EVALUATION THE EFFECT OF THREE HOME-MADE MICRONEEDLE ELECTRODES ARRAY FOR IONTOPHORETIC METHYLENE BLUE DYE AND INSULIN TRANSDERMAL DELIVERY IN DIABETES-INDUCED MICE SKIN

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Abstract. Diabetic patients are in rapid increase globally. An efficient drug delivery may decrease the disease burden. In the current experimental research design, the effect of three home-made microneedle (MN) electrodes arrays (parallel, triangular and circular) for iontophoretic methylene blue (MB) dye and insulin transdermal delivery was evaluated on diabetes-induced mice. The methods involve a short time (5 s) exposure of high voltage square pulsed (50 V), followed by applying a direct current (DC) up to 0.5 A of 6, 9 and 12 V for 5 minutes on mice skin. The circular micro needle (MN) electrode array showed a relative significant ($p > 0.05$) higher response that emphasized by the decrease in glucose level of treated groups compared with control, and histomorphometrical analysis for microscopic photo of MB dye expansion in mice skin layers.

Key words: AC ionotophoresis, microneedle electrodes, transdermal deleivery, diabetes.

INTRODUCTION

The adults living with diabetes are increasing globally, with increasing in associated risk factors such as overweight or obese. Diabetes prevalence is faster in low- and middle-income countries than in higher ones. Guidelines and protocols to improve management of diabetes are lacking funding and implantation. As a result, the early diagnosis is the main key for treatment and health-care of diabetes [21].

According to diabetes type, targeting insulin has the crucial role for diabetes management. Most of insulin administration routes, such as syringes, insulin pens, and insulin pumps are invasive. They are associated with injection pain, non-compliance, and peripheral hyperinsulinemia [15]. The need of minimal or non-invasive routes for insulin administration is the challenge. Inhaled insulin was the first approved non-invasive tool. Other administration routes (like oral, buccal, nasal, peritoneal and transdermal) are under investigation [10].

Received: June 2018;
in final form October 2018.

ROMANIAN J. BIOPHYS., Vol. 29, No. 1, P. 000–000, BUCHAREST, 2019

Transdermal drug delivery (TDD) is an administration route of drug through skin. It found a good compliance with patients over conventional oral or invasive intramuscular or intravenous routes [10]. Beside a controlled release, (TDD) bypasses the first bypass of gastrointestinal tract and liver. Moreover, (TDD) has other advantages such as a non-invasive tool, can be ceased by the patch off on application site, improve bioavailability, provides steady plasma levels, suitable for paediatrics, and affordable for unconscious or vomiting patient [10,16].

Although the previous advantages of (TDD), a limited drug release is caused by skin horny outer layer *stratum corneum* [10, 20]. Consequently, (TDD) system can be affected by drug nature and partition coefficient or pH degree. Also, system composition affects mainly the functional permeability of the drug like hydrophilicity or hydrophobicity. Enhancers for (TDD) system act a synergistic effect without affecting the drug actions. (TDD) enhancers can be chemical (alcohols, esters, lipids), mechanical (ultrasound) or electrical (electroporation and iontophoresis) [7, 9].

In electroporation (EP) technique, the membrane is excited with a suitable sufficient electric pulse (>100 V) for milliseconds; it exhibits transient pores which pass otherwise impermeable molecules though cell membrane [14]. EP can be either reversible (for short time permeation and cells survive), or irreversible (cells die by necrosis or apoptosis) [3]. Iontophoresis (IP) exploits an electrical permeablization of ionized drugs by the use of electrical impulse of 0.5 mA/cm^2; the pH value is crucial for effectiveness of TDD system [3].

According to direct current iontophoresis (DC-IP), small direct electric current is used as a source external energy. Theoretically, there are few relationships that governing the DC-IP process: Ohm's law and Faraday's law. There are many variables affecting the DC-IP process, such as donor (electrode system), and receptor cell (*in vitro*) or patient skin (*in vivo*). Other physicochemical parameters also contribute, including: drug concentration, ionization form, micro-environment pH, current intensity and duration, stability, current dosage and density, and patient anatomical factors [3, 12].

