LONG-LASTING LEVOTHYROXINE SODIUM MICRONEEDLE PATCH FOR HASHIMOTO'S THYROIDITIS TREATMENT

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ABSTRACT

This paper reports first-of-its-kind, long-lasting treatment technology using transdermal, minimally invasive, biocompatible, dissolving Polyethylene Glycol Diacrylate (PEGDA) microneedles (DMNs) loaded with levothyroxine sodium (LT4) hormone for Hashimoto's Thyroiditis (HT) treatment. This promising solution ensures the continuity of treatment, controlled dosage, sustained release, and efficient drug administration without absorption limitations. Additionally, a COMSOL model of the MN dissolution is demonstrated. The microneedle array has been engineered to provide sustained treatment for over 10 days (modeling/*in vitro* studies; *in vivo* experiments planned). Successful patch implementation could potentially alleviate patients' need to rely on daily synthetic hormone pills, offering a more convenient and precise therapeutic alternative.

KEYWORDS

Dissolvable Microneedles, Levothyroxine, Hashimoto's Thyroiditis treatment, COMSOL Modeling, Sustained Drug Release.

INTRODUCTION

Thyroid disease is the second most prevalent endocrine condition affecting women—an incidence of 3.5 per 1000 women per year. Traditional Hashimoto's Thyroiditis treatment involves oral medication that must be consumed daily, which can result in inconsistent hormone levels and side effects **[1]**. Current treatment comprises levothyroxine at the recommended dose of 1.6 to 1.8 µg/kg/day. LT4 is the synthetic option for T3, the hormone produced by the thyroid gland. The human body synthetizes and transforms LT4 to T3. Excessive hormone administration can lead to deleterious and morbid effects, such as arrhythmias, and osteoporosis **[2]**. HT is frequently associated with gastric disorders in between 10% to 40% of patients with the disease **[3]**. Additionally, celiac disease, atrophic gastritis, lactose intolerance, and pyloric infection in Helicobacter are some of the diseases that could limit the oral absorption of LT4 in patients **[4]**. Alternatively, the injection route for levothyroxine is used only in hospitals if the patient presents myxedema coma (severe hypothyroidism) **[5]**. Dissolvable Microneedles (DMNs) with LT4 embedded in the dissolving polymer matrix offered in the form of a patch could be a promising solution to all these difficulties. DMNs offer a more efficient drug delivery mechanism to different anatomical sites, including the transdermal delivery of drugs of varying sizes **[6,7]**, intracellular delivery, and ocular drug delivery. In this work, the proposed dissolving drug-loaded DMNs are microfabricated from a hydrogel matrix mixed with the hormone. The polymer used is PEGDA, a biocompatible hydrogel **[8]**, which has a composition that can be used to modify drug release properties with an ideal matrix for delivering levothyroxine sodium and could result in a highly controlled and sustained drug release alleviating issues with oral administration of the drug. Acrylate presence in PEGDA allows the material to be crosslinked producing large mesh size capable of storing the hormone **[9]**. This method further overcomes issues associated with the percentage of prescribed doses of the medication taken by the patient, oral limitation pill absorption, and non-adherence in 92% of treatments **[10]**.

MATERIALS AND METHODS

Materials

The materials used in this paper: L-Thyroxine sodium salt pentahydrate (LT4) (purity: ≥ 98%; HPLC, powder) was obtained from Sigma-Aldrich (USA); PEGDA was obtained from Sigma-Aldrich (USA); Phosphate-Buffered Saline (PBS) was obtained from Fisher Scientific (USA), and EcoFlex™ 00-30 was obtained from Amazon (USA).

COMSOL Multiphysics Model Development

Control drug release devices are commonly fabricated to maintain the delivery of drugs for a specific amount of time. This release can occur through two processes: surface erosion and bulk erosion. In surface erosion, the drug-loaded device starts degrading from its external surface to its internal structures over time, changing its shape and size. Conversely, bulk erosion uses water to diffuse into the polymeric matrix, changing external and internal surfaces equally **[11]**. The developed Multiphysics model uses surface erosion to simulate the DMN's degradation and shape change inside the skin. To simulate the drug diffusion into skin, a cylinder with a 500 µm radius and 1185 µm height was designed to emulate the skin dimensions using COMSOL software as shown in **Figure 1**. For skin properties, the following was used: porosity of 70%, 0.48 for Poisson ratio, and Young's modulus of 8 MPa **[12,13]**. In addition, a DMN design (singular MN with a cone geometry of 120 µm radius, 700 µm height, and 7 µm tip radius) was used. The material properties used for PEGDA DMN are: dynamic viscosity of 5.373 Pa*s, a density of 1.12 g/ml, a diffusion coefficient of $4.86 \times 10^{-9} \pm 1.86 \times 10^{-12}$ cm^2/s, Poisson ratio of 0.5, and Young's modulus of 20 MPa **[14-17]**.

