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An idea of using drug combination therapy through double-layered dissolving microneedles to treat streptozotocin-nicotinamide induced diabetic rats

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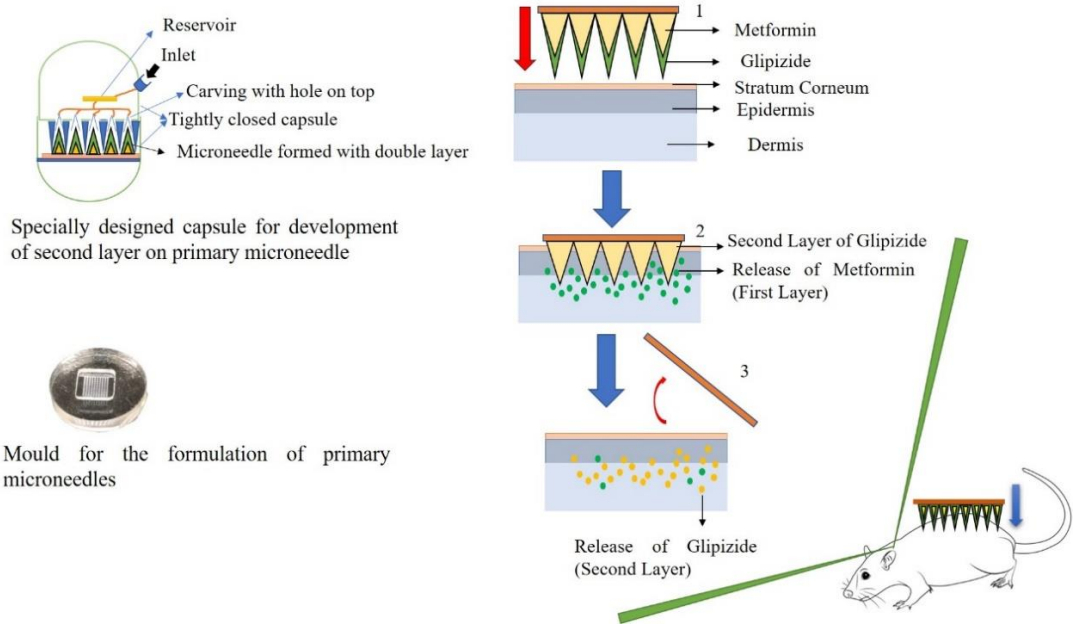
Abstract: Microneedle transport approach has been used from past few a long time as a method to break the stratum corneum layer of skin and to perform the effective transport of drug across the skin and it is especially used for delivery of peptides, protein, DNA, oligonucleotides, molecular mass medicine and inactivated viruses across the dermal layer of the skin. In this hypothetical paper, we specifically targeted on how we will develop double-layer microneedle which can deliver drug-mixture therapy efficaciously. Because there are many diseases together with cancer, tuberculosis, diabetes, leprosy, HIV, and AIDS, which may be efficiently handled by way of drug-combination therapy however this remedy is particularly confined to the tablet, capsule or another form of dosage. If, we can develop double-layered microneedle that can an opportunity to treat these illnesses with painless delivery. In the paper, we have tried to deal with type-2 diabetes with double-layered microneedle and that is formulated with the aid of hypothetically designed capsule which has the projection in it and which may be connected with projection of primary microneedle and can form cone-like cavities and from these cavities we will deliver our drug and materials for the development of secondary layer on a primary layer of microneedle and this technique can be utilized in different disease remedy and it is going to reduce fee of treatment, increase in patient compliance, site directed drug-delivery, increase in bioavailability of drug in the blood stream and increase in therapeutic index with less side effects.



Keywords: Microneedles; double-layer microneedles; type-2 diabetes; hypothetically designed capsule; therapeutic index

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Introduction

From the past few decades, the employment of syringe injection for delivery of drug particularly for delivering anti-diabetic medicine has been magnificently magnified in variety. However, we've got some limitations related to the standard syringe, the chance of infection and inflammation at the location of injection related to pain and anxiety. Another additional disadvantage includes manufacturing waste, the value in production, safety protocol needed for disposal and alternative disease associated with the employment of unsterilized syringe over the amount [1].