A novel modification to increase transdermal transport involves the use of microscopic needle electrodes that pierce the skin and create micrometer-scale openings without reaching nerve. These needles are 200–750 μm in length, in form of an array of 150–650 microneedles/cm^2 and have diameter of tip 25 mm, interfacial area of 490 mm^2, with an insertion force of 0.058 N. These can be made of silicon, metal, sugar and plastics [12].

There are four kinds of microneedles; (a) poke with patch approach, (b) coat and poke approach (needles incorporated with drug, release of pharmacological active moeity takes place by dissolution), (c) biodegradable microneedle (insertion of drug incorporated polymeric microneedle which is biodegradable) and (d)

hollow microneedle (puncturing epidermal surface with needles with a hollow bore) [1].

Combining iontophoresis with microneedle electrode arrays to enhance transdermal drug delivery was investigated earlier [3, 7, 11]. The current exploratory experimental research was designed to study the role of three microneedle electrodes arrays (circular, parallel, and triangular) for DC-IP transdermal delivery of insulin through albino mice skin.

MATERIALS AND METHODS

MATERIALS

Animal subjects

In the current work, ~~a~~ hairless albino male mice were incorporated from the animal house of the Medical Research Institute, Alexandria University. All participant animals were gathered and treated according to international ethical guidlines The ethical review board of Medical Research Institute, Alexandria University approved the study. Diabetes is induced by the method adopted from Hassan *et al*. [3] using streptozotocin [STZ], a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia by its alkylating property corresponding to that of cytotoxic nitrosourea compounds. The diabetic mice were feed on a high fat diet with average weight of ≈35 g. They were kept at controlled temperature (25±2 °C) and ambient humidity. The mice were injected intraperitoneally with a single high dose of 180±10 mg/kg STZ to achieve consistently a diabetic state with limited morbidity and mortality.

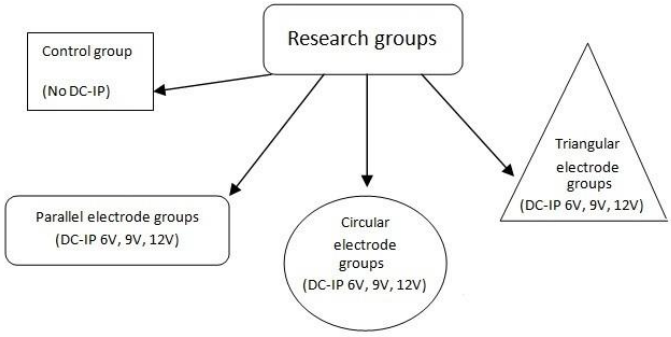

Fig. 1. Distribution graph of mice groups incorporated in the current experimental work. Control group without DC-IP application, and test groups for parallel, circular, triangular DC-IP electrodes at 6 V, 9 V, 12 V.

Body weight and blood glucose concentration were monitored once before and daily after STZ injection until a diabetic state was confirmed by the glucose dehydrogenase method (*Vitro*Scient Diagnostics) [18]. Mice exceeding 300 mg/dL glucose level were considered diabetic. The mice were categories in test and control groups (each group $n = 10$) as shown in Figure 1.

Insulin and methylene blue dye preparation

Methylene blue (MB) dye, a heterocyclic aromatic compound, was used as an experimental drug due to its simplicity of detection in tissues and its moderate molecular weight, that is compatible with several actual drugs. MB dye was incorporated in a hydrogel to give a concentration of 1 mg MB/g gel and used as transdermal drug release indicator [17]. Insulin used were purchased from local pharmacy as Insulin H Mix; Recombinant human insulin / protamine insulin mixture, Sedico pharmaceutics, Egypt. The ratio of insulin to MB gel was 1.5 unit of insulin, 1 mg MB/g gel. This mixure of (insulin/MB) was equally spread over part the abdomen part of mice skin portion o using flate surface plastic spatula. This porcedure was conventinally termed skin painting.

