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Original Article

Evaluation of the Feasibility of Transdermal Delivery of Neratinib (NB) Loaded Microneedles to Treat Breast Cancer

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ABSTRACT

Background: Breast cancer is the world's second-largest leading cause of death in women. Targeting highly localized tumors using transdermal microneedle drug delivery can be highly advantageous in treating breast cancer. **Aim:** The present study evaluates the feasibility of transdermal delivery of promising chemo preventive agent tyrosine kinase inhibitor Neratinib (NB) and physicochemical properties through breast skin and mammary papilla. **Materials and Methods:** The Microneedles (MNs) were fabricated by photo-polymerization method using PEGDA as a biopolymer and TPO as a photo initiator. Optimized (F3) MNs were characterized for stereomicroscopy, Scanning Electron Microscopy (SEM), and mechanical testing. The *in vitro* permeation studies were carried out on a vertical Franz diffusion cell using porcine skin. **Results:** The cytotoxicity of the optimized formulation on MCF-7 cell lines was carried out using an MTT assay. The FT-IR compatibility studies showed no chemical interaction between drug and excipients used, and an increase in NB solubility decreased the epidermal/vehicle partition coefficient and vice-versa. Microneedles' pitch and total base diameter were evaluated and found acceptable for the study. The mechanical test confirmed that >30% of the needles penetrated the 3rd and <30% penetrated the 4th layers. The *in vitro* permeation studies showed that in breast skin and mammary papilla, the highest skin retention of NB was observed with 64% alcohol compared to 32 and 48% alcohol. Microneedles with 32% alcohol significantly increased the permeation of NB, and microneedles increased the cumulative amount of NB permeated through breast skin by 2.9-fold and decreased the lag time by 3.6-fold. However, there was no significant difference in the skin retention amount after pretreatment with microneedles. **Conclusion:** The results showed that the neratinib-loaded microneedles can be used as an effective transdermal delivery for preventing and treating breast cancer.

Keywords: Breast cancer, Microneedles, Tyrosine kinase inhibitors, Transdermal delivery, Neratinib.

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INTRODUCTION

The second most common malignancy among women is breast cancer. The American Cancer Society (ACS) says that every one of the eight women may get breast cancer at some point. In the United States, 252,710 new instances of breast cancer were predicted to have occurred in women in 2017, and 40,610 breast cancer-related deaths were also estimated.[1] By 2050, it is anticipated that there will be about 3.2 million additional instances of breast cancer in women worldwide.[2] Creating effective treatment and prophylactic strategies is vital since breast cancer is one of the severe health issues, particularly for women.

A wide range of variables influences the emergence of breast cancer. Genetic, hormonal, environmental, familial history and lifestyle choices are some risk factors for breast cancer. BRCA1 and 2 (Breast cancer 1 and 2) are the genes that majorly get mutated in breast cancer.[3] Mutations in these two genes are considered to be responsible for 5-10% of breast cancer cases. Women with the BRCA1 mutation had a lifetime chance of developing breast cancer of 55-65%, compared to 45% for those with the BRCA2 mutation.[4] There are further unidentified mutations that could result in breast cancer. In addition to genetics, lifestyle choices, and environmental variables also affect the development of breast cancer. Targeted treatment is a successful strategy for treating breast cancer because it targets particular cancer cells. Targeting the overexpressed receptors in the cancer cell, immune-targeted

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treatment is a subset of several targeted therapies that use monoclonal antibodies.[5]

The harmful side effects, the emergence of chemical agent resistance, and the requirement for additional treatments to be administered in addition to chemotherapy are all drawbacks of chemotherapy. Due to these drawbacks, molecular-based therapies are highly beneficial.[6] Small and large molecules can be successfully delivered to the subjects needing them by MNs. This study aims to understand different alcohol concentrations' effects as a permeation enhancer and vehicle for breast-specific localized transdermal medication delivered using microneedles to minimize any side effects of the therapy and precisely target locally advanced tumors. To increase the drug's effectiveness for treating or preventing breast cancer, this study uses neratinib, a tyrosine kinase inhibitor used to treat hormone receptor-positive breast cancer, administered locally in the form of dispensable microneedles.

MATERIALS AND METHODS

Materials

Neratinib was a gift sample from Shilpa Medicare, Bangalore. All other solvents, materials, and excipients used were of Pharmacopeial grade purchased from Sigma Aldrich, Mumbai.