In this constraint, we tend to encourage to use microneedles over the quality of syringe and thanks to microneedle posses' bound blessings over standard syringes like: The administration of huge molecule is feasible, Painless administration, First-pass metabolism is avoided, Compared to hypodermic/ standard injection, the speed of healing at the injection web site is quicker, Ease of administration, Enhanced drug effectuality might cause dose reduction, Rapid drug delivery will be achieved by microneedles plus associate degree electrically controlled micro-pump, The rate of drug delivery is additional manageable compared with drug delivery via the stratum[2].

Microneedles (MNs) are microscopic needles and size ranges from one to a hundred metric linear units and one micron in diameter and organized on a pad that's robust enough to specific into the skin and transports the drug across the skin. it's little to avoid nerve stimulation. These microstructure devices consist of microstructure projection coated with a drug or with a vaccinium and applied on the skin to supply intradermic delivery of the drug [3].

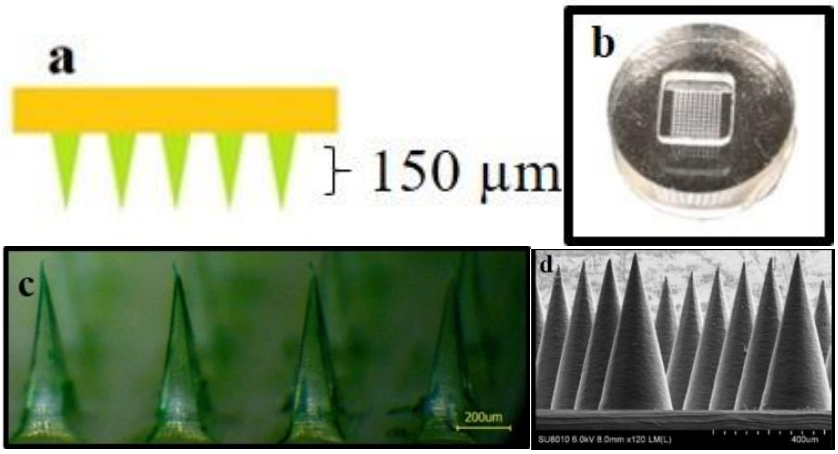

Figure 1: a. Basic Structure of Microneedle, b. Microneedle mould, c. Bright field micrographs of dissolving Microneedle, d. SEM image of Microneedle[4]

Types of Microneedle

Based on the excellence, we are able to classify microneedles as solid, hollow, coated, and dissolving microneedles.

Solid microneedles: These area unit a set of projections with arrays which will be used for making holes in corneum and area unit applied before the appliance of a drug and so removed. It applies these microneedles for a precise amount and these will develop micro-channels within the skin to assist in transportation of the drug across the stratum layer [5] [6].

Coated microneedles: These micro needles are encircled by a drug dispersion layer or with a drug resolution. the number of drugs is loaded depends upon the thickness of the coating and also the size of microneedles and these microneedles is explored for delivery of the higher derma layer. High mass compounds like proteins, vaccines, and oligo nucleotides is delivered by the assistance of those microneedles [7].

Hollow microneedles: These microneedles contain a hollow bore within the middle. Hollow bore bypasses the horny layer of the skin and

permits the medications to taste these hollow bores and reaches the opposite lower layers of the cuticle. These microneedles area unit in the main utilized to move drug solutions directly into the skin layer by making an immediate channel for the drug to pass [8].

Dissolving microneedles: These microneedles get utterly dissolved within the skin and leave no residue behind it. These microneedles usually form up of soluble materials like polymers and sugars that area unit inert, safe and can get utterly dissolve within the skin. Medication area unit incorporated within these microneedles to urge free into the skin [9]. Dissolution and bio-acceptability of compound within the skin create it best for semi-permanent medical care with improved patient compliance. Whereas developing dissolving microneedles, we must always pay a lot of specialise in the drug distribution across the needle. Thus, polymer-drug mixture may be an essential step whereas developing dissolving microneedles [7].