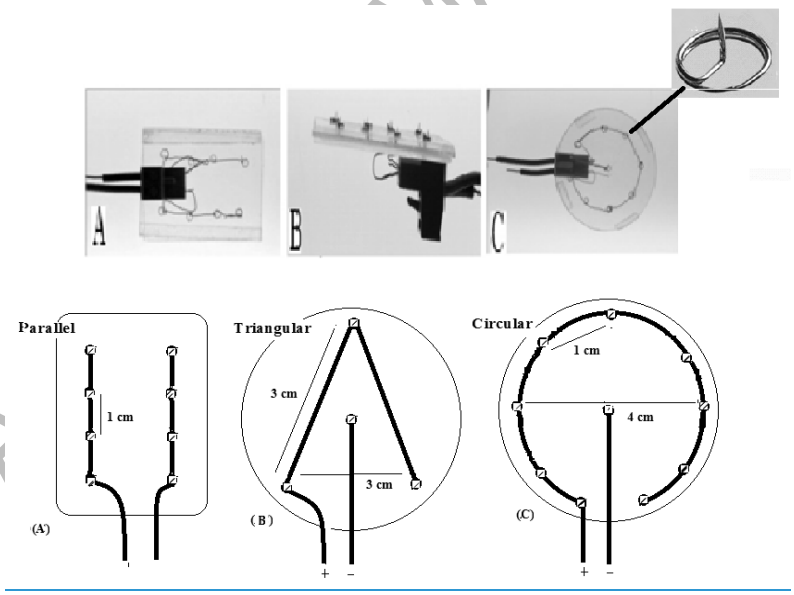

Fig. 2. Photographs and corresponding schematic dimensions of the three home-made electrode arrays, A: parallel, B: triangular, and C: circular with the serine needle electrode that involved in the DC-IP transdermal work.

Two physical techniques were used to enhance the delivery of methylene blue and insulin through mice skin. These techniques are electroporation [EP], and direct current iontophoresis [DC-IP] using microneedles array [MNs].

Designing microneedle electrode arrays

In the current work, three types of MNs arrays were considered. They were manufactured in our lab using printed circuit board and acupuncture needles model (seirin acupuncture needles p-type) (0.22×1.6 μm) which have a 2.8 mm diameter ring handle and 10×10 mm hypoallergenic and waterproof skin tape, used for both ear and acupuncture. The area enclosed by the MN array was equal ~ 450 mm^2 for all shapes (parallel, triangular array and circular) array MN electrodes**.** The dimensional description of the electrode arrays is shown in Figure 2.

Electroporation and iontophoresis

The mice were trapped in a home-made trapping tube as in Figure 3. MN electrode coated with MB hydrogel and insulin was fixed to the selected mice skin with a weight of 1 N. Transient (EP) was peformed by alternating current (AC) square wave function generator at 50 V for 5 seconds. It followed by applying (DC-IP) of 6, 9 and 12 V at current up to 0.5 A for 5 minutes. The experiment setup is shown in Figure 4. This procedure was done for all study groups.

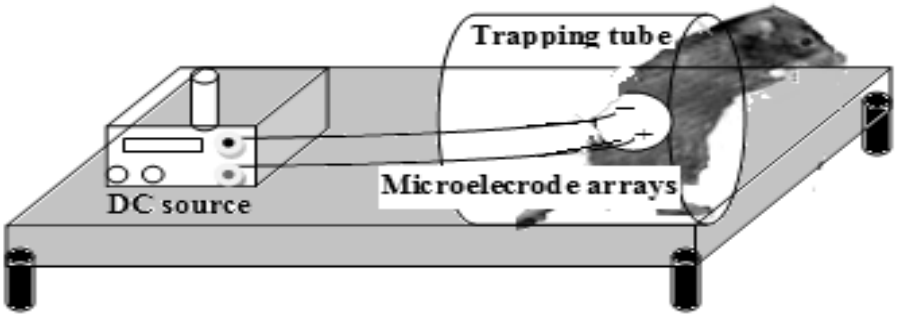

Fig. 3. Diagram of home-made setup of animal trapping tube for iontophoresis microelecrode arrays attachment.