Methods

Pre-formulation studies

HPLC method development for neratinib

An HPLC method was developed to quantify Neratinib (NB) using 0.01 M disodium hydrogen orthophosphate (pH 3.0) and acetonitrile with 0.1% formic acid as the mobile phase. Before the sample analysis, the column must be equilibrated for 25 to 30 min. The eluents were observed for 6 min at 215 nm. The system appropriateness, specificity, linearity, accuracy, precision, and robustness of the created method are all tested as part of the validation process. The developed procedure's ability to obtain test results proportional to the concentration of the measured sample. The method is considered valid if the correlation coefficient R^2 is greater than 0.998. The linearity of Neratinib was tested at concentrations ranging from 30-180µg/mL.[7]

Drug characterization by FTIR spectroscopy

Drug and physical mixtures were characterized using an FT-IR spectrophotometer by the KBr pellet method. Three MNs from the drug-coated MNPs were separated using a scalpel, for which pellets were formed by mixing with potassium bromide. An average of 64 scans were taken at a resolution of 4.0 cm^{-1} at 4000-500 cm^{-1} for each spectrum.[8]

Solubility studies

Neratinib solubility tests were conducted using alcohol concentrations of 32, 48, and 64%. Briefly, 1 mL of the 32% alcohol was used as a vehicle, and excess NB was added. The mixture was shaken for 24 hr at 37°C and 100 rpm and was stored at 37°C for an additional 24 hr without shaking to ensure a proper phase separation. The samples were filtered using a membrane filter (0.2 µm), and analyzed the drug concentration using HPLC after necessary dilutions.[9]

Epidermal Partition Coefficient studies (EPC)

The EPC studies of NB used alcohol concentrations of 32, 48, and 64% carried on porcine breast epidermal membrane was separated from the skin using the heat method, after which the obtained skin was immersed in 60°C water for 2 min. Forceps were then used to separate the epidermis from the dermis, and the epidermis was then washed with PBS, pH 7.4. Kim wipes were used to dry the epidermal sheet, which was then put in a desiccator and maintained at -20°C until required. Before investigating the PC, the epidermal sheets were hydrated in a phosphate buffer solution for 6 h. After hydration, the epidermal sheet was placed in a vial with 1mg of the drug and 1 mL of a particular concentration of alcohol (vehicle). It was shaken for 24 hr at 37°C for equilibration. After being digested, the epidermal sheet was placed in a shaker water bath set at 37°C for one night. Kim wipes were used to gently blot the epidermis dry the following day to remove any leftover pharmaceutical solutions. The drug concentration in the epidermal sheet and the vehicle was quantified using HPLC, from which the epidermal partition coefficient's values were calculated.[10]

Formulation and fabrication of NB-loaded microneedles

Polymer Solution Preparation

The first step comprises mixing PEGDA with ethanol in a 3:4 ratios, then mixing it on the magnetic stirrer at 200°C for 60 min or until a clear solution. PO (0.25% v/v) was then gradually added to this mixture while magnetically stirring for 60 min or until the TPO was completely dispersed in the polymer solution. Lastly, the z-axis stage and the resin solution are maintained ready and placed immediately underneath the solution's surface for printing of Micro Needle Patches (MNPs) in a 3D printer. The stage should have a high resolution and 50 mm stroke precision.

Loading modeled Micro Needle Patches (MNPs)

3D models (CAD) of MNPs are equipped with the system. The MNP support is intended to keep the MNs stable during manufacture.[11] They are transformed into CAD files after development. The control unit further divided the STL files into several cross-sections of the necessary thickness. The PSL system (SUKSHM 3D®) is then given with the image files as input and

managed by LabVIEW software. The component is rinsed with isopropyl alcohol to remove any leftover uncured photopolymer, and any surplus solvent is blown away using a primary air blower. After being cleaned, the pieces were post-cured under UV light to solidify. The cured MNPs were subjected to additional mechanical, optical stereo microscopy imaging, and SEM characterization procedures.

Evaluation of MNPs

Stereomicroscopy and Scanning Electron Microscopy

The morphology and size of the prepared MNPs are analyzed using stereo microscopy from ZEISS SteREo Discovery.V20, Germany, and Scanning Electron Microscopy (SEM) from Carl Zeiss, Neon Crossbeam, Germany. 5 to 10 nm of Gold was applied to the polymeric MNPs to increase the conductivity for SEM.[12]

Mechanical testing

Using a texture analyzer (TA.XT, Stable Micro Systems Ltd., UK), the potential for compression and insertion of the blank MNPs was investigated. Parafilm (8 layers), with a thickness of 126 µm, was used to mimic the skin to examine the compressions. MNPs/array were inserted into the film at 1.19 mm/s, 32 N of force, and held there for 30 sec. The height of the needle before and after the microneedle insertion into the film was done to quantify compression. The results are frequently expressed as a height reduction in percentage. The polymeric film's layers were microscopically analyzed to tally the number of holes created.[13]

