Delivery of Denosumab via Hollow Microneedle

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

This experiment has the ethical permission for conducting the research on animal (mice).

Abstract

The emergence of transdermal drug delivery via hollow microneedle can be used to enhance the distribution of medications delivered through the skin. In this current study, *in-vivo* animal studies of denosumab were carried out which involved the use of hollow microneedle to draw a comparison with the painful injection for the treatment of osteoporosis. The *in-vivo* study of the hollow-microneedle mediated delivery of denosumab, a monoclonal antibody was conducted to investigate if there is any possibility of improvement in osteoporosis, which affects a significant segment of post-menopausal women. Hollow microneedles mediated denosumab deliveries showed a remarkable improvement as compared to traditional injections. In recent times, hollow microneedle has managed to receive special attention as a novel drug delivery system due to its nature of being minimally invasive across the skin. In conclusion, the hollow microneedles may prove to be of great assistance for widening the feasibility of delivery of a wide range of therapeutic entities.

Keywords: Transdermal; Hollow microneedle; Osteoporosis; Denosumab; Skin.

Dedication

I want to dedicate this project to my respectable supervisor Dr. Md. Jasim Uddin, Assistant Professor in Department of Pharmacy, Brac University for his continuous guidance throughout my project.

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I would like to proceed by thanking the Almighty who is the source of our strength and knowledge which have enabled me to complete this project with full diligence.

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3D	Three-dimensional
MN	Microneedle

Chapter 1

Introduction

The oral administration is the preferred method as a conventional drug delivery system. However, when a drug is administered orally, sometimes it may not be feasible enough to generate a fast therapeutic response due to some limitations (Zhu, Wang, Liu, & Guo, 2016). However, poor absorption of drugs and rapid enzymatic degradation in the gastrointestinal tract or liver are some of the reasons behind this low feasibility of oral administration (Prausnitz, 2017). The use of hypodermic needles can be helpful to overcome these limitations. Even though, the use of hypodermic needles in the treatment was successful in improving the absorption of drugs, it has quite a number of limitations, such as, induction of pain and invasiveness and production of bio-hazardous wastes. The administration through these pain-inducing needles also requires training (Yun, Kim, & Lee, 2017). At present, to solve the problems associated with these two methods, extensive research is going on in the arena of transdermal drug delivery system for delivery of drugs (Ma & Wu, 2017). However, the main limitation of transdermal drug delivery route is less permeability of drug molecules through the stratum corneum (Sharma, Hatware, Bhadane, Sindhikar, & Mishra, 2019). Stratum corneum is formed with 15-20 corneocytes layer having a thickness of 10-15 μm (Ita, 2015). The skin is basically a three-layered structure, the outermost of which is the epidermis, the middle layer is the dermis and the innermost is hypodermis (Waghule et al., 2019). Beneath the stratum corneum, the avascular layer epidermis is present which is 50-100 μm thick. The stratum corneum and the outermost epidermis together form the full epidermis layer. Then next comes the dermis layer having a depth of 4 mm which consists of hair follicles, lymph vessels, sweat glands and nerve endings. Below the dermis, lies the hypodermis comprising fat micro-lobules and fibrous collagen, it also contains blood vessels, lymphatics and nerves having a thickness of up to several millimeters (Ita, 2015). As the

stratum corneum acts as a major barrier by allowing only the lipophilic and low molecular weight drug molecules (less than 500 Da), so researches are being conducted throughout the world to improve the permeation of drugs across the skin with a view to reducing difficulties in designing topical formulations(Marshall, Sahm, & Moore, 2016). During the administration of therapeutic entities across the stratum corneum, scientists have come up with various strategies to increase the permeability (Milanetti, Raimondo, & Tramontano, 2016). Among these approaches, microneedle mediated delivery has emerged as an evolving area in transdermal drug delivery system (Pandey, Shukla, Skoog, Boehm, & Narayan, 2019). To make the delivery of macromolecules and hydrophilic drugs from the skin into the systemic circulation more efficient, microneedle technology is currently a great technique (Alhnan et al., 2016). Studies are going on all over the world to make the utilization of microneedle mediated delivery more efficient so that in the near future, it leads to a ,it leads to widespread acceptance among the people(Konta, García-Piña, & Serrano, 2017).

1.1 Microneedle

Microneedles are basically micron-scale needles having the lengths within the range of 50-900 µm designed to improve the permeation of drugs across the rate limiting layer of stratum corneum (Bodhale, Nisar, & Afzulpurkar, 2010). In the 21st century, this delivery tool is drawing attention to expand the sectors of application in the pharmaceutical and biomedical sciences (Amodwala, Kumar, & Thakkar, 2017).

1.2 Mechanism of microneedle mediated delivery

The needles embedded in microneedles have sharper tips in comparison to the hypodermic needles which allow the disruption of stratum corneum, the major barrier. This disruption leads to the formation of micro-scale delivery channels without interrupting the nerve fibres and blood vessels located in the outermost epidermis and vascular dermis layer (Lane et al.,

2018). As a result, the enhancement of delivery efficiency is possible through the transportation of drugs via this delivery tool (Ma & Wu, 2017).