HISTOMORPHOMETRY ANALYSIS FOR STAIN DIFFUSION

The mice skin samples were collected after exposure to the different voltage and then were detached of mice body immediately and preserved in deep freeze to further histological analysis as frozen sections. The lab setting for image analysis is shown in Figure 4. The images were captured by digital program provided by the

manufacture company Image J. Software (MathWorks, USA, Matlab version 5.5 [6]. Dye transdermal penetration was calculated by the mean of skin pores diameter and the length of the blue color penetration on captured images. Histomorphometrical quantification of MB stain expansion was calculated on screen for different captured microscopic photographs.

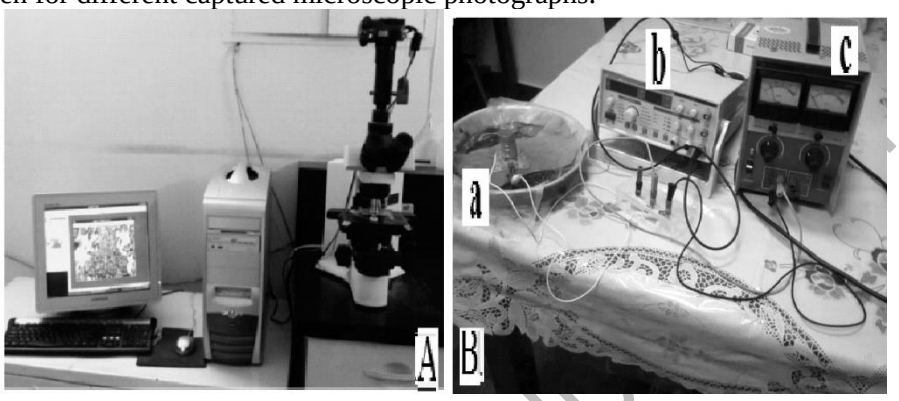

Fig. 4. The experiment set-up **(A)** histomorphometric photo analysis system, **(B)** EP, and DC-IP instruments involved **a**: electrode-mice skin dish, **b**: square pulse AC function generator (model CA1640 p-02, multi waveforms with frequency counter up to 20 MHz), and **c**: DC source (model DYNASCAN CORP, model 1652, can give maximum output voltage of 240 V, and maximum DC current of 0.8 A).

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 22.0 [5]. Quantitative data were described using minimum and maximum, mean, median and standard deviation. The correlation between values in groups was done using Spearman Correlation. Correlation was significant at the levels $p > 0.05$.

RESULTS AND DISCUSSION

We can notice that the glucose levels (before and after insulin skin painting on the mice skin) are significantly higher ($p > 0.05$) than control group in both parallel and triangular MN electrodes for almost all test groups (Fig. 5). Meanwhile, in the circular MN electrode, the glucose levels are relatively little higher than control group before painting. Otherwise, after insulin painting, the circular electrode shows the most effective in insulin delivery, rendering to decrease in glucose level in relation to control group.

The histomorphometric analysis of MB dye expansion in skin layers is shown in Figure 6. The circular MA electrodes showed the greatest effect on MB dye

expansion, with operating voltage of 6 V, which is more effective than other values (9 and 12 V).

Nowadays, transdermal drug delivery is riding a crucial role in the field of drug therapy. Topical drug administration is the most affordable drug delivery route for patients. Although, this route is non-invasive, *stratum corneum* stands still the rate-limiting barrier for many drug types. Unlike non-ionizing drugs, ionizing one cannot cross this barrier easily. An external driving force is required to overcome the skin barrier. In the case of EP and DC-IP, electric energy is the external force [10, 12, 15].