Preparation of swine tissues

Obtained the female swine breast tissues from a nearby slaughterhouse and used a knife to remove the underlying fat tissue and a hair clipper to remove the hair on the skin. 700 µm thick breast skin was obtained dermatomed and enclosed by the nipple. The mammary papilla and breast skin were clearly shown to be damage-free. The cleaned tissues are stored at -18°C after removing the keratin plug using 60% alcohol. The stored tissues have to be used within 3 months of storage. Different alcohol concentrations were used to assess the drug penetration through the breast skin using sub- and saturated NB concentrations. Different concentrations of alcohol are used for sub-saturated (50, 100, and 180 mg/mL) and saturated (10 mg/mL) permeation studies for vehicles containing 32, 48, and 64% (v/v) of alcohol.

In vitro permeation studies

A vertical *in vitro* Franz diffusion cell was used for the mammary papilla and breast skin penetration tests. The stratum corneum of the breast skin, also known as the mammary papilla, faced the donor compartment and was positioned between the receptor and donor compartments. 0.64 cm^2 of surface area was open to penetration. Phosphate-buffered saline (5 mL) was placed into the receptor compartment (PBS, pH 7.4). The receptor compartment was kept at 37°C and agitated with a magnetic stirrer at 100 rpm. In the donor compartment, 500 µL of the NB-containing formulation was placed. In the case of the MN trials, the NB formulation was applied after the permeation region of the breast skin had been prepared with MN arrays. Parafilm was used to cover the sampling port and donor compartment to prevent the fluid from evaporating. With aluminum foil, the Franz diffusion cells were shielded. 200 µL samples were taken out of the receptor compartment at predetermined intervals (6, 12, 24, 30, 36, and 48 hr), and the same volume of fresh receptor media system was added to keep the volume constant. The duration of each trial was 48 hr. The mammary papilla and breast skin were digested after the trial to evaluate the drug content in the tissue. HPLC was used in all studies to determine the drug concentration.[14]

MTT assay

The MTT test was used to evaluate the cytotoxicity of the optimized formulation F3 on MCF-7 human breast cancer cells. By exposing the various nanomolar doses of NB (100-800 nM) from the optimized formulation F3 and free NB, the cytotoxicity (%) was calculated. The control was the F3 formulation without NB. MCF-7 human breast cancer cells were grown, supplemented, and incubated for the MTT assay following published guidelines. After that, the cells were seeded onto 96-well plates and set according to the steps outlined in the literature. Each well received 10 µL of the MTT reagent following a 72 hr incubation period. The cultivated plates underwent a second incubation at 37°C for 4 hr. Using a plate reader set to 450 nm, the amount of formazan crystals produced was discovered.[15]

RESULTS AND DISCUSSION

Pre-formulation studies

HPLC method development for neratinib

Results were tabulated after injecting five standard solutions of Neratinib at concentrations ranging from 30-180 µg/mL (Figure 1A). With a slope of 6008.1 and r^2 (correlation coefficient) of 0.9937, a graph of concentration vs. peak area is plotted. Neratinib's regression equation was y=6008.1X.

FTIR Study

Figure 1B includes the FT-IR spectra of pure NB and an improved formulation (f3). The characteristic peaks of NB in formulation F3 and pure NB did not undergo any significant changes. However, in formulation F3, the intensity of the NB's distinctive peaks was diminished. This finding revealed that the NB in formulation F3 may be partially amorphous, which may improve the NB's solubility and rate of dissolution when compared to pure NB. Overall, the FTIR analysis's findings indicated that the drug employed in the microneedle formulation and the polymers used to make them had no chemical interaction.