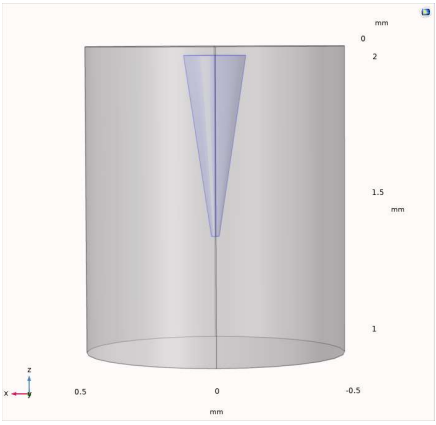

Figure 1: *COMSOL 3D model of microneedle and skin to simulate levothyroxine diffusion rate within the skin and PBS. A skin cylinder of 500 µm radius and 1.185 mm height coupled with a microneedle of 700 µm height and 120 µm radius are observed. The same 3D model dimensions were used for in-skin and in-water dissolution simulations.*

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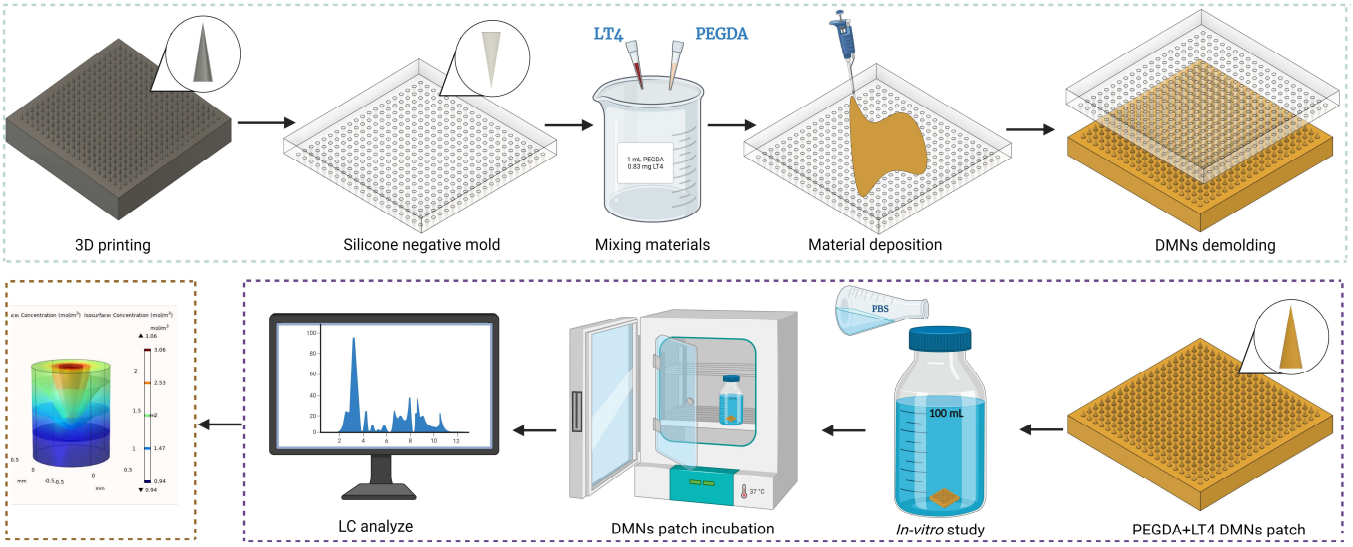


***Figure 2**: Schematic fabrication process of a 3D dissolvable microneedle patch CAD design of a 1.5 cm² base for a matrix of 20x20 microneedles (H: 700 µm; R: 120 µm). In an in-vitro study, the MNs array was submerged in 100 ml of PBS. The microneedles were incubated at 37ºC for 4 days. Experimental results were compared with in-water microfluidic computational simulation. Created with BioRender.com*