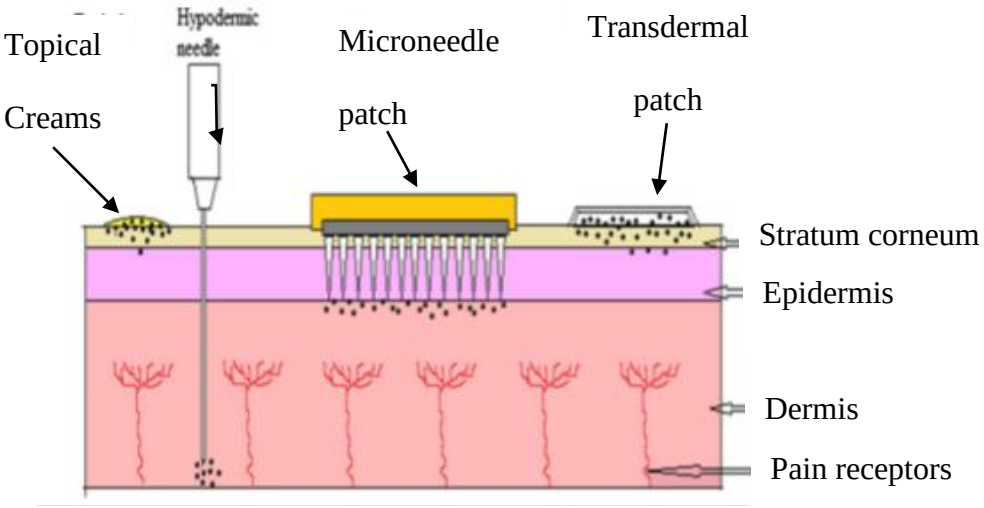

Figure 1:Comparison of topical cream, hypodermic needle, microneedle patch and transdermal patch (Waghule et al., 2019).

In Figure 1, it clearly shows that the mechanism of delivery of microneedles doesn't involve the pain receptors. This is why; it doesn't induce pain unlike hypodermic needles. This diagram also shows that the microneedle mediated delivery ensures high bioavailability on reaching the site of action unlike the formulations designed for topical use. The drugs, when administered through the microneedles, are placed directly on the layer of epidermis or upper epidermis from where it gets into the systemic circulation (Waghule et al., 2019).

1.3 Different parameters for the classification of microneedles

The classification of microneedles is based on a number of factors:

- **Applications**: diagnostic tool, cosmetic tool, therapeutic tool.
- **Structure**: hollow, coated, solid, dissolving.
- **Techniques for fabrication**: dry etching, micro molding, lithography, wet etching.

▪ **Shape of the tip**: cylindrical, volcano, snake fang, canonical, micro hypodermis, cylindrical.

▪ **Use of material**: polymers, glass, silicon, metals.

▪ **Shape**: cylindrical, canonical, candle, pyramid Spear, Square, spike, hexagonal, octagonal, rocket, pentagonal, star (Sharma et al., 2019).

For fabricating different types of microneedles, the wide variation of materials is used (Nejad, Sadeqi, Kiaee, & Sonkusale, 2018). The substances that are involved in developing a microneedle should satisfy the characteristics stated below:

- ✓ bio-compatibility
- ✓ cost-effective
- ✓ easy to use
- ✓ availability
- ✓ high durability
- ✓ non-corrodible
- ✓ good mechanical strength (Jeong, Lee, Choi, & Park, 2017).

1.4 The fabrication materials for different types of microneedles

The extensive variety of materials ranging from metal to polymers is utilized for fabricating different types of microneedles (Nejad, Sadeqi, Kiaee, & Sonkusale, 2018). The materials which are used in the development of microneedles should meet the following characteristics (Jeong, Lee, Choi, & Park, 2017):

- ✓ compatibility
- ✓ economic
- ✓ user friendly

- ✓ easily available
- ✓ high tensile strength
- ✓ non-corrosive
- ✓ good mechanical strength.

1.4.1 Silicon

For the first time, in the 1990s, microneedle was made using silicon. The time-consuming complex process of fabrication and the high cost limits its use in microneedle (Nejad et al., 2018). Moreover, there are biocompatibility issues due to brittle nature of silicon, some part may break and remain in the skin causing health hazards (Sharma et al., 2019).

1.4.2 Metals

Metals exhibit strong mechanical properties and good biocompatibility. These metals are tougher and less fragile than silicon which make these microneedles more convenient in comparison to silicon (Chen, Chen, Wang, Jin, & Guo, 2017). In the production of microneedles, stainless steel was the first used metal (Sharma et al., 2019). Titanium is preferred over stainless steel(Kearney, Caffarel-Salvador, Fallows, McCarthy, & Donnelly, 2016).

1.4.3 Ceramic

Alumina (Al_2O_3) is mainly used because of its chemical resistance (Boks et al., 2015).

1.4.4 Silica glass

Glass microneedles are not used now commercially, but only for experimental purposes. Borosilicate glass which is made up of silica and boron trioxide is more elastic. The fabrication is done manually, thus are time consuming (Serrano-Castañeda, Escobar-Chávez, Rodríguez-Cruz, Melgoza-Contreras, & Martínez-Hernández, 2018).

1.4.5 Carbohydrate

Though carbohydrates are safe for the human health and available at a cheap price, they degrade at elevated temperatures making the process of fabrication complicated. The drug-loaded carbohydrate mixture is casted into the moulds to get the microneedles. The drug release is controlled by the time-based dissolution of carbohydrate inside the skin (Waghule et al., 2019).

1.4.6 Polymers

The toughness, excellent biocompatibility, low cost, biodegradability and minimal toxicity are the features of polymers that render them more appropriate for the fabrication process (Nayak & Das, 2013). Fabricating materials like silicon, metals etc. are better than polymers in terms of strength but it is tougher in comparison to glass (Kim et al., 2017). Poly carbonate, PLA (polylactic acid), PLGA (poly lactic-co-glycolic acid), PGA (polyglycolic acid), PMMA (polymethyl methacrylate), PVA (polyvinyl alcohol), PVP (polyvinyl pyrrolidone) etc. are some of the examples of polymers employed for fabrication of different types of microneedles(Donnelly et al., 2011).

1.5 Types of microneedles

Different applications require the use of different types of microneedles. Each category of microneedle has its particular mechanism of action for delivering the drug. The microneedles can be of the following categories:

1.5.1 Solid microneedles

At first, this microneedles is applied on the skin to cause the formation of a pore in the skin(Katsumi et al., 2017).The passive diffusion of drug occurs through the preformed channels(Kearney et al., 2016).