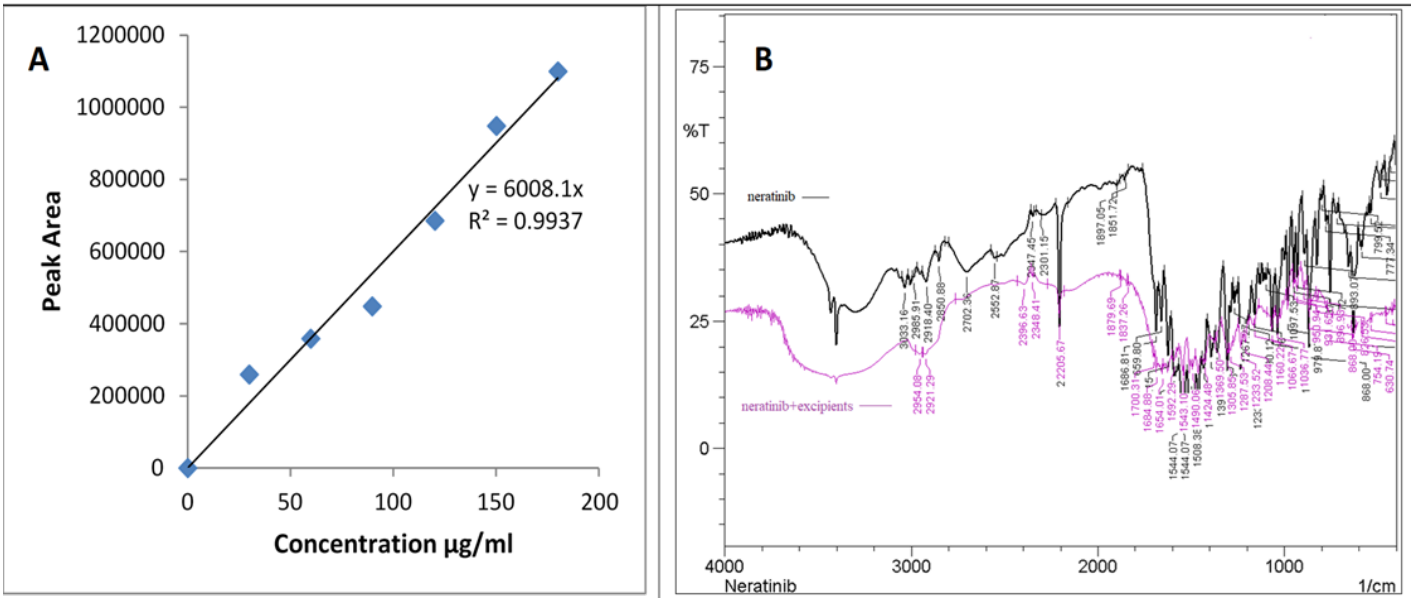

Figure 1: A) Linearity graph of Neratinib (NB) B) FT-IR spectra of pure drug NB and Physical Mixture.

Solubility and Epidermal Partition Coefficient (EPC)

The solubility experiments showed that as the amount of alcohol in the car grew, so did the solubility of NB. The solubility of NB increased by 2.3 times with 50% alcohol and by 5.2-fold with 64% alcohol as compared to 32% alcohol. In addition, NB was 1.9 times more soluble in 64% alcohol than in 48% alcohol. The results demonstrate that the solubility of NB is influenced by the amount of alcohol in the car. Studies on the EPC show that as the amount of alcohol in the vehicle rose, the EPC of NB dropped.

When 48% alcohol was present compared to 32% alcohol, the NB partition coefficient reduced by 1.4 times; when 64% alcohol was present, it decreased by 2.1 times. According to the results of the PC testing, the alcohol content of the car has an impact on the NB EPC. The results show that the PC and solubility of NB have an inverse relationship, with the latter decreasing as NB solubility increased and the former increasing (Table 1).

Table 1: Solubility and EPC of NB in the presence of alcohol at varying concentrations as a vehicle.

Vehicle (Alcohol %)	Solubility (mg/mL)±SD*	Epidermal partition coefficient±SD*
32	27.32±0.12	0.165±0.002
48	65.43±0.16^{x}	0.109±0.003^{x}
64	149.23±0.54^{x,y}	0.071±0.001^{x,y}

Data presented in mean±SD, SD-Standard Deviation, *n=3A substantial difference from 64% alcohol is indicated by "x" and a significant difference from 48% alcohol is indicated by "y."

Table 2: MNs dimensions.

Parameter	Predicted Dimensions (µm)	Actual dimensions(µm)
Base diameter	152	159
Tip diameter	6	9
Height	205	192
Pitch	810	825

Fabrication of MNPs

PEGDA and TPO were used as a biopolymer and photo initiators in the photo-polymerization process to create the MNPs. Popular biocompatible natural polymer PEGDA exhibits hydrophilic properties after polymerization and can imitate the characteristics of a tissue's extracellular matrix. Free radicals are generated when the mixture absorbs UV LED light (λ=385nm), which triggers the start of polymerization. TPO, a photo initiator with better absorption at 385 nm and readily soluble in ethanol, breaks down to form 2 free radicals. These free radicals mix and react with PEGDA, opening the chain at the C-C bond, which initiates the cross-linking. Vinyl linkages left behind by PEGDA monomer act as a source of polymer chain initiation, causing the merging of 2 chains, thus forming a dead polymer chain that prevents the growth of the polymer chain by varying specific process parameters such as the concentration of the polymer and initiator, intensity of light and time of exposure.