Uniquely PEGDA was used for DMNs due to its material properties and prior utilization in dissolving matrices in the body. Further levothyroxine represents only 0.0741% of the total DMN weight. After the two objects were built in COMSOL, they were assembled using the "union" function, and the singular DMN was placed completely inside the skin for a greater contact surface area between the needle and the skin. The assembly included just one DMN instead of a complete patch because of the similar behavior assumption of all DMNs in the patch. Finite Element Modeling (FEM) evaluating the transport of diluted species in porous media using COMSOL software was performed to predict the dissolution time of the DMN patch. Additionally, in-water and in-skin models were simulated. The first model was initiated using skin as the solvent, after, in-water was simulated to validate the DMNs dissolution and compare it with experimental results obtained from dissolution in PBS. Assuming the skin model is isotropic, diffusion was simulated through Fick's first and second laws and Millington & Quirk's effective diffusivity model **[18]**.

Microfabrication Process Development

Figure 2 shows the schematic process flow for DMN development depicting a 1.5 cm^2 microneedle patch that was designed using Fusion360 (Autodesk), and subsequently 3D printed in high-temperature resin (FormLabs) (exposure: 8s; intensity: 30-34.13 mJ/cm^2; layer thickness: 5-50 µm). The 3D printed master mold was fabricated with an ASIGA Max X UV385, followed by an IPA bath sonication for 15 min and UV curing at 60ºC for 15 minutes. Subsequently, the master mold was sputter coated with gold (Quorum Technologies Q150) and used to fabricate silicone (EcoFlex™ 00-30) negative mold cured at standard room temperature for 4 hours prior to DMN micromolding. Micromolding fabrication process involved mixing 1 mL of PEGDA with 0.83 mg of levothyroxine sodium (LT4; initial concentration of 3.32 mol/m^3) followed by casting on the negative micromold. The EcoFlex+PEGDA DMNs patch complex is cured in the oven for 1 hour at 150ºC. A surface profile was obtained after demolding the PEGDA DMNs patch using Confocal Microscopy (**Figure 3**). ***The microneedles had an experimental height of 665 µm and a 21 µm tip radius***. The small difference compared to design values is due to material losses during the demolding process.

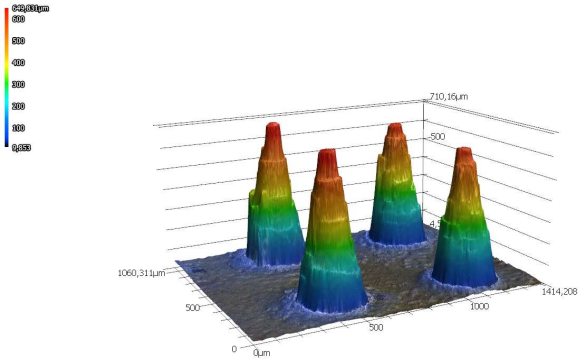
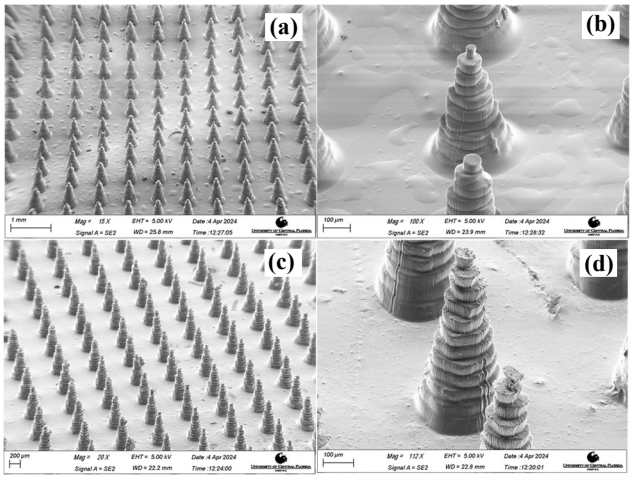


***Figure 3:** Laser confocal characterization of PEGDA MNs loaded with LT4 hormone. A height of 665 µm and a tip radius of 21 µm were obtained.*