1.5.2 Coated Microneedles

This type of microneedles is enclosed with dispersion layer or a drug solution which causes faster drug dissolution. This facilitates the delivery to a great extent (Rajabi et al., 2016).The exploration of this type of microneedles is evident in DNA, drug delivery, bio-molecules and vaccines (Pandey et al., 2019).

1.5.3 Dissolving/biodegradable microneedles

This type of microneedle is a better choice for continuous therapy due to biodegradability which makes it a widely accepted category of microneedle (Baek, Shin, & Kim, 2017). It is a one-step process of application and so it requires no physical removal. Effective needle drug distribution is one of the challenges encountered with the use of dissolving microneedles (Mistilis, Bommarius, & Prausnitz, 2015).

1.5.4 Hydrogel-forming microneedles

This category of microneedles has been developed lately which requires the use of super swelling polymers. The polymers make up the hydrophilic structure (Kearney et al., 2016). When inserted into the skin, interstitial fluid cause these polymers to swell up (Ita, 2015). Eventually, channels are formed between the drug patch and capillary circulation (Cao et al., 2019).

1.5.5 Hollow Microneedles

Usually, this type of hollow microneedle is used for delivering liquid formulation through the subcutaneous layer of the skin (Nguyen et al., 2019). In comparison to the other types of microneedles, these microneedles allow the administration of larger doses of the drug. These microneedles have an empty space positioned inside that can accommodate large amount of drug solution or dispersion. Using various techniques like pressure, electrical assistance or diffusion, delivery of the drug substances is possible continuously through these type of

microneedles (Sharma et al., 2019). If the drug is to be given by a rapid bolus injection, the release pressure and drug flow rate can be controlled (Katsumi et al., 2017). Using a variety of materials including ceramic, silicon, glass, metal and polymers, the fabrication of hollow microneedles is done (Mansoor et al., 2015). Commonly, for inserting proteins, antigens, oligonucleotides and other high molecular weight substances, this hollow microneedle is a good choice (Lyon, Aria, & Gharib, 2014). The major challenge encountered here is the probability of clogging of needle openings when the skin is pierced and thus the flow of drug is hindered(Waghule et al., 2019).

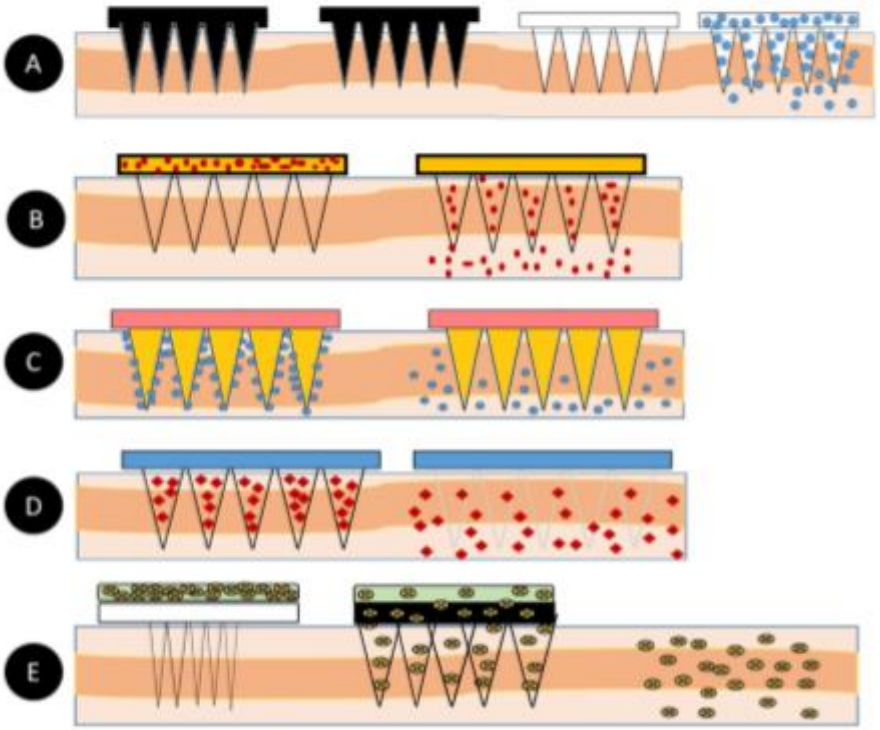

Figure 2:A-Solid microneedles, B-Hollow microneedles ,C-Coated microneedles ,D: Dissolving microneedles, E: Hydrogel forming microneedles (Sharma et al., 2019).

In Figure 2, it shows the method of drug delivery for each kind of microneedle is different. The drug that is delivered gets attached to the microneedle in various ways depending on the type of microneedle. In Table 1 it shows that each microneedle has its own strengths and limitations but the number of benefits derived from the microneedles outweighs the limitations.

Table 1:Benefits versus limitations of different microneedles (Sharma et al., 2019).

