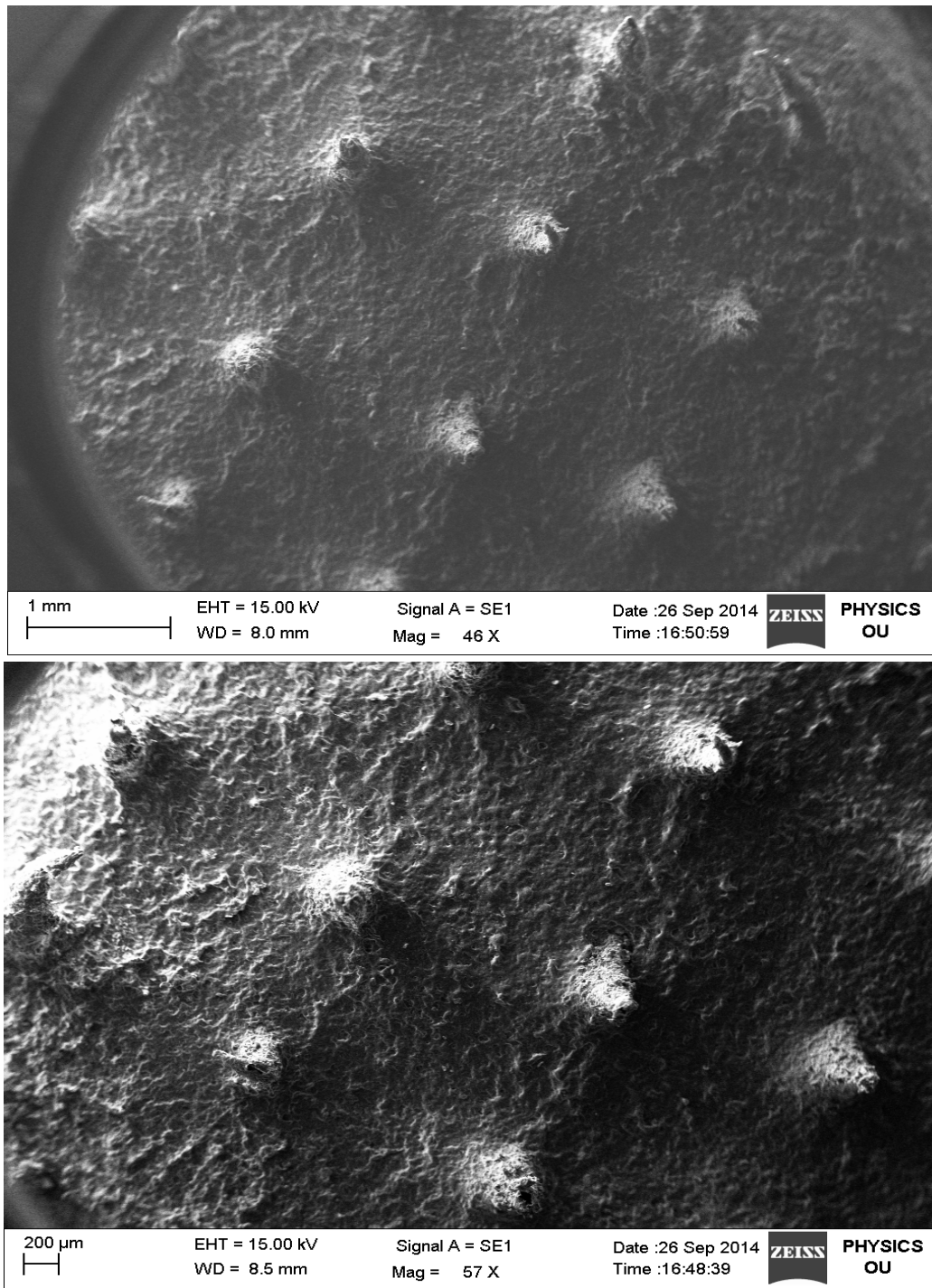


Figure 5. Scanning electron microscope image of the patch showing microneedles with height less than 1 mm and thickness of the base around 200 μm and the tip at 20 μm thickness

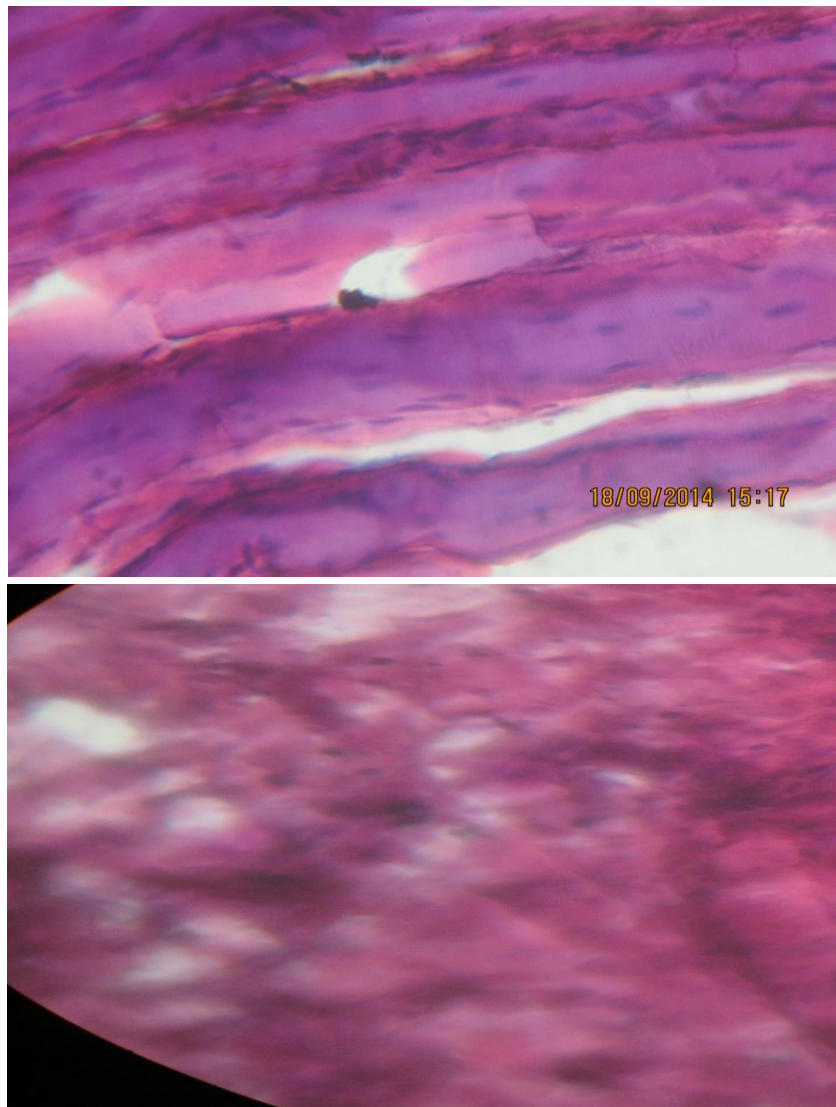


Figure 6. Histology image showing the penetration of microneedles into rat skin