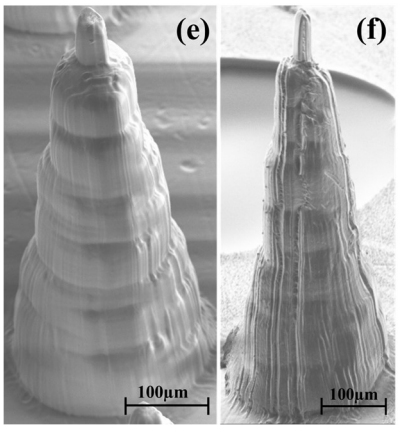


Figure 4: *(a): Scanning electron microscope (SEM) image of 3D printed 20x20 microneedles array resin master mold. (b): SEM close-up of a singular high-temperature resin microneedle. (c): SEM images of the PEGDA microneedle patch loaded with LT4. (d): SEM image of a single PEGDA microneedle loaded with LT4 hormone. (e): SEM 3D printed MN with 5 µm layer thickness. (f): SEM DMN with 5 µm layer thickness.*

Figures 4 (a-b) depict SEM images of the 3D-printed master mold array and magnified single DMN with a light intensity of 34.13 mJ/cm^2 and layer thickness of 50 µm. **Figures 4 (c-d)** showcase SEM of the microfabricated DMN patch of PEGDA loaded with LT4. **Figure 4 (e)** illustrates a singular 3D printed MN in the master mold with a light intensity of 30 mJ/cm^2 and layer thickness of 5 µm. Lastly, **Figure 4 (f)** showcases an SEM image of a singular PEGDA DMN with an optimized tip.

In-Vitro Release Study

In-vitro release experiments involved incubating the DMNs patch at 37ºC in a closed beaker with 100 mL PBS for 4 days collecting a sample of 1 mL every 24 hours and replacing it with 1 mL of fresh PBS **[19, 20]**. Using a liquid chromatography method, described in the following section, it was possible to detect and analyze the concentration of LT4 in the *in-vitro* study.

Liquid Chromatography Method

The analysis of levothyroxine was performed following previously reported methods **[21, 22]** with some modifications. Quantification of LT4 was determined using an Agilent 1260 Infinity coupled with a Time-of-Flight mass spectrometer (6230 Q-TOF, Agilent Technologies Inc.). A zorbax RX-C18 column (5 µm, 4.6 x 150 mm, Agilent Technologies Inc.) was used at a set temperature of 25 °C. The injected volume was 5 µL with a flow rate of 0.70 mL/min was used as a gradient mobile phase of eluent A (water with formic acid 0.1%) and eluent B (methanol with formic acid 0.1%) for 10 minutes of total run time. The LC mobile phase gradient started with 20% of eluent B which was maintained for 1.2 min and then ramped to 60% until 2.5 min. Subsequently, it ramped to 80% until 6.2 min and increased to 100% until 8.5 min. Finally, it was returned to 20% at 10 min. The mass spectrometer was operated under positive ionization mode with a dual electrospray ionization source (ESI). The nebulizing, drying, and collision gas used was nitrogen and the ESI settings were gas temperature 350 °C, capillary 5500 V, drying gas 10 L/min, nebulizer 35 psi, fragmentor 175 V, and mass range 100 to 3000 m/z. The quantification analysis was performed using the Mass Hunter workstation Qualitative Analysis Software version 10.0 (Agilent Technologies Inc.). A calibration curve was prepared using Levothyroxine at a concentration range from 100 to 1000 ppb with extracted ion chromatograms (EIC) mode.

RESULTS AND DISCUSSIONS

Figures 5 (a-b) collectively illustrate a computed temporal concentration progression of DMN dissolution within the skin model. ***Inside of 24 hours, the patch delivered 0.7 mol/m^3 constituting 21.08% of the total concentration***. In **Figure 5 (b)**, ***the COMSOL simulation reveals a concentration of levothyroxine sodium amounting to 3.3 mol/m^3 (99.4% of total concentration) after 10 days***. A comparative analysis between the computational in-water 3D model results and experimental PBS findings serves to validate simulated results of the penetration of LT4 into the skin. **Figures 5 (c-d)** show the in-water dissolution model. **Figure 5 (c)** depicts the release of a ***solution concentration at 24 hours of 0.03 mol/m^3 which indicates a 2.2*10^{-5} mol/m^3 LT4 concentration***. In contrast, the liquid chromatography results for the PBS experiment showed no perceptible LT4 (0 mol/m^3) at 24 hours. However, ***for the 3D in-water model at 3 days the concentration of LT4 was 3.1*10^{-4} mol/m^3 in comparison with 2.7*10^{-4} mol/m^3 for the PBS in-vitro experiment resulting in a standard deviation of 0.2828 mol/m^3***.

Experimental results of PBS after 4 days showed a decrease in hormone concentration from 3.3*10^{-4} mol/m^3 to 1.18*10^{-4} mol/m^3 in 24 hours because of degradation during the incubation. ***After the sixth day of the PBS in-vitro study, the concentration remained unchanged which indicates the hormone completely degraded***. Consequently, an *in-vitro* study adding bovine albumin serum (BSA) to the solvent (PBS) will be performed in a future study to avoid hormone degradation during the incubation **[23]**. **Figure 6** depicts the concentration changes over four days predicted by the computational model employing water as solvent and comparison with values obtained with PBS *in-vitro* release experiment depicting similar trends of release.

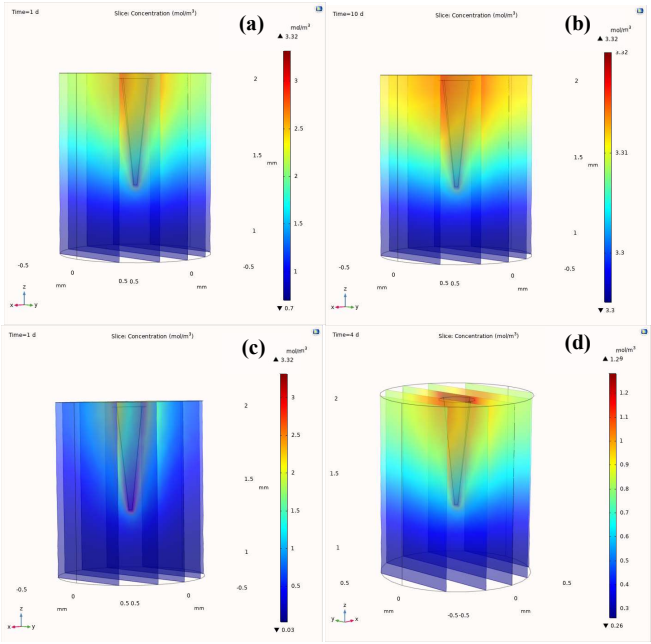


Figure 5: *Transport of diluted species in porous media using COMSOL. (a): PEDGA MNs dissolution in skin model at 24 hours (concentration: 0.7 mol/m^3). (b): Computational simulation of PEDGA MN dissolution in skin model at 10 days (concentration:3.3 mol/m^3). c): Simulation of PEGDA needle in water to validate COMSOL model and emulate experimental dissolution with PBS at 24 hours. d): PEGDA in-water 3D model microneedle for four days.*

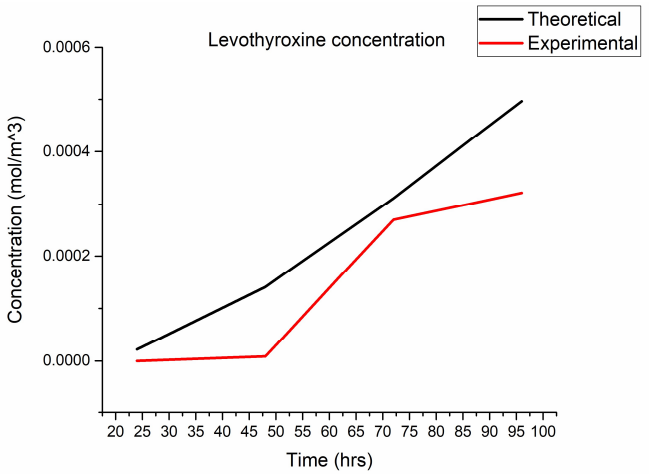


Figure 6**:** *Graph of levothyroxine concentration in liquid chromatography analysis of MNs patch immersed in PBS for 4 days (experimental, N=1 at this stage; in the process of increasing N) and computation model simulated in water solution (theoretical). The difference between values is due to changes in PBS and water solvent characteristics as the hormone degrades due to temperature (37℃) during incubation.*

CONCLUSIONS

This study presents for the first time a long-lasting dissolving microneedles patch for Hashimoto's Thyroiditis. Microfabrication process development resulted in the successful demonstration of hormone integrated PEGDA DMNs using a micromolding process with 3D printed master molds and an Eco-Flex silicone negative mold. Computational results predicted by COMSOL indicate an LT4 sustained release in the skin for 10 days. However, experimental results show a dissolution in PBS of four days attributed to degradation of hormone. Future works could pursue *in-vitro* studies using hormone stabilizers to avoid degradation and *in vivo* studies in mouse models.

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