Hydrogel-forming microneedle: These microneedles are the most recent development within the series of microneedles [7]. The arrays of those microneedles comprise super-swelling polymers. This chemical compound will take an oversized quantity of water into three-dimensional compound network as a result of this chemical compound is deliquescent [10]. These arrays absorb ECF when insertion into the skin and tumefy to make continuous channels between dermal capillary circulation and also the drug patch-reservoir resulting in the diffusion of the drug into the skin [11].

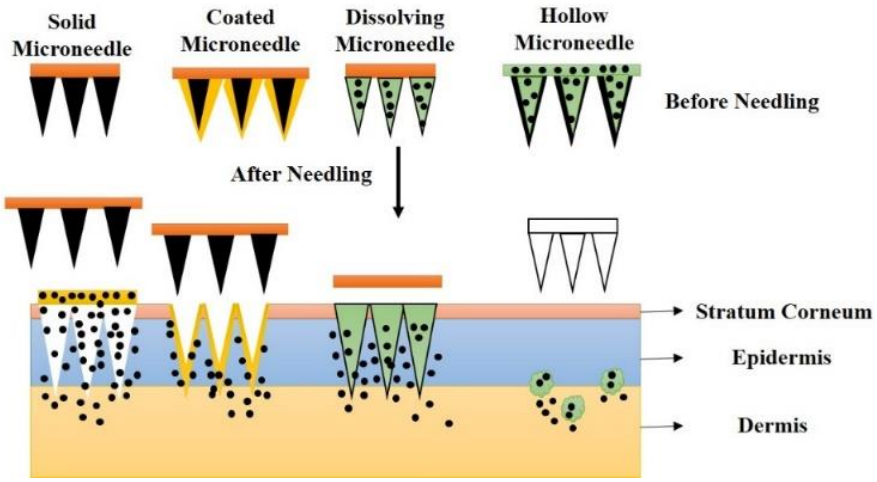

Figure 2: Different types of microneedles on skin layer

Diabetes Mellitus

We could outline DM as a bunch of metabolic diseases that are characterised by symptoms thanks to defects of endocrine secretion and/or multiplied cellular resistance to endocrine [12]. Such deformities arise because of irregular functions of the regulative systems chargeable for the mobilization of metabolic fuels and storage of it, which incorporates the organic process and catabolism of lipids, carbohydrates, and proteins, emanating from the defective hormone secretion, hormone action, or both [13]. We can classify diabetes into 2 varieties i.e. insulin-dependent diabetes (IDDM, kind I) and ketoacidosis-resistant diabetes mellitus (NIDDM, Type II). The kind I polygenic disorder is an associate degree autoimmune disorder that's characterised by an area inflammatory reaction in and around cells of the isle and later ends up in the destruction of insulin-secreting cells. Whereas in kind II polygenic disorder, it is characterised by peripheral endocrine resistance and impairment of endocrine secretion [14]. Chronic and different metabolic disturbances of DM might cause long - run tissue and organ injury [12] and alternative complications like vessel diseases, peripheral tube illness, kidney failure, neuropathy, stroke, retinopathy, blindness, and amputations, etc. [14]. Drugs available to treat type-II diabetes such as sulfonylurea (glyburide, glimepiride, glipizide), metformin, alpha-glucosidase inhibitor (acarbose, miglitol, voglibose), thiazolidinedione (pioglitazone, rosiglitazone) [15].