The PµSL system, specifically created by our research team and is today marketed as SUKSHM 3D® with the tagline "micro fast prototyping," was used for the whole fabrication process. The optical system's main component is a UV LED light source with a peak intensity wavelength of 385 nm and a wavelength range of 340-410 nm. The UV LED light is projected onto the dynamic mask after passing through several optical components. Light patterns are produced by positioning several micromirrors following the projected images as input. This study's main objective was to create MNPs that were dimensionally accurate utilizing

Table 3: ***In vitro*** **permeation parameters of NB in the sub-saturated and saturated concentration of alcohol through breast skin.**

Vehicle (%)	LT (h)±SD*	F (µg/cm²/hr) ±SD*	CA (µg/cm²)±SD*	RA (µg/cm²)±SD*	TAR
Sub-Saturated Concentration					
32	1.98±0.23[a]	6.98±0.53[a,b]	289.76±29.95	102.12±18.98	0.36
48	3.32±0.65	4.64±0.54[a]	199.87±12.11	79.98±13.87	0.40
64	4.12±0.32	2.56±0.34	109.23±29.41	63.54±6.87	0.58
Saturated Concentration					
32	11.98±0.23[a]	5.45±0.83[a,b]	292.52±18.63	92.18±15.76	0.41
48	2.31±0.72	6.25±0.84[a]	208.77±15.18	68.72±14.91	0.49
64	6.57±0.52	1.89±0.58	129.32±17.57	109.64±5.28	0.62

the SUKSHM 3D® approach, which uses suitable processing parameters to adjust the optical reduction ratio. The system's visual reduction factors were calculated and checked using a stereomicroscope for dimensional errors. The average amount of error in the dimensions of manufactured MNs was discovered to be 1.375%. As a result, the SUKSHM 3D® technology assisted in obtaining MNPs with precise measurements that were within acceptable tolerances.

Optical Stereomicroscopy (OS)and Scanning Electron Microscopy (SEM)

The created conical-shaped MNPs (blank) had 30 (6 by 5) MNs and were around 1 mm in size. Table 2 displays the actual v/s predicted dimensions of MNs.

The MNs on the MNPs were parallel to the patch's base. Figure 2A displays OS images of constructed Nms. Single MN's height is 193.7 µm along with the MN's pitch and the base's total diameter. The dimensions of one MNP are thoroughly characterized.

Overall, the base and tip diameter and pitch of the manufactured MNs were slightly larger than the desired value. On the other hand, the MNs' height was a little bit lower than the desired specifications. The fabricated MNs were nevertheless deemed suitable for the required study.

Mechanical testing

The mechanical strength of MNs is an important consideration when testing blank MNPs mechanically because they must pierce the stratum corneum to deliver the medication painlessly. Utilizing the TA.XTS texture analyzer, mechanical strength, in particular, and the impact of compression were determined. A steady and perfect force of 32 N was applied to MNP for 30, which simulates the usual pressure that people employ while applying MNPs to subjects who are in need. Eight film layers were used for the insertion investigation and compression analysis. Less than 30% of the needles and more than 30% penetrated the third and fourth layers. Penetration is successful if it creates more than 20% of the holes on each film layer. The MNPs of the current investigation were thus found to be located between the 3rd and 4th layers.

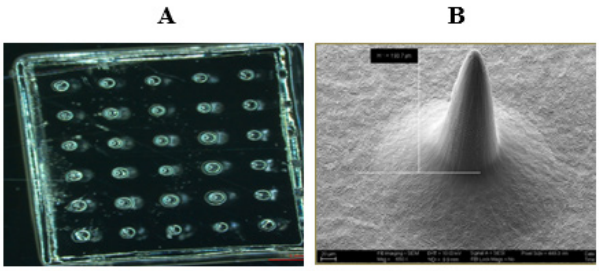

Figure 2: Images of MNs in A) Optical Stereomicroscopy B) Scanning electron microscopy.

In vitro permeation studies

Breast skin

Sub-saturated and saturated (Table 3) NB drug concentration was used to test drug penetration. In sub-saturated concentration, the 32% alcohol vehicle demonstrated the maximum NB penetration through the breast skin in the NB permeation trials utilizing 32%, 48%, and 64% different alcohol as a vehicle. 32% alcohol showed the highest Flux (F) (6.98 µg/cm²/h), and 64% alcohol showed the lowest F among the various percentages of alcohol as a vehicle studied. Also, 64% alcohol was found to have the highest Lag Time (LT) (4.12 hr). With 32% alcohol, NB was retained at its highest concentration. NB permeation investigations' saturation concentrations were also put to the test. Compared to 48 and 64% alcohol, the 32% alcohol vehicle had the maximum NB infiltration through the breast skin. The vehicle with the highest NB flux (11.98 g/cm²/hr) was 32% alcohol. With 64% alcohol, the maximum skin retention of NB was noted at the end of 48 hr.