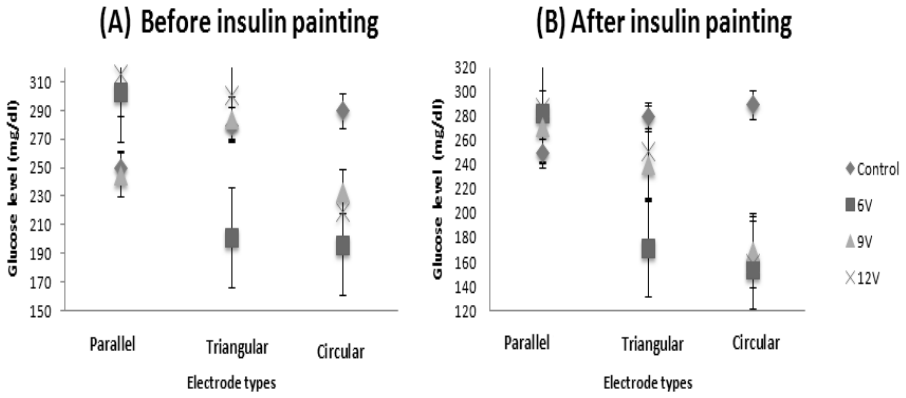

Fig. 5. Plot scattering representation of the mean values of glucose levels before [A] and after [B] insulin painting on mice skin with DC-IP application of 6V, 9V, and 12V, $p > 0.05$.

There is a linear dependence of skin resistivity to applied voltage. During DC-IP application, the receptor cells resistance decreases, consequently as the current, in milliampere, increases until the source compensates to deliver a constant current. At the same time, voltage varies to compensate the resistance changes (Ohm's law). From earlier studies, as the skin resistivity decreases, the permeability rate increases [1, 8, 12].

Skin structure reorientation is affected from the starting of the electrical stimulation, as explained by Prausnitz *et al.* [12]. In our work, a slow response was experienced by applying a short square wave alternating current within 5 seconds. The mechanism of skin response can be explained by the rearrangement in the lipid bilayer structure of *stratum corneum* layer. This effect leads to the formation of pore-like structures enhancing transdermal delivery of the ionizable drugs by iontophoretic mechanism via further application of direct current for 5 minutes with an average current of 0.3 A [12, 20].

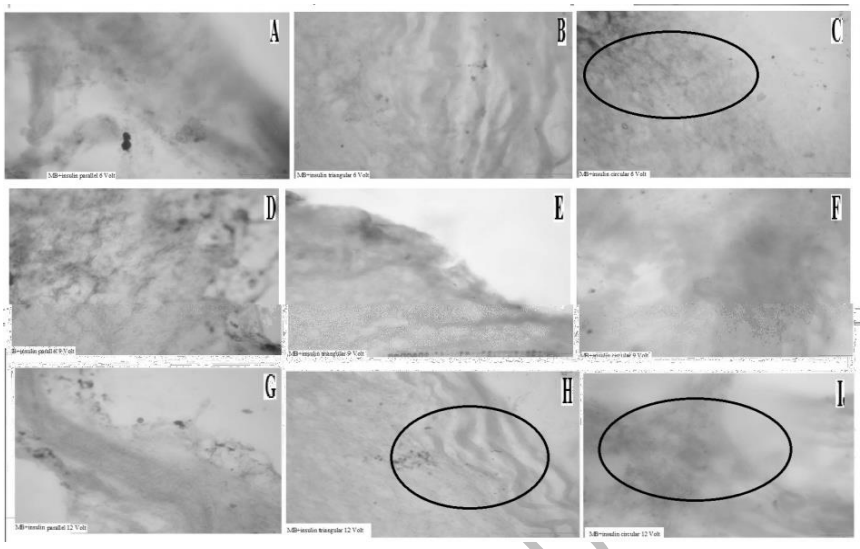

Fig. 6. Histomorphometrical analysis of transdermal penetration of insulin and MB stain through mice skin by DC-IP, by parallel, triangular, and circular electrodes of 6 V (A, B, C), 9 V (D, E, F), and 12 V (G, H, I).