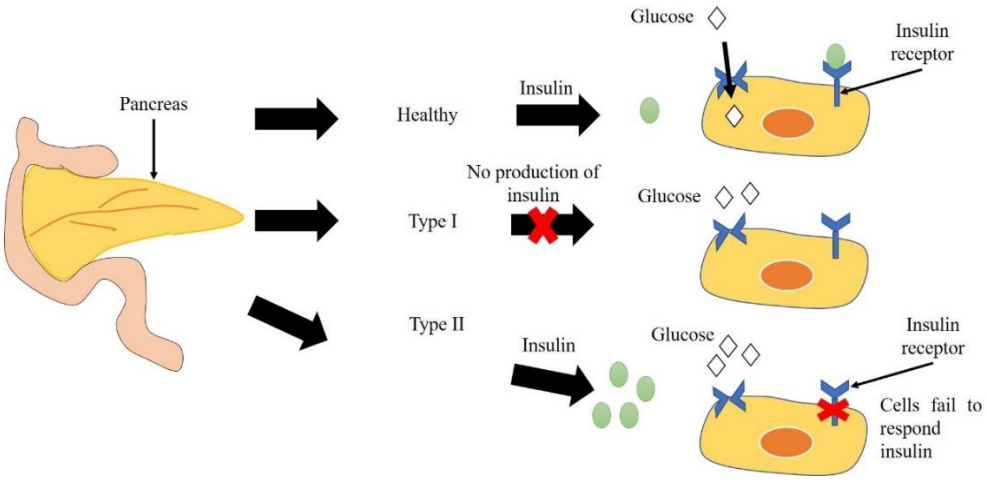

Figure 3: Comparison between healthy, type I & type II diabetes mellitus

Drug combination medical aid has bound benefits over mono-drug medical aid like two medication, every drug functioning at a separate site, block different effector pathways; thus, once two drug categories are co-administered, then there'll be increased lowering result of blood pressure. Drug combination medical aid might increase effectiveness by counteracting counter-regulatory mechanisms of one drug that's triggered by the opposite drug. Combination medical aid has fewer adverse effects as compared to high-dose monotherapy that results in increasing patient compliance and preventing treatment failure which may result from mono-therapy. Together medical aid, adverse effects of one drug are effectively neutralized by another drug [16].

Hypothesis

Drugs to be used in Drug-combination therapy: Normally in oral preparation (tablets), we tend to want to offer glipizide and metformin in 2.5 mg and 250 mg severally together medical care. We are going to use a similar quantitative relation of 1:100 for developing our microneedles for our first-line medical care [17].

Formulation of Double-layered microneedles: We are exploitation our base material for developing microneedle is Hyaluronan (HA) (sodium hyaluronate, average Mw 150 kD which might be purchased from Lifecore Biomedical), a collection of mould for formation of microneedle during which there's one special style mould, and our medication which is able to be employed in combination medical care in a very totally different proportion to see it best result (glipizide and metformin).

Table 1: Overview of the different formulation composition used to prepare the double-layered dissolving microneedles. The compositions refer to the liquid formulations before double-layered dissolving microneedles.

Formulation	Layer	Composition	Formulation approach	Layer	Composition	Formulation approach
A	**First Layer**	Metformin/ Hyaluronan (% w/v)	24.75 mg/ 50 ml	**Second Layer**	Glipizide/ Hyaluronan (% w/v)	0.25 mg/ 50 ml
B	**First Layer**	Metformin/ Hyaluronan (% w/v)	49.50 mg/ 50 ml	**Second Layer**	Glipizide/ Hyaluronan (% w/v)	0.50 mg/ 50 ml
C	**First Layer**	Metformin/ Hyaluronan (% w/v)	69.30 mg/ 50 ml	**Second Layer**	Glipizide/ Hyaluronan (% w/v)	0.70 mg/ 50 ml

D	**First Layer**	Metformin/ Hyaluronan (% w/v)	99.0 mg/ 50 ml	**Second Layer**	Glipizide/ Hyaluronan (% w/v)	1.00 mg/ 50 ml
Control	**First Layer**	Metformin/ Hyaluronan (% w/v)	0.00 mg/ 50 ml	**Second Layer**	Glipizide/ Hyaluronan (% w/v)	0.00 mg/ 50 ml