Serial No.	**Microneedle type**	**Materials used for fabrication**	**Benefits**	**Limitations**
1.	Solid	Silicon, stainless steel, acrylic	High mechanical strength	• Two step process. • Poor patient compliance.
2.	Hollow	Silicon, metal ,glass, ceramic and polymers	• Large administration of dose is possible. • Can be used for large molecular weight substances. • Single step process	• Chances of needle blockage • Critical fabrication process • Costly
3.	Coated	Stainless steel, titanium, polymer	Single step process	Limited drug can be coated.
4.	Dissolving	PVP,PLGA,PVA	• Easy fabrication process • Low cost	• High temperature is needed which may affect payload. • Chances of polymer deposition in skin.
5.	Hydrogel-forming	PVA,PLGA	• Intact removal is possible	• Less mechanical strength

1.6 Advantages of hollow microneedles

The use of hollow microneedles is preferred over other forms of delivery for various reasons. As the depth of penetration of microneedles is limited (typically <500 μm), so the piercing of the skin in a minimally invasive way is possible (Dragicevic & Maibach, 2017). Apart from this, a major advantage derived from hollow microneedle is, it allows large administration of doses. Large molecular weight substances can also be injected through it (Li, Zeng, Shan, & Tong, 2017). As it is a single step process, the patient compliance is better when drug is transported through the microneedles, as a result it is considered to be a better choice over the other types (Marshall et al., 2016). The onset of action is also fast which manages to have sufficient bio-availability on reaching the site of action (Waghule et al., 2019). The microneedle mediated delivery doesn't require the assistance of others during the administration of drugs (Zhu et al., 2016). So, the self-administration allows the patient to be adherent to the treatment regimen without being dependent on others (Cao et al., 2019).

1.7 Limitations

Even though the transportation of drugs through the microneedle is quite advantageous, it has a few limitations as well. In case of sensitive skin, there is the chance of skin allergy or irritation (Linec & Mušič, 2019). As the size of the needle is very small and in terms of thickness, it is even thinner compared to hair, breakage of the tips of microneedles may occur which can cause biocompatibility problems if these remained inside. There are few limitations which can be counteracted with advanced selection of materials while developing microneedles (Waghule et al., 2019).

1.8 Applications of hollow microneedles

The application of hollow microneedles to deliver drugs is evident in various aspects of biomedical advancement due to its non-invasive nature, multi-faceted use and prime selectivity (Xu et al., 2019). Furthermore, currently, research on hollow microneedles is going on to expand the range of applications to a greater extent, such as: vaccine therapy (Arya & Prausnitz, 2016; Mansoor et al., 2015; Fernando et al., 2016), peptide delivery (Gupta, Felner, & Prausnitz, 2009; Sohn et al., 2016),targeted delivery of phenylephrine for the treatment of fetal incontinence (Jun, Han, Kang, Park, & Park, 2015),hormone delivery (Davis, Martanto, Allen, & Prausnitz, 2005; Sharma et al., 2019),cosmetic ingredients (Yang et al., 2019),lidocaine therapy (Yu, Tay, Guo, Xu, & Yap, 2009;Baek et al., 2017;Caffarel-Salvador et al., 2015), ocular delivery (John R.Giudicessi, BA.Michael J.Ackerman., 2008), cancer therapy (Martin, Allender, Brain, Morrissey, & Birchall, 2012;Tawde, Chablani, Akalkotkar, & D'Souza, 2016; Psimadas, Georgoulias, Valotassiou, & Loudos, 2012).

1.9 Prevalence of osteoporosis

The fractures resulting from osteoporosis constitute about 80% of total fractures which mostly happen to post-menopausal women. These fractures put an individual to 25% risk of death within the next year, with continued elevated mortality in following year after the event (Hanley, Adachi, Bell, & Brown, 2012). As per the estimation, throughout the world, the number of patients with osteoporotic hip fractures exceeds 200 million. Reports say that in both Europe and the United States, 30% women are osteoporotic, and it was estimated that 40% post-menopausal women and 30% men will experience an osteoporotic fracture at some point in the rest of their lives (Sozen et al., 2017).

1.10 Treatment options for osteoporosis

Almost all pharmacological agents for osteoporosis specifically target the bone resorption component of bone remodeling pathways; they are therefore classified as anti-catabolic or anti-resorptive agents (e.g. the bisphosphonates etidronate, alendronate, risedronate and zoledronic acid; estrogen and the selective estrogen receptor modulator (SERM) raloxifene; salmon calcitonin; and denosumab) (Iv, 2012).The only anabolic agent currently available is teriparatide. These treatments reduce the risk of osteoporotic fractures and stabilize bone mass and strength. These drugs are available as various dosage forms like oral solution, tablet or injectable solutions injected through hypodermic needles.

To make the treatment for osteoporosis more efficient, the use of hollow microneedle can be of great advantage eliminating the problems associated with other traditional approaches of the therapy.

1.11 Objectives of the study

The objectives of this research work are as follows:

- Introduction of novel 3D printed hollow microneedle for the delivery of denosumab for the treatment of osteoporosis.
- To investigate the release profile of hollow microneedle mediated denosumab delivery in blood *in vivo*.

Chapter 2

Materials and Methodology

2.1 Materials

Denosis 60 (Denosumab) was purchased from Incepta Pharmaceuticals Ltd. which contains 1 ml of sterile solution of denosumab 60 mg. This drug is a monoclonal antibody having a molecular weight of 147 kD (EMA, 2010) indicated mainly to be used for the treatment of osteoporosis in post-menopausal women. The recommended dose of denosumab is 60 mg administered as a single subcutaneous injection once every 6 months. The bio-availability of this SC injection is 61% with an elimination half-life of approximately 32 days (Hanley et al., 2012).

2.2 3D printed hollow microneedles

3D printed hollow microneedles, which were previously developed with the help of engineering software (Sophia et al., 2020), were the tools employed for the delivery of denosumab.

2.3 Optical coherence tomography (OCT)

For the assessment of the depth and width of the penetration of each microneedle, OCT has been used. Initially, excision of the skin of the albino mice was done (<24 hours after birth) followed by trimming up to 500μm with the help of a dermatome. The storage of the skin was maintained at nearly −20°C and defrosted at normal room temperature. To prepare the sample of skin for the OCT analysis, the stratum corneum was shaved to avoid the issue of hair follicles. The scanning of the skin was done at a rate of up to 15B-scans (2D cross-sectional scans) per second A total of microneedles were used for the evaluation through an OCT microscope (Uddin et al., 2020)

2.4 Animal models

Swiss albino hairless female mice (6-7 weeks; weighing 20–25g), were selected for conducting the *in-vivo* trials for the drug therapy of denosumab.