The electrode geometry is crucial in cell impedance measurement. The electric field distribution is mainly related to electrode shape. Microneedles are prepared in low cost patch, simple to patients, available to macromolecules and provide precise localization. They can be categorized as solid for tissue pretreatment, drug-coated, dissolving, and hollow microneedles [3, 6, 193].

Jin *et al.* [8] use an equivalent circuit model to measure HeLa cell impedance. This model shows that the circle and parallel electrodes provide higher electric field strength compared to cross and standard electrodes at the same operating voltage. These finding are matching with our work. The parallel and triangular electrodes ensure a greater insulin transdermal delivery that is emphasized by decreasing in glucose level of mice blood.

From previous works [7, 17, 20], increasing the operating voltage decreases the impedance of the cell in all electrode shapes. This finding was matched with our finding as presented in the histomorphometrical analysis that emphasizes that circular MN electrodes shows the greatest effect on MB dye expansion, and operating voltage of 6V experiences the most dye expansion than the other values (9 and 12 V).

CONCLUSIONS

All of all, according to our finding, we can conclude that iontophoresis is a promising tool for enhancing drug transdermal drug delivery. The mechanism depends on the applied voltage and the electrode shape and geometry. In the current work we evaluate the effect of three home-made microneedle electrodes array (parallel, circular and triangular) for iontophoretic methylene blue dye and insulin transdermal delivery in diabetes-induced mice skin. The parallel and triangular electrodes are of greater insulin transdermal delivery that is emphasized by decreasing in glucose level of mice blood.

The histomorphometrical analysis emphasized that circular microneedle electrodes show the greatest significant ($p > 0.05$) effect on MB dye expansion, and operating voltage of (6 V) experiences the most dye expansion than the other values (9 and 12 V).

Acknowledgements. I would like to appreciate the valuable guidance and enthusiasm of Prof. Dr. Ehab Ibrahim Abdo, head of Medical Biophysics Department, Medical Research Institute, Alexandria University, Egypt, for scientific retrieving for the work.

R E F E R E N C E S

1. BANGA, A.K., S. BOSE, T.K. GHOSH, Iontophoresis and electroporation: comparisons and contrasts, *Int. J. Pharm*., 1999, **179**(1), 1–19, doi:10.1016/s0378-5173(98)00360-3.
2. DINH, S.M., C.-W. LUO, B. BERNER, Upper and lower limits of human skin electrical resistance in iontophoresis, *AIChE Journal*, 1993, **39**(12), 2011–2018, doi:10.1002/aic.690391211.
3. GUJJAR, M., A.K. BANGA, Iontophoretic and microneedle mediated transdermal delivery of glycopyrrolate, *Pharmaceutics*, 2014, **6**(4), 663–671, doi:10.3390/pharmaceutics6040663.
4. HASSAN, Z., A. DEWA, M.Z. ASMAWI, M.Z.A. SATTAR, Assessment of vascular reactivity at different time-course on streptozotocin-induced diabetic rats, *J. Exp. Integr. Med*., 2011, **1**(3), 175–183, doi:10.5455/jeim.250411.or.007.
5. IBM SPSS STATISICS, http://ibm.com/products/spss-statistcs, Accessed May 31, 2018.
6. IMAGJ, http://imagej.net/welcome, Accessed May 31, 2018.
7. ITA, K. Transdermal delivery of drugs with microneedles potential and challenges. *Pharmaceutics*, 2015, **7**(3), 90–105, doi:10.3390/pharmaceutics7030090.
8. JIN, L.H., B. YANG, Y. ZHANG, P.L. LIN, C. CUI, J. TANG, Patterning of HeLa cells on a microfabricated Au-coated ITO substrate, *Langmuir*, 2009, **25**(9), 5380–5383, doi:10.1021/la804297x.
9. MARTANTO, W., S.P. DAVIS, N.R. HOLIDAY, J. WANG, H.S. GILL, M.R. PRAUSNITZ, Transdermal delivery of insulin using microneedles *in vivo*, *Pharm. Res.* 2004, **21**(6), 947–952, doi:10.1023/b:pham.0000029282.44140.2e.
10. MARWAH, H, T. GARG, A.K. GOYAL, G. RATH, Permeation enhancer strategies in transdermal drug delivery, *Drug Deliv*., 2014, **23**(2), 564–578, doi:10.3109/10717544.2014.935532.

11. PILLAI, O., R. PANCHAGNULA, Transdermal delivery of insulin from poloxamer gel: *ex vivo* and *in vivo* skin permeation studies in rat using iontophoresis and chemical enhancers, *J. Control Release*, 2003, **89**(1), 127–140, doi:10.1016/s0168-3659(03)00094-4.
12. PRAUSNITZ, M.R., The effects of electric current applied to skin: A review for transdermal drug delivery, *Adv. Drug Deliv. Rev.*, 1996, **18**(3), 395–425, doi:10.1016/0169-409x(95)00081-h.
13. RIEMMA PIERRE, M.B, F.C. ROSSETTI, Microneedle-based drug delivery systems for transdermal route, *Curr. Drug Targets*, 2014, **15**(3), 281–291, doi:10.2174/1389450111314660232.
14. ROUSTIT, M., S. BLAISE, J.L. CRACOWSKI, Trials and tribulations of skin iontophoresis in therapeutics, *Br. J. Clin. Pharmacol*., 2014, **77**(1), 63–71, doi:10.1111/bcp.12128.
15. SHAH, R.B., M. PATEL, D.M. MAAHS, V.N. SHAH, Insulin delivery methods: Past, present and future, *Int. J. Pharm. Investig*., 2016, **6**(1), 1–9, doi:10.4103/2230-973x.176456.
16. SINGH, D., M. PRADHAN, M. NAG, M.R. SINGH, Vesicular system: Versatile carrier for transdermal delivery of bioactives, *Artif. Cells Nanomed. Biotechnol*., 2015, **43**(4), 282–290, doi:10.3109/21691401.2014.883401.
17. SILVA, Z.S. Jr., Y.Y. HUANG, L.F DE FREITAS, C.M. FRANÇA, S.B. BOTTA, P.A. ANA, R.A. MESQUITA-FERRARI, K.P. SANTOS-FERNANDES, A. DEANA, C.R. LIMA LEAL, R.A. PRATES, M.R. HMBLIN, S.K. BUSSADORI, Papain gel containing methylene blue for simultaneous caries removal and antimicrobial photoinactivation against *Streptococcus mutans* biofilms, *Sci. Rep*., 2016, **6**(1), 33270, doi:10.1038/srep33270.
18. VITROSCIENT, http://vitroscient.com/?=glucose, Accessed May, 2018.
19. WANG, M.-H, W.-H. CHANG, Effect of electrode shape on impedance of single HeLa cell: A COMSOL simulation, *BioMed. Res. Int.*, 2015, 871603, 1–9, doi:10.1155/2015/871603.
20. WANG, M.H., L.S. JANG, A systematic investigation into the electrical properties of single HeLa cells *via* impedance measurements and COMSOL simulations, *Biosens. Bioelectron*., 2009, **24**(9), 2830–2835, doi:10.1016/j.bios.2009.02.012.
21. WORLD HEALTH ORGANIZATION, *Diabetes*, 2018, Available at: http://www.who.int/en/news-room/fact-sheets/detail/diabetes, Accessed May 31, 2018.