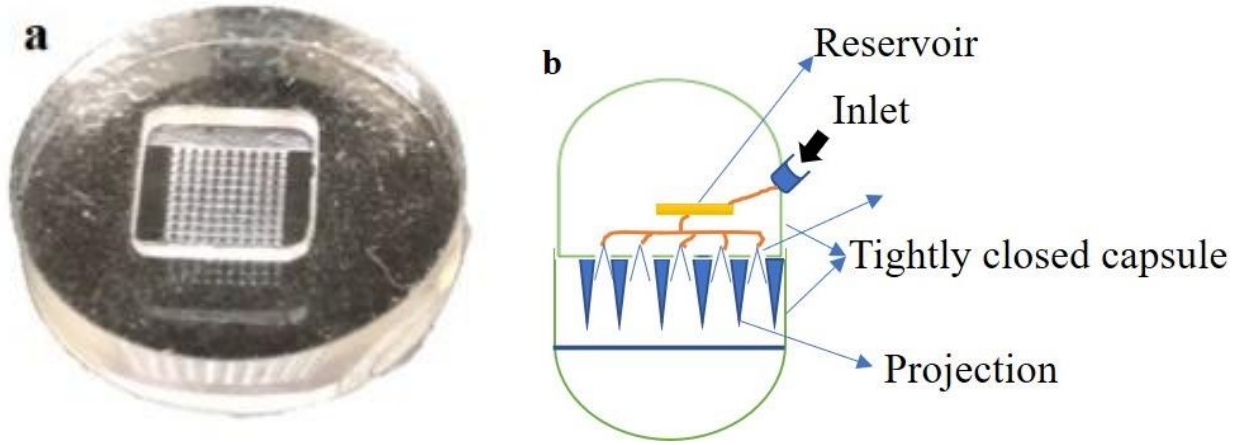

Figure 4: a: Primary mould used to form first-layer microneedle, **b:** Hypothetical-design capsule for developing double-layer microneedles.

Steps for formulation of single-layer microneedles

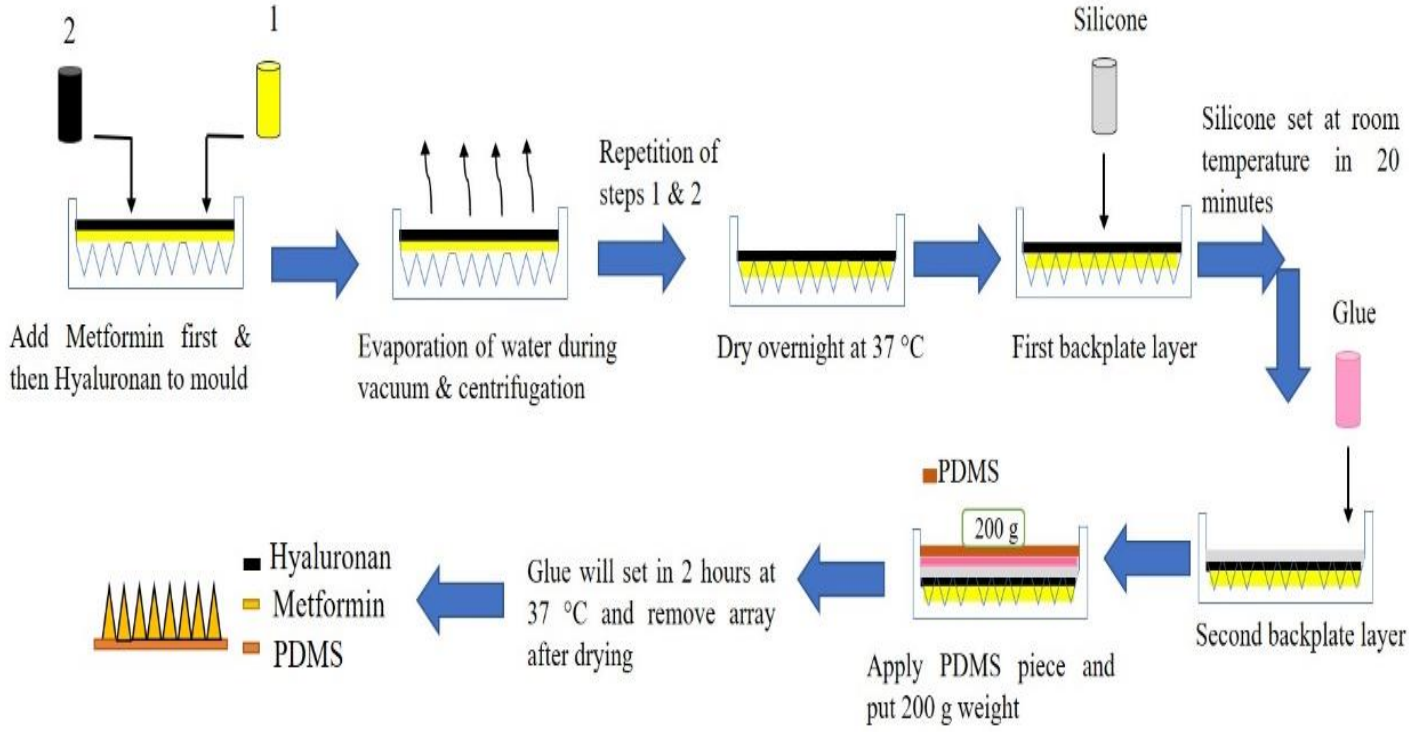

Figure 5: Preparation of single layer dissolving microneedle [18]
PDMS: Polydimethylsiloxane

Steps for formulation of double-layer microneedles

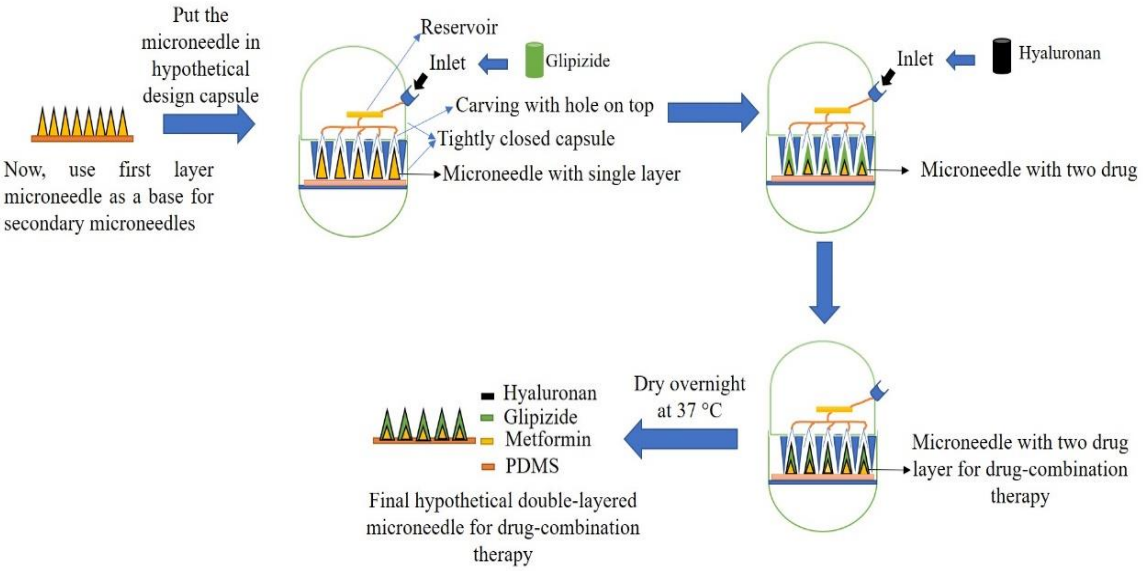

Figure 6: Preparation of double layer dissolving microneedle

- ✓ We have used our primary dissolving microneedle as our base for the event of double-layer dissolving microneedles.
- ✓ Now we've got opened our theoretic capsule in 2 components, one half having projection which is able to be fastened with our basic primary dissolving microneedle and permit to develop cavities higher than primary microneedle. Gap that is found higher than the opening of cavities from their medication and microneedle forming material is passed with high pressure and speed through an inlet. With the assistance of pressure and speed of fabric flow, we will manage the speed of formation of secondary layer on the first layer. We will fix the second part of a capsule having a holding plate that's accountable to connect our primary microneedle structure in position and with a projection of the primary layer and making a lock condition.
- ✓ After the developing microneedle, leave the microneedle within the capsule for nightlong at thirty-seven °C to dry and once waterlessness then takes away microneedle from the array.
- ✓ Now repeat the first layer and second layer process development method for developing the different variation of metformin and glipizide double layer microneedles.