2.5 Quantification and isolation of animal models

In total, 18 albino mice were taken for the *in-vivo* studies of denosumab, which was then subdivided into 3 groups, each comprising of 6 mice. Group 1 (Untreated, negative control), Group 2 (subcutaneously injected) and Group 3 (by using SC injection). All mice were in the age range of 6-7 weeks, housed in solid bottom cages and were fed with a SDS VRF1 diet.

2.6 Loading Formulations on to the Hollow Microneedle

The drug solution was dispensed onto the array of hollow microneedles in the form of fine droplets using syringe. The liquid formulation of the drug was deposited into the drug reservoir positioned within the hollow microneedle. This allowed the release of the drug through the barriers of skin in a controlled manner. The hollow microneedle mediated delivery of denosumab allowed the administration without leaving any piercing remark within 5 minutes.

2.7 *In-vivo* study in mice

At first, three groups of mice were made (for each group, n=6); (1) group 1 is left untreated (negative control); (2) group 2 treated with subcutaneously administered injection (positive control); (3) group 3 treated with the 3D printed hollow microneedles. Before initiating the experiment, the animals were then subjected to shaving under careful observation to avoid any kind of injury to the skin. The arrays of the hollow microneedles were applied on the dorsal side of one group of the animals. Following the drug delivery, the 3D printed microneedles patches were removed and plasma concentration of the drug was monitored to observe the release pattern through a hollow microneedle mediated delivery.

Chapter 3

Results and Discussion

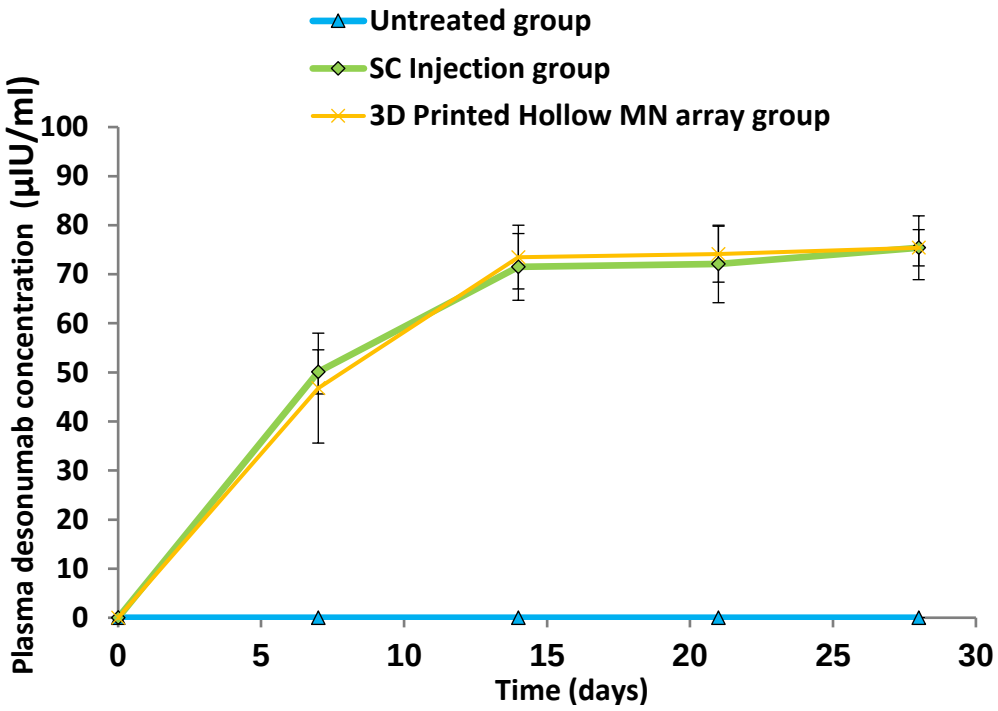

Figure 3: Release profile of denosumab

3.1 Evaluation of the release pattern of hollow microneedle mediated delivery

The graphical representation establishes a relationship between plasma concentrations of denosumab (µIU/ml) versus time (days).This shows a comparison of the release profile from which it is found that, when denosumab is delivered through a hollow microneedle, it was able to mimic the release profile obtained through the subcutaneous administration of denosumab. The observation was made in three groups after carrying the study for 28 days.

In the group treated with subcutaneously administered injection, the plasma concentration was found to be 50.1 µIU/ml at the end of a week, which reached to 71.5 µIU/ml by 2

weeks. In a span of three weeks, it attained 72.1µIU/ml and finally at the end of 28 days, the concentration of 75.4µIU/ml was achieved.

Now, in the group treated with 3D printed hollow microneedles, in the first week, a plasma concentration of 46.8 µIU/ml was attained. In a couple of weeks, it reached a concentration of 73.5 µIU/ml. In the following week, it reached to a point of 74.1 µIU/ml. After a period of 28 days, the plasma concentration of denosumab managed to attain 75.4 µIU/ml.

In terms of delivery strategies, the comparative studies between the delivery of drug through hollow microneedle and subcutaneous administration of drug are shown in this study. Even though, subcutaneously injected drug managed to facilitate the release of denosumab, a comparable release profile was evident between the group treated with subcutaneously administered injection and the group treated with hollow microneedle. In comparison to the SC group, similar rate of release was observed with the 3D printed hollow microneedle without inducing any stimuli of pain. The use of 3D printed hollow microneedles to carry out the in vivo studies of the animals was successful in delivering the drug without interfering with the expected outcome of the treatment strategy. The findings when investigated exhibit rapid release profile of Denosumab providing fast dissolution rate.