To calculate the tissue affinity ratio, the RA was divided by CA.10 mg/mL drug was employed. Data is displayed as mean±SD (n=3). A substantial variance from 64% alcohol is indicated by "a" and a

Table 4: *In vitro* permeation parameters of NB in the sub-saturated and saturated concentration of alcohol through mammary papilla.

Vehicle (%)	LT (h)±SD*	F (µg/cm²/hr)±SD*	CA (µg/cm²)±SD*	RA (µg/cm²)±SD*	TAR
Sub-Saturated Concentration					
32	5.53± 0.12	0.48±0.02	22.98±043	782.12±28.43	34.03
48	6.48± 0.34[a]	0.35±0.01[a]	19.76.12±0.54	658.21±19.87	33.31
64	6.39± 0.09	0.68±0.04[a,b]	34.32±0.12	887.32±19.77	25.84
Saturated Concentration					
32	3.28 ±0.12	1.68±0.02	78.34±2.01	918.65±39.87	11.73
48	1.98±0.32[a]	1.78±0.04	82.98±2.09	796.44±19.21	9.60
64	1.48±0.31[a]	2.19±0.02[a,b]	101.27±2.53	998.65±40.12	9.86

Table 5: Permeation parameters of NB MNs (Breast skin).

Vehicle (%)	LT (h)±SD*	F (µg/cm²/hr) ±SD*	CA (µg/cm²)±SD*	RA (µg/cm²)±SD*	TAR
32	2.17±0.32	6.98±0.76	297.65±23.17	99.13±18.76	0.33
MN	0.57±0.12[a]	19.43±0.43 [a]	892.54±26.12	104.32±11.98	0.17

*Standard deviation (n=3).

significant variance from 48% alcohol is indicated by "b" p-value <0.05 indicates that the values are significant.

Mammary papilla

When NB (sub-saturated concentration) was permeated through the mammary papilla using various percentages of alcohol as a carrier, 64% alcohol demonstrated the maximum NB permeation. The full flow was found at 64% alcohol as a vehicle (0.68 g/cm²/hr) out of all the alcohol concentrations examined. The vehicle with the longest LT (6.48 hr) was 48% alcohol. With 64% alcohol, the highest tissue retention of NB was noted. Additionally, the saturation concentration of the NB permeation trials (Table 4) was examined. Compared to 32% and 48% alcohol as vehicles, 64% alcohol as a vehicle had the maximum NB penetration via the mammary papilla. The results from the sub-saturated concentration are similar in that the vehicle with the highest F of NB (2.19 g/cm²/hr) was with 64% alcohol. Similarly, 64% alcohol was shown to have the maximum tissue retention of NB. The values are significant at a p<0.05, and the data is presented as mean±SD (n=3).

A substantial difference from 64% alcohol is indicated by "a" and a significant difference from 48% alcohol is indicated by "b" p-value <0.05 indicates that the values are significant.

For saturated concentrations, 32, 48, and 64% hydroalcoholic vehicles were used, respectively, 30, 80, and 170 mg/mL. A substantial variance from 32% alcohol is indicated by "a" and a significant variance from 48% alcohol is indicated by "b" p-value <0.05 indicates that the values are significant.

Effect of MNs on NB permeation

MNs were exclusively tested on breast skin since the mammary papilla contains built-in ductal apertures for medication delivery. The 32% alcohol was tested with microneedles because it had the greatest penetration into the breast skin. MNs with 32% alcohol dramatically boosted the quantity of NB that permeated breast skin, increasing the total amount by 2.9-fold and decreasing the lag time by 3.6-fold (Tables 5 and 6). After pretreatment with microneedles, the amount of skin retention did not significantly differ.

To calculate the tissue affinity ratio, RA was divided by CA. It contained 10 mg/mL of the drug. Data is displayed as mean±SD (n=3). The letter "a" denotes a considerable departure from 32% alcohol. When the p-value is less than 0.05, the values are significant.

MTT Assay

A microneedle formulation is used for local administration of drugs and treatment for breast cancer treatments. So, the Microneedle formulation was tested for breast cancer cells. Using the MTT assay with free NB concentrations of 100, 200, 300, 400, 500, 600, 700, and 800 nM and the optimized formulation F3, the cell survival (%) was determined. Figure 3 overviews the concentration-dependent cell survival results (%). At an

Table 6: Neratinib delivery rate with MNs through breast skin.