Testing of hypothesis

Induction of Experminental Diabetes in Wistar rats

Rats were unbroken in a very controlled temperature and lightweight condition (i.e. 37°C and 12:12 hour light: dark cycles) [19] and permit free access to water and food more or less for two weeks to adopt the experimental conditions. Rats were fasted for twelve hours to avoid failure of induction and to avoid aldohexose competition with streptozotocin at GLUT2 transporters. Through an experiment, we are able to induce polygenic disorder in rat by giving the only dose of streptozotocin through intraperitoneal route (i.e. sixty-five mg/kg weight of streptozotocin dissolved in a very 0.1 M freshly ready change state buffer having pH 4.5), once the quarter-hour we'll provide the second dose of nicotinamide (120 mg/kg body weight) and dissolved in traditional saline. To counter drug-induced hypoglycaemia, rats got a five-hitter aldohexose resolution long. Blood samples were collected once seventy-two hours of induction from the tail vein of rats by a puncture to work out the glucose with a glucometer. If the glucose level is over 250 mg/dl, then polygenic disorder is confirmed and currently we tend to check our different quantitative relation

of metformin and glipizide double-layer microneedles on diabetic rats [20].

Figure 7: a: Wistar Rat (approxiamtely 150-200 g for experiment), **b:** Hair removed by clipper and hair removal cream for insertion microneedles into diabetes induced wistar rat, **c:** Insertion of double-layer microneedle for treatment of type-2 diabetic wistar rat

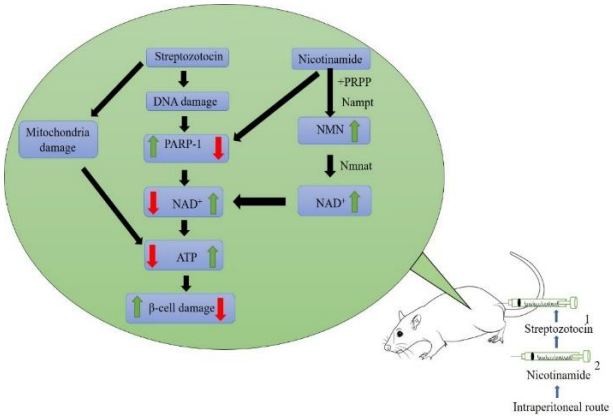

Figure 8: Cytotoxic aand protective action of streptozotocin & nicotinamide on B-cells

respectively. High nicotinamide intake could result in a rise within the production of reactive element species and result in aerophilous stress and hypoglycaemic agent resistance [21].

PARP-1: Poly (adenosine triphosphate [ADP]-ribose) polymerase-1, PRPP: 5-phosphoribosylpyrophosphate, NMN: Nicotinamide mononucleotide, Nampt: Nicotinamide phosphoribosyltransferase, Nmnat: Nicotinamide/ nicotinic acid mononucleotide adenyltransferase.

Implication of hypothesis

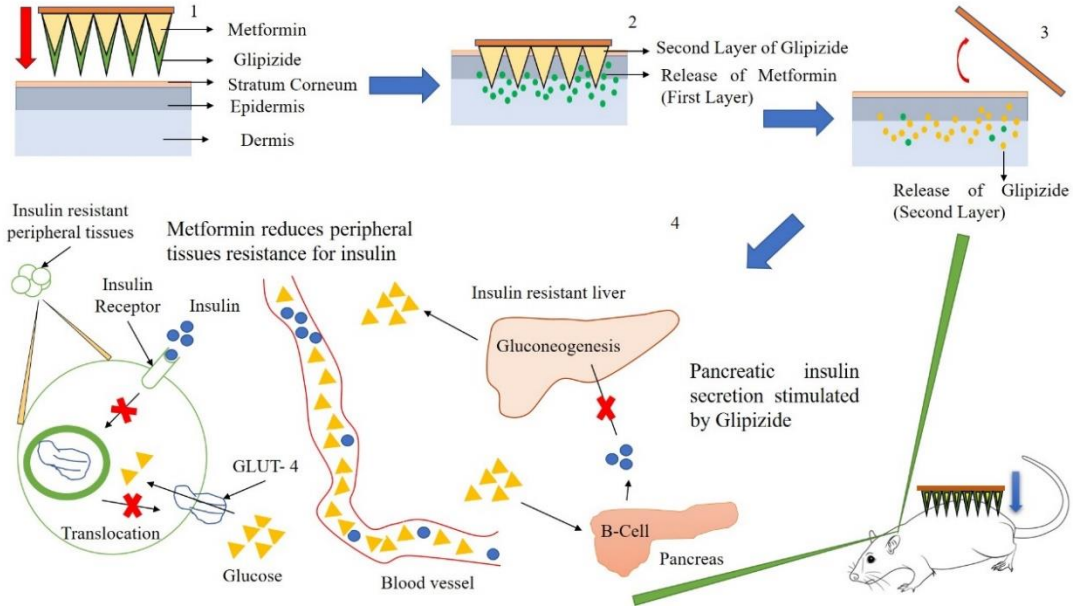

Figure 9: Mechanism of action of metformin & glipizide through double-layered microneedle [22]

After, the made development of double-layered microneedle that is comprised of metformin and glipizide and separated by two layers of hyaluronan. During this paper, we've hypothetic tried to make double-layered microneedles by the assistance of hypothetically

designed capsule which might be worked on a basis of lock and key theory as a result of its own projection which might be fitted adjacently to microneedle projection of primary microneedle or we are able to say single layer microneedle and by this technique, it permits to developed cup-like cavities on top of the primary or single layer microneedle. From these cup-like cavities our drug and material will simply flow with the assistance of a hole that is given on top of the cavities and by the assistance of an inlet, we are able to simply monitor and manage the speed of drug and material and, it helps us in developing the second layer on primary layer in a very controlled manner. We tend to create the various formulation of metformin and glipizide through variation in their formulation unit and we have hypothetically given procedure for testing on Wistar rat, once causing polygenic disease to Wistar rat through intraperitoneal route by injecting streptozotocin and nicotinamide combination. Once testing each formulation on Wistar rat we are able to check the aldohexose level by glucometer and that we can compare the results of each formulation and we are able to calculate serum level of total cholesterol, glyceride, and high-density lipoprotein-cholesterol by collection of blood sample from tail vein of every Wistar rat and that we will study microscopic anatomy of normal, diabetic and drug-treated exocrine gland of Wistar rat.

Conclusion

In summary, until we've got a single-layer microneedle nearly for each unwellness however still, it's a dream to use an inspiration of drug combination medical aid in microneedle to treat diseases like polygenic disorder, T.B., cancer, malaria, leprosy, and HIV/AIDS, rather than the pill, capsule or the other indefinite quantity forms. Size of the microneedle may be a gift for humans owing to its size we will get painless treatment however this size is additionally a limitation for developing the second layer on the first layer of the microneedle. Double-layer microneedle can face a retardant owing to its size, form and double layer, the realm can become slim to carry the drug within and it'll become tough to deliver the additional quantity of the drug to a patient and that we will improve the indefinite quantity by increasing the number of applications of the microneedle to a patient. Within the future maybe it'll do to develop this kind of microneedle and it'll facilitate patients and in addition, doctor to induce additional precise and site-targeted delivery of drug and by the help of double-layer microneedle, we'll scale back price, medical specialty waste, and improve patient compliance.

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Competing Interests

The authors declare that they have no competing interest.

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