3.2 Improved patient compliance

A comparable release profile similar to the SC group is obtained without inducing pain which makes the choice of a hollow microneedle to deliver drugs a tool of well tolerability and safety. As a result, the patient is more likely to adhere to the treatment plan designed for a patient with osteoporosis. Thus, this approach involving the use of hollow microneedle for drug therapy will facilitate the patient care and lead to the expected outcome of the treatment ensuring an improved quality of life (Alhnan et al., 2016).

Chapter 4

Conclusion

The increased capacity of loading drug formulations and drug consistency make hollow microneedles a desirable tool of delivering therapeutic entities in an optimized way. Previously, various drugs have been delivered successfully with the help of hollow microneedles to combat the challenges linked to the conventional delivery strategies. In this study, for the first time, the use of 3D printed hollow microneedle has been introduced to deliver denosumab which will open up new treatment options for patients diagnosed with osteoporosis facilitating a better approach to patient care. The *in-vivo* studies of the animals proved that 3D printed hollow microneedles allow quick release profile being minimally invasive and thus have a significant contribution to achieve the therapeutic goal.

Chapter 5

The Future Direction of Hollow Microneedle

Exploration of hollow microneedle for transdermal drug delivery system will expand the horizon of applications in local and systemic therapy. Combination of micro-pumps, ultra-sound or electroporation with hollow microneedles will enable increased permeation of the drugs ensuring better drug release profile of drugs. In the near future, the use of hollow microneedles integrated with biosensor can be used for accurate diagnosis of disease. Early detection will also help to design treatment plan using targeted drug delivery by hollow microneedles. Even though it is an effective drug delivery system, there are many hurdles that this device encounters during its translation from the laboratory to the present market. Desirable delivery of drugs at controlled rate, production cost and complicated fabrication process calls for the need to conduct extensive research to counteract these challenges to make it cost-effective. By developing this tool and promoting its usability, soon this hollow microneedle will be used for emergency health conditions. Pediatric drug delivery is also another aspect where the successful usage of this device will be prominently evident. A lot of pre-clinical trials have already been carried out but only a few have been found to be successful in human subjects. Taking this study ahead to clinical subjects in the coming future will reinforce the utilization of hollow microneedles in a widespread manner making a revolution in the field of health science.

References

Alhnan, M. A., Okwuosa, T. C., Sadia, M., Wan, K. W., Ahmed, W., & Arafat, B. (2016). Emergence of 3D Printed Dosage Forms: Opportunities and Challenges. *Pharmaceutical Research*, *33*(8), 1817–1832. https://doi.org/10.1007/s11095-016-1933-1

Amodwala, S., Kumar, P., & Thakkar, H. P. (2017). Statistically optimized fast dissolving microneedle transdermal patch of meloxicam: A patient friendly approach to manage arthritis. *European Journal of Pharmaceutical Sciences*, *104*(51), 114–123. https://doi.org/10.1016/j.ejps.2017.04.001

Arya, J., & Prausnitz, M. R. (2016). Microneedle patches for vaccination in developing countries. *Journal of Controlled Release*, *240*, 135–141. https://doi.org/10.1016/j.jconrel.2015.11.019

Baek, S. H., Shin, J. H., & Kim, Y. C. (2017). Drug-coated microneedles for rapid and painless local anesthesia. *Biomedical Microdevices*, *19*(1). https://doi.org/10.1007/s10544-016-0144-1

Bodhale, D. W., Nisar, A., & Afzulpurkar, N. (2010). Design, fabrication and analysis of silicon microneedles for transdermal drug delivery applications. *IFMBE Proceedings*, *27*, 84–89. https://doi.org/10.1007/978-3-642-12020-6-21

Boks, M. A., Unger, W. W. J., Engels, S., Ambrosini, M., Kooyk, Y. Van, & Luttge, R. (2015). Controlled release of a model vaccine by nanoporous ceramic microneedle arrays. *International Journal of Pharmaceutics*, *491*(1–2), 375–383. https://doi.org/10.1016/j.ijpharm.2015.06.025

Caffarel-Salvador, E., Tuan-Mahmood, T. M., McElnay, J. C., McCarthy, H. O., Mooney, K., Woolfson, A. D., & Donnelly, R. F. (2015). Potential of hydrogel-forming and

dissolving microneedles for use in paediatric populations. *International Journal of Pharmaceutics*, *489*(1–2), 158–169. https://doi.org/10.1016/j.ijpharm.2015.04.076

Cao, J., Zhang, N., Wang, Z., Su, J., Yang, J., Han, J., & Zhao, Y. (2019). Microneedle-assisted transdermal delivery of etanercept for rheumatoid arthritis treatment. *Pharmaceutics*, *11*(5), 1–12. https://doi.org/10.3390/pharmaceutics11050235

Chen, Y., Chen, B. Z., Wang, Q. L., Jin, X., & Guo, X. D. (2017). Fabrication of coated polymer microneedles for transdermal drug delivery. *Journal of Controlled Release*, *265*(March), 14–21. https://doi.org/10.1016/j.jconrel.2017.03.383

Davis, S. P., Martanto, W., Allen, M. G., & Prausnitz, M. R. (2005). Hollow metal microneedles for insulin delivery to diabetic rats. *IEEE Transactions on Biomedical Engineering*, *52*(5), 909–915. https://doi.org/10.1109/TBME.2005.845240

Donnelly, R. F., Majithiya, R., Singh, T. R. R., Morrow, D. I. J., Garland, M. J., Demir, Y. K., … Woolfson, A. D. (2011). Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. *Pharmaceutical Research*, *28*(1), 41–57. https://doi.org/10.1007/s11095-010-0169-8

Dragicevic, N., & Maibach, H. I. (2017). Percutaneous penetration enhancers physical methods in penetration enhancement. *Percutaneous Penetration Enhancers Physical Methods in Penetration Enhancement*, 1–508. https://doi.org/10.1007/978-3-662-53273-7

EMA. (2010). CHMP Assessment Report for Prolia. *Epar*, (March). https://doi.org/10.1136/bjsm.2009.060731

Fernando, G. J. P., Zhang, J., Ng, H. I., Haigh, O. L., Yukiko, S. R., & Kendall, M. A. F. (2016). Influenza nucleoprotein DNA vaccination by a skin targeted, dry coated, densely

packed microprojection array (Nanopatch) induces potent antibody and CD8+ T cell responses. *Journal of Controlled Release*, *237*, 35–41. https://doi.org/10.1016/j.jconrel.2016.06.045

Gupta, J., Felner, E. I., & Prausnitz, M. R. (2009). Erratum: Minimally invasive insulin delivery in subjects with type 1 diabetes using hollow microneedles (Diabetes Technology and Therapeutics (2009) 11:6 (329-337)). *Diabetes Technology and Therapeutics*, *11*(7), 471. https://doi.org/10.1089/dia.2009.0729

Hanley, D. A., Adachi, J. D., Bell, A., & Brown, V. (2012). Denosumab: Mechanism of action and clinical outcomes. *International Journal of Clinical Practice*, *66*(12), 1139–1146. https://doi.org/10.1111/ijcp.12022

Hao, Y., Dong, M. L., Zhang, T. Y., Peng, J. R., Jia, Y. P., Cao, Y. P., & Qian, Z. Y. (2017). Novel Approach of Using Near-Infrared Responsive PEGylated Gold Nanorod Coated Poly(l-lactide) Microneedles to Enhance the Antitumor Efficiency of Docetaxel-Loaded MPEG-PDLLA Micelles for Treating an A431 Tumor. *ACS Applied Materials and Interfaces*, *9*(18), 15317–15327. https://doi.org/10.1021/acsami.7b03604

Ita, K. (2015). Transdermal delivery of drugs with microneedles—potential and challenges. *Pharmaceutics*, *7*(3), 90–105. https://doi.org/10.3390/pharmaceutics7030090

Iv, I. (2012). *P Atient I Nformation on Bisphosphonates*. (July), 2011–2013.

Jeong, H. R., Lee, H. S., Choi, I. J., & Park, J. H. (2017). Considerations in the use of microneedles: pain, convenience, anxiety and safety. *Journal of Drug Targeting*, *25*(1), 29–40. https://doi.org/10.1080/1061186X.2016.1200589

Ji, M.-X., & Yu, Q. (2015). Primary osteoporosis in postmenopausal women. *Chronic Diseases and Translational Medicine*, *1*(1), 9–13.

https://doi.org/10.1016/j.cdtm.2015.02.006

John R.Giudicessi, BA.Michael J.Ackerman., 2013. (2008). NIH Public Access. *Bone*, *23*(1), 1–7. https://doi.org/10.1038/jid.2014.371

Jun, H., Han, M. R., Kang, N. G., Park, J. H., & Park, J. H. (2015). Use of hollow microneedles for targeted delivery of phenylephrine to treat fecal incontinence. *Journal of Controlled Release*, *207*, 1–6. https://doi.org/10.1016/j.jconrel.2015.03.031

Katsumi, H., Tanaka, Y., Hitomi, K., Liu, S., Quan, Y. S., Kamiyama, F., … Yamamoto, A. (2017). Efficient transdermal delivery of alendronate, a nitrogen-containing bisphosphonate, using tip-loaded self-dissolving microneedle arrays for the treatment of osteoporosis. *Pharmaceutics*, *9*(3). https://doi.org/10.3390/pharmaceutics9030029

Kearney, M. C., Caffarel-Salvador, E., Fallows, S. J., McCarthy, H. O., & Donnelly, R. F. (2016). Microneedle-mediated delivery of donepezil: Potential for improved treatment options in Alzheimer's disease. *European Journal of Pharmaceutics and Biopharmaceutics*, *103*, 43–50. https://doi.org/10.1016/j.ejpb.2016.03.026

Kim, S., Dangol, M., Kang, G., Lahiji, S. F., Yang, H., Jang, M., … Jung, H. (2017). Enhanced Transdermal Delivery by Combined Application of Dissolving Microneedle Patch on Serum-Treated Skin. *Molecular Pharmaceutics*, *14*(6), 2024–2031. https://doi.org/10.1021/acs.molpharmaceut.7b00111

Konta, A. A., García-Piña, M., & Serrano, D. R. (2017). Personalised 3D printed medicines: Which techniques and polymers are more successful? *Bioengineering*, *4*(4). https://doi.org/10.3390/bioengineering4040079

Lane, C. A., Hardy, J., Schott, J. M., Assadeck, H., Toudou Daouda, M., Adehossi Omar, … Douma Maiga, D. (2018). Inflammatory demyelinating diseases of the central nervous

system in Niger. *Revue Neurologique*, *175*(4), 261–268. https://doi.org/10.1111/ene.13439

Li, J., Zeng, M., Shan, H., & Tong, C. (2017). Microneedle Patches as Drug and Vaccine Delivery Platform. *Current Medicinal Chemistry*, *24*(22), 2413–2422. https://doi.org/10.2174/0929867324666170526124053

Linec, M., & Mušič, B. (2019). The effects of silica-based fillers on the properties of epoxy molding compounds. *Materials*, *12*(11), 1–11. https://doi.org/10.3390/ma12111811

Lyon, B. J., Aria, A. I., & Gharib, M. (2014). Fabrication of carbon nanotube—polyimide composite hollow microneedles for transdermal drug delivery. *Biomedical Microdevices*, *16*(6), 879–886. https://doi.org/10.1007/s10544-014-9892-y

Ma, G., & Wu, C. (2017). Microneedle, bio-microneedle and bio-inspired microneedle: A review. *Journal of Controlled Release*, *251*, 11–23. https://doi.org/10.1016/j.jconrel.2017.02.011

Mansoor, I., Lai, J., Ranamukhaarachchi, S., Schmitt, V., Lambert, D., Dutz, J., … Stoeber, B. (2015). A microneedle-based method for the characterization of diffusion in skin tissue using doxorubicin as a model drug. *Biomedical Microdevices*, *17*(3). https://doi.org/10.1007/s10544-015-9967-4

Marshall, S., Sahm, L. J., & Moore, A. C. (2016). The success of microneedle-mediated vaccine delivery into skin. *Human Vaccines and Immunotherapeutics*, *12*(11), 2975–2983. https://doi.org/10.1080/21645515.2016.1171440

Martin, C. J., Allender, C. J., Brain, K. R., Morrissey, A., & Birchall, J. C. (2012). Low temperature fabrication of biodegradable sugar glass microneedles for transdermal drug delivery applications. *Journal of Controlled Release*, *158*(1), 93–101.

https://doi.org/10.1016/j.jconrel.2011.10.024

Milanetti, E., Raimondo, D., & Tramontano, A. (2016). Prediction of the permeability of neutral drugs inferred from their solvation properties. *Bioinformatics*, *32*(8), 1–7. https://doi.org/10.1093/bioinformatics/btv725

Mistilis, M. J., Bommarius, A. S., & Prausnitz, M. R. (2015). Development of a thermostable microneedle patch for influenza vaccination. *Journal of Pharmaceutical Sciences*, *104*(2), 740–749. https://doi.org/10.1002/jps.24283

Nayak, A., & Das, D. B. (2013). Potential of biodegradable microneedles as a transdermal delivery vehicle for lidocaine. *Biotechnology Letters*, *35*(9), 1351–1363. https://doi.org/10.1007/s10529-013-1217-3

Nejad, H. R., Sadeqi, A., Kiaee, G., & Sonkusale, S. (2018). Low-cost and cleanroom-free fabrication of microneedles. *Microsystems & Nanoengineering*, *4*(1), 1–7. https://doi.org/10.1038/micronano.2017.73

Nguyen, T. T., Choi, J. ah, Kim, J. S., Park, H., Yang, E., Lee, W. J., … Park, J. H. (2019). Skin immunization with third-generation hepatitis B surface antigen using microneedles. *Vaccine*, *37*(40), 5954–5961. https://doi.org/10.1016/j.vaccine.2019.08.036

Pandey, P. C., Shukla, S., Skoog, S. A., Boehm, R. D., & Narayan, R. J. (2019). Current advancements in transdermal biosensing and targeted drug delivery. *Sensors (Switzerland)*, *19*(5). https://doi.org/10.3390/s19051028

Prausnitz, M. R. (2017). Engineering Microneedle Patches for Vaccination and Drug Delivery to Skin. *Annual Review of Chemical and Biomolecular Engineering*, *8*(1), 177–200. https://doi.org/10.1146/annurev-chembioeng-060816-101514

Psimadas, D., Georgoulias, P., Valotassiou, V., & Loudos, G. (2012). Molecular

Nanomedicine Towards Cancer : *Journal of Pharmaceutical Sciences*, *101*(7), 2271–2280. https://doi.org/10.1002/jps

Rajabi, M., Roxhed, N., Shafagh, R. Z., Haraldson, T., Fischer, A. C., Van Der Wijngaart, W., … Niklaus, F. (2016). Flexible and stretchable microneedle patches with integrated rigid stainless steel microneedles for transdermal biointerfacing. *PLoS ONE*, *11*(12), 1–13. https://doi.org/10.1371/journal.pone.0166330

Serrano-Castañeda, P., Escobar-Chávez, J. J., Rodríguez-Cruz, I. M., Melgoza-Contreras, L. M., & Martínez-Hernández, J. (2018). Microneedles as enhancer of drug absorption through the skin and applications in medicine and cosmetology. *Journal of Pharmacy and Pharmaceutical Sciences*, *21*(1), 73–93. https://doi.org/10.18433/jpps29610

Sharma, S., Hatware, K., Bhadane, P., Sindhikar, S., & Mishra, D. K. (2019). Recent advances in microneedle composites for biomedical applications: Advanced drug delivery technologies. *Materials Science and Engineering C*, *103*(May), 109717. https://doi.org/10.1016/j.msec.2019.05.002

Sohn, W., Lee, E., Kankam, M. K., Egbuna, O., Moffat, G., Bussiere, J., … Slatter, J. G. (2016). An open-label study in healthy men to evaluate the risk of seminal fluid transmission of denosumab to pregnant partners. *British Journal of Clinical Pharmacology*, *81*(2), 362–369. https://doi.org/10.1111/bcp.12798

Sozen, T., Ozisik, L., & Calik Basaran, N. (2017). An overview and management of osteoporosis. *European Journal of Rheumatology*, *4*(1), 46–56. https://doi.org/10.5152/eurjrheum.2016.048

Tawde, S. A., Chablani, L., Akalkotkar, A., & D'Souza, M. J. (2016). Evaluation of microparticulate ovarian cancer vaccine via transdermal route of delivery. *Journal of Controlled Release*, *235*, 147–154. https://doi.org/10.1016/j.jconrel.2016.05.058

Turner, A. S. (2001). Animal models of osteoporosis - Necessity and limitations. *European Cells and Materials*, *1*, 66–81. https://doi.org/10.22203/ecm.v001a08

Uddin, M. J., Scoutaris, N., Economidou, S. N., Giraud, C., Chowdhry, B. Z., Donnelly, R. F., & Douroumis, D. (2020). 3D printed microneedles for anticancer therapy of skin tumours. *Materials Science and Engineering C*, *107*(September 2019), 110248. https://doi.org/10.1016/j.msec.2019.110248

Waghule, T., Singhvi, G., Dubey, S. K., Pandey, M. M., Gