ULTRA FLEXIBLE MACROPOROUS MICRONEEDLES FOR SUSTAINED DELIVERY OF (2R,6R)-HYDROXYNORKETAMINE

Riddha Das[1,2], Aydin Sadeqi[1,2], Caroline A. Browne[3], Irwin Lucki[3] and Sameer Sonkusale[1,2]*
[1] Department of Electrical and Computer Engineering, Tufts University, USA
[2] Nano Lab, Advanced Technology Laboratory, Tufts University, USA
and [3]Uniformed Services University of Health Sciences, USA

ABSTRACT
Transdermal delivery using microneedles provides an alternative method to locally administer drug molecules with controlled release rate. In this study, our goal was to develop an ultra-flexible macro porous patch with hard microneedles that penetrate the skin. We demonstrated encapsulation of a non-hallucinogenic derivative of a dissociative anesthetic pharmaceutical, which is approved as a therapy for treatment resistant depression. The microneedles are made from biocompatible photo-curable resin. Solid formulation of this drug is encapsulated in the resin, which ensures high drug loading capacity. A flexible substrate is used as the backing to ensure skin conformability. The encapsulated drug molecules are released by diffusion in contact with interstitial fluid. Our findings demonstrate uniform encapsulation of the drug molecules into the resin and sufficient robustness of the microneedles to withstand the skin penetration forces.

KEYWORDS
Microneedle, laser cutting, laser lithography, post-traumatic stress disorder, drug delivery.

INTRODUCTION
Post-traumatic stress disorder (PTSD) is a mental health disorder occurring in susceptible individuals exposed to serious injury and traumatic life events. Despite the widespread availability of therapies to counteract PTSD, current treatments are only marginally effective. Sub-anesthetic doses of ketamine are indicated for the treatment of major depressive disorder. In addition, ketamine is also reported to alleviate chronic pain and PTSD [1]. However, administration of ketamine via intranasal and IV infusion limits its widespread utility due to restricted administration by trained medical practitioners in specialized clinics and status as a scheduled compound. In this work, we report a non-invasive microneedle (MN) transdermal drug delivery device for sustained delivery of ketamine and ketamine's non-hallucinogenic metabolite (2R,6R)-hydroxynorketamine (HNK) to avoid the psychotomimetic side effects and potential abuse liability of ketamine [2].

Existing MNs have issues with reliable penetration (e.g. hydrogel based MN) and/or insufficient drug dosing (e.g. drug coated solid MN) [3,4]. They require expensive cleanroom methods to manufacture and are usually limited in size. Recently, our group developed a cross-over-lines (COL) laser lithography approach to make large area microneedles [5]. It utilizes laser micromachining with user-defined geometric patterns to make high aspect ratio molds. The molds are then used to make ultra-flexible conformal large area microneedle patches. Use of the COL approach enables fabrication over a large area format previously not possible. Another innovation is the direct use of solid drug formulation encapsulated in porous matrix of photocurable resin. We recently demonstrated release of ibuprofen and lidocaine using such macroporous solid hard microneedles (macroPOSH) [6].

DESIGN AND FABRICATION
COL laser lithography was used to make negative polymethyl methacrylate (PMMA) molds [5]. This mold was first duplicated into a super-elastic ecoflex mold. Next, a polymeric mixture solution of photocurable biocompatible dental resin and finely powdered drug (HNK) was cast into the mold and stretched for loading. A flexible substrate such as polyethylene terephthalate (PET) or paper was then applied, and the entire construct was UV cured to realize large area MN patches. The resultant patch exhibited flexible backing with solid cured hard tips. The drug was essentially encapsulated into the porous matrix of the resin and released

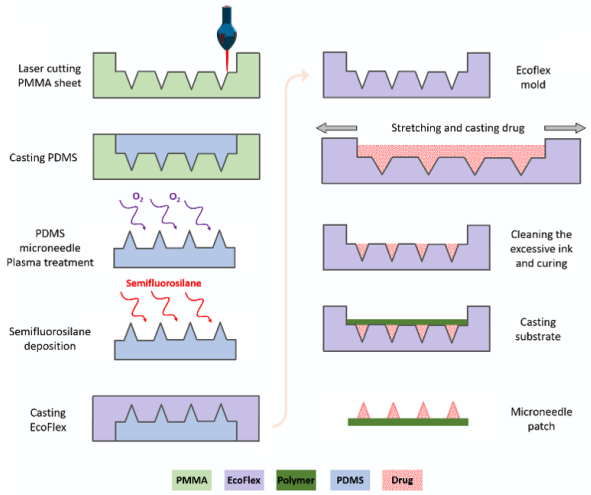

Figure 1: Fabrication of microneedle mold and microneedle patches: CO2 laser cutter was used to fabricate microneedle PMMA mold using the proposed cross-over lines (COL) technique, the PMMA mold was used to fabricate polydimethylsiloxane (PDMS) microneedles mold, which was used to fabricate ecoflex mold. This ecoflex mold was then used to fabricate microneedle patches.

when it came in contact with interstitial fluid. The ability to load drug in powdered form ensures high drug loading capacity in the macroporous solid hard (macroposh) microneedles. Moreover, its hard tips facilitate reliable penetration in all skin types.

978-1-940470-04-7/HH2022/$25©2022TRF
DOI 10.31438/trf.hh2022.107

RESULTS AND DISCUSSION

The microneedle patches were fabricated from HNK encapsulated into dental resin. HNK was grinded to fine powder and was mixed uniformly with the resin solution with the weight ratio of 1:1. The drug solution was then pressed against the mold. The mold was stretched from four sides to ensure maximum penetration of the drug solution. A blade removed the excess drug solution, and the top part of the mold was covered with blank resin solution. The samples were cured using ultraviolet light with wavelength of 405 nm. After a few minutes, the patches get hard enough to be removed from the microneedle molds. The patches were then stored at room temperature. For proof of concept, we made patches of 1x1 cm^2 with 400 microneedles each of base width: 300±30 µm, height: 1500±20 µm, tip: 10±3 µm and resin to drug ratio of 1:1. Scanning Electron Microscopic (SEM) image confirmed the formation of conical microneedles (figure 2a).

Next, HNK release study was performed from the

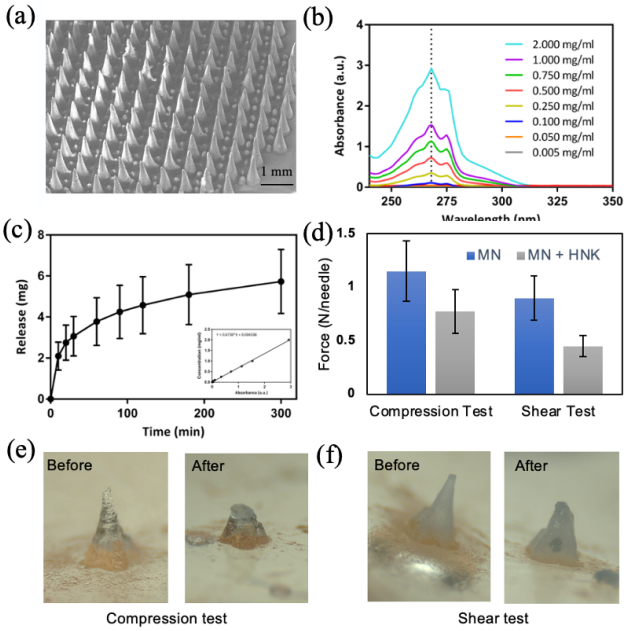

Figure 2: (a) SEM image of microneedle patch (b) Different concentration of HNK measured by UV-vis Spectrophotometer (c) HNK release profile in PBS (inset: Calibration curve of HNK) (d) Comparison of robustness of microneedle patches with and without HNK

microneedle patches in Dulbecco's Phosphate Buffered Saline (PBS). The absorbance was measured between 240 – 350 nm as shown in Figure 2b. We calibrated the HNK solution based on the absorbance amplitude at 268 nm (figure 2c inset). The drug capacity of each microneedle in the patch was estimated to be 18 µg from the release study in buffer solution assuming equal distribution of drug in each microneedle.

The compression and shear tests were performed with an Instron tensile tester (figure 2d). The microneedle without drug started to break down at the force of ~1.1N per needle and ~0.85N per needle on average during compression and shear test respectively. The microneedles with drug started to break down at the force of ~0.75N/needle and ~0.5N/needle

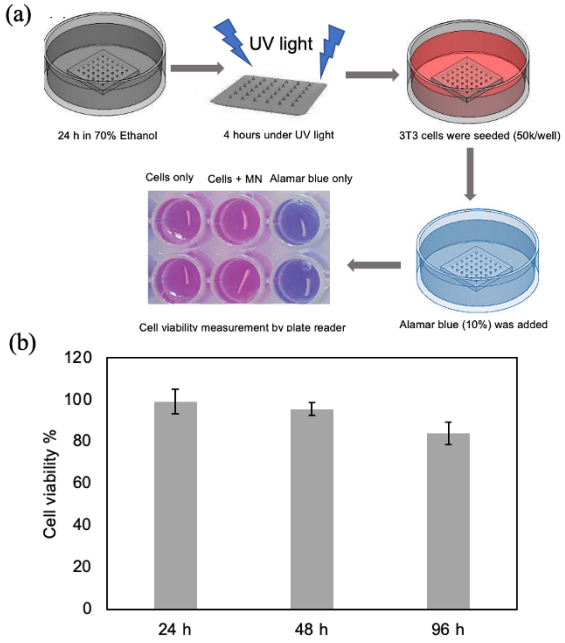

Figure 3: (a) Flow diagram of cell viability measurement experiment with fibroblast 3T3 cells in presence of MN patches (b) cytotoxicity of MN patches

during compression and shear test respectively. Although, there was a significant reduction in robustness after HNK addition, there was an almost ten-fold margin of safety over the force (0.058 N per needle) needed for insertion into skin using microneedles of this geometry.

Cytotoxicity of microneedles was investigated in fibroblast cells (3T3) using a standard protocol. Briefly, the microneedle patches were sterilized by keeping them immersed in 70% ethanol for 24 h in a biosafety cabinet followed by irradiation by UV light for several hours. The sterilized microneedle patches were placed in each of the wells of a 6 well plate. 3T3 cells (50k cells/well) were seeded in all the wells (with and without patches). Alamar blue test was done after 24, 48 and 96 hours at Ex/Em wavelength of 560/590 using a plate reader. The cells in the presence of microneedles grew at a rate similar to the control cells as shown in the Figure 3. Negligible reduction in cell viability was observed even after 96 h. This data demonstrates excellent biocompatibility of the microneedle patches.

To assess the drug capacity and release profile of the microneedle patch, an *in vitro* skin model was developed (Figure 4a). The model was comprised of 10% gelatin in PBS (modeling dermis layer of the skin) covered by a thin layer of parafilm (modeling epidermis layer of the skin). The patch was pressed against the polymer layer with a maximum force not exceeding 20N to achieve penetration. After placing the microneedles patch, the model was placed in an incubator at 37˚C. At different time intervals, the patch was removed and

placed on a fresh *in vitro* set to avoid saturation of the gelatin solution with the release content from the patch. The release content from the patch was qualitatively and quantitatively evaluated for different time intervals.

To evaluate the release content from the patch, the gelatin

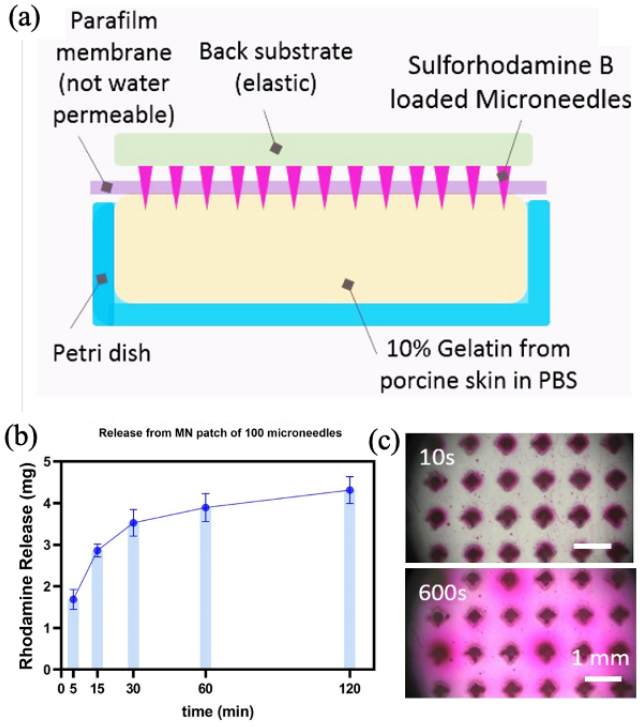

Figure 4. (a) in-vitro skin model (b) quantitative evaluation (c) qualitative evaluation of sulphorhodamine B release profile.

was melted, and the concentration of the released dye was measured using a UV-Vis spectrometer. The dye used in our *in vitro* analysis was sulphorhodamine B. A relatively uniform release pattern was observed from individual microneedles in the patch suggesting uniform fabrication of the needles.

CONCLUSION

In this study, we fabricated HNK loaded macroporous microneedles (MN) patches with ultra-flexible PET sheet backing. The drug is essentially encapsulated into the porous matrix of the resin and released when it comes in contact with interstitial fluid. The ability to load powdered form of a drug ensures high drug loading capacity in the macroporous solid hard (macroposh) microneedles. The ultra flexible backing ensures conformability and its hard tips facilitate reliable penetration in all skin types. According to shear and compression test results of the hard tips, the patches were sufficiently robust to withstand the force during skin penetration. The relatively uniform release profile of the cargo in our *in vitro* skin model confirmed uniform fabrication of each microneedle. MN patches demonstrated excellent biocompatibility in the *in* vitro cytotoxicity experiment. To summarize, we developed a biocompatible ultra flexible transdermal delivery platform for treatment of PTSD using a non-hallucinogenic analog of ketamine, namely HNK. Future studies will involve studying the effects of the extended drug release on behavior in rat models of PTSD symptom clusters.

ACKNOWLEDGEMENTS

The authors would like to acknowledge financial support from TTW program at USUHS under award number HU0001-20-2-0014. The content and conclusions do not necessarily represent the official position or policy of the Uniformed Services University of the Health Sciences, the Department of Defense, or the U.S. Government.

REFERENCES

[1] J. W. Murrough, D. V. Iosifescu, L. C. Chang, R. K. Al Jurdi, C. E. Green, A. M. Perez, S. Iqbal, S. Pillemer, A. Foulkes, A. Shah, D. S. Charney, and S. J. Mathew, "Antidepressant Efficacy of Ketamine in Treatment-Resistant Major Depression: A Two-Site Randomized Controlled Trial", *Am. J. Psychiatry* 170, 1134 (2013).

[2] J. N. Highland, P. Zanos, L. M. Riggs, P. Georgiou, S. M. Clark, P. J. Morris, R. Moaddel, C. J. Thomas, C. A. Zarate, E. F. R. Pereira, and T. D. Gould, "Hydroxynorketamines: Pharmacology and Potential Therapeutic Applications", *Pharmacol. Rev.* 73, 763 (2021).

[3] K. Lee, C. Y. Lee, and H. Jung, "Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose", *Biomaterials* 32, 3134 (2011).

[4] S. F. Lahiji, M. Dangol, and H. A. Jung, "patchless dissolving microneedle delivery system enabling rapid and efficient transdermal drug delivery", *Sci. Rep.* 5, 1 (2015).

[5] H. R. Nejad, A. Sadeqi, G. Kiaee, S. Sonkusale, "Low-Cost and Cleanroom-Free Fabrication of Microneedles", *Microsystems Nanoeng.* 4, 17073 (2018)

[6] A. Sadeqi, G. Kiaee, W. Zeng, H. Rezaei Nejad and S. Sonkusale, "Hard polymeric porous microneedles on stretchable substrate for transdermal drug delivery", *Sci. Rep.* 12, 1853 (2022).

CONTACT

*sameer.sonkusale@tufts.edu