Formulation	C_{24} (µg/cm²)±SD*	F(µg/cm²/hr)±SD*	Area (cm²) required to del3iver 1 mg in 24 hr (1000/Q24)	DR (µg/hr) per g of tissue
32% alcohol (Nipple)	197.32±9.43	7.56±0.13	3.87	2.16
MN+32 % alcohol (skin)	387.45±28.32	18.65±1.09	1.89	5.32

*Standard deviation (n=3).*The average human breast is 200 cm², hence the delivery rate estimates are computed as the flow *200cm²/700 g of tissue. Microneedles-MN.

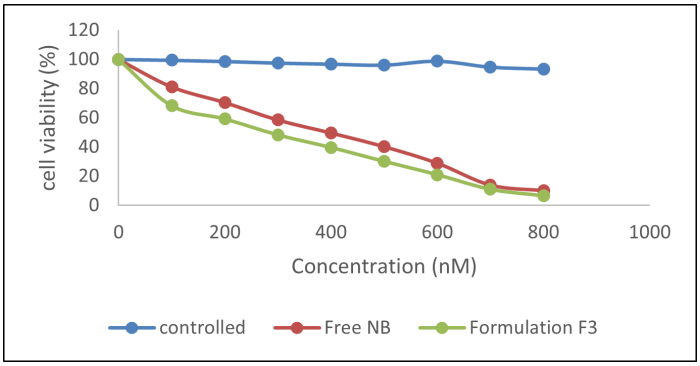

Figure 3: Concentration-dependent cell viability (%) curve for free NB, control, and optimized formulation F3 on MCF-7 human breast cancer cells.

exposure level of 800 nM, the maximal cytotoxicity of free NB was determined to be 89.13±0.7809%. However, the quantity of NB (800 nM) in optimized formulation F3 demonstrated 93.87±0.3490% cytotoxicity, which was shown to be significant than free NB (p<0.05). Overall, it was discovered that NB in an optimized formulation F3 was significantly more effective on MCF-7 cells than free NB. The concentration-dependent cell viability curves were also used to determine the concentrations of free NB and the optimized formulation F3 that cause 50% of cell death (IC_{50}) (Figure 3). It was determined that the free NB IC_{50} value was 379.32 nM. However, it was discovered that NB's IC_{50} in formulation F3 was 259.87 nM. The IC_{50} value of the improved formulation F3 was much lower than that of free NB. Based on these findings and outcomes, it was determined that NB in the optimized formulation F3 was more effective than free NB.

DISCUSSION

The current study aimed to create and assess transdermal microneedles to deliver the anti-cancer medication Neratinib (NB). Microneedles are a promising therapy for treating, managing, and preventing breast cancer because they target chemo preventive drugs to the mammary papilla or breast surface. Neratinib (NB), a tyrosine kinase inhibitor, was used to stop the growth of cell lines that express high amounts of HER-2 (3T3/neu, SK-Br-3, and BT474). It is far less effective against cell lines that do not express HER-2 or EGFR.[16] This study aims to better understand the effects of varying alcohol concentrations as a permeation enhancer and delivery system for targeted transdermal medication administration to the breast utilizing microneedles.[17]

Pre-formulation studies were conducted to understand the distinctive qualities of the medicine and the excipients. The FT-IR spectra revealed that the distinct peaks of NB in formulation F3 and pure NB did not change significantly. However, in formulation F3, the intensity of the NB's distinctive peaks was diminished. This finding revealed that the NB in formulation F3 may be partially amorphous, which may improve the NB's solubility and rate of dissolution when compared to pure NB. The solubility tests revealed that the solubility of NB increased as the amount of alcohol in the vehicle rose. When compared to 32% alcohol, the solubility of NB rose by 2.3-fold with 48% alcohol and by 5.2-fold with 64% alcohol. According to studies on the epidermal partition coefficient, the EPC of NB decreased when alcohol concentration in the vehicle increased. Compared to 32% alcohol, 48% alcohol reduced the partition coefficient of NB by 1.4 times, and 64% alcohol reduced it by 2.1 times.

The photo-polymerization approach was used to create the microneedle patches (MNPs) using PEGDA and TPO as biopolymer and photo initiator, respectively (158). Variations in the process parameters, such as light intensity, concentration of PEGDA and TPO, and exposure time, can regulate the complete photo-polymerization reaction. The PµSL system, specifically created by our research team and is today marketed as SUKSHM 3D® with the tagline "micro fast prototyping," was used for the whole fabrication process. Stereomicroscopy was used to describe the manufactured MNPs and revealed that the microneedles were perpendicular to the MNPs. According to the SEM characterization, the height of a single MN is 193.7 m, and the entire base diameter is 237.9 m. The characterization results demonstrated that the MNs' heights were appropriate for the proposed investigation. Compression analysis was used for the mechanical testing, and eight film layers were used for the insertion investigation. Less than 30% of the needles and more than 30% of the needles penetrating the third layer, respectively. Penetration is effective when more than 20% of each film layer

has holes.[18] Therefore, the MNPs of the current investigation were inferred to be located between the third and fourth film layers.

The most significant penetration through the breast skin was seen in a vehicle with 32% alcohol content, % alcohol showed the maximum flux (6.98 g/cm^2/hr), and 64% alcohol showed the lowermost flux among various percentages of alcohol as a vehicle studied. Studies on NB permeation's saturation concentrations were put to the test. Compared to 48 and 64% alcohol, the 32% alcohol vehicle had the maximum NB penetration through the breast skin. The vehicle with the highest NB flux (11.98 g/cm^2/hr) was 32% alcohol. With 64% alcohol, the highest skin retention of NB was noted. The highest rate of NB penetration was seen when 64% alcohol was used as the vehicle in investigations on the permeation of NB through the mammary papilla. The highest flux was found at 64% alcohol as a vehicle (0.68 g/cm^2/hr), out of all the alcohol concentrations examined. The car with the longest lag (6.39 hr) was 48% alcohol. With 64% alcohol, the highest tissue retention of NB was noted. NB permeation studies' saturation concentration was also put to the test. Compared to 32% and 48% alcohol as vehicles, 64% alcohol as a vehicle had the maximum NB penetration via the mammary papilla. The results from sub-saturated concentration were similar in that the vehicle with the maximum flow of NB (2.19 g/cm^2/hr) was 64% alcohol.

Similarly, 64% alcohol was shown to have the maximum tissue retention of NB. Microneedles have only been tested on breast skin. The amount of alcohol that penetrated the breast skin the most, 32%, was evaluated using microneedles. The amount of NB that permeated through the breast skin cumulatively increased by 2.9-fold, and the lag time decreased by 3.6-fold when administered by microneedles containing 32% alcohol.

The IC_{50} value of NB in an optimized formulation F3 was determined from the MTT assay on MCF-7 cell lines and was found to be 259.87 nM. The IC_{50} value of the improved formulation F3 was much lower than that of free NB. Based on these findings and outcomes, it was determined that NB in the optimized formulation F3 was more effective than free NB. When NB was injected into 4T1 tumor-bearing mice, *in vivo* effectiveness studies revealed that the tumor volume grew till the 6th day.

Then, a reduction in tumor size and weight of animals was seen, and by day 9, the animals had passed away. The tumor volume in the NB microneedles group rapidly decreased until all the animals perished on day 11. The control animal group scarcely exhibits any apoptotic nuclei. Both the intra-tumoral injections and the MN groups had apoptotic nuclei. Notably, the NB injection and NB-MNs group demonstrate more apoptotic cells. This apoptotic pattern aligns with the outcomes of tumor inhibition *in vivo*. Hematoxylin and eosin staining of tumor sections was used to determine the effectiveness of the new formulation's tumor inhibition. In the NB injection and NB-MNs treatment, particularly the NB intra-tumoral injection and NB-MNs group, we observed a decline in cell density, a loss of nuclear stain intensity, cell shrinkage, the emergence of spindle-shaped nuclei, clumping of the nucleoplasm, and even the elimination of cells. Necrotic regions also appeared. It was determined that the MN-based administration is an efficient method for treating and caring for breast cancer based on the positive findings from the research.

CONCLUSION

The MN-based administration is an efficient method for treating and managing breast cancer, according to the studies' promising outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LT: Lag time; **F:** Flux; **CA:** Cumulative Amount; **RA:** Retained Amount; **TAR:** Tissue Affinity Ratio; **DR:** Delivery rate; **C_{24}:** Cumulative amount at 24 hr.

SUMMARY

The objective of this research was to examine the viability of transdermal distribution of the promising tyrosine kinase inhibitor Neratinib (NB) and its physicochemical properties through the mammary papilla and breast skin. The goal of this study was to better understand how alcohol at various concentrations affects penetration and how it can be delivered locally to the breast via a transdermal route utilizing microneedles. It was determined that the MN-based administration is an efficient method for treating and caring for breast cancer based on the positive findings from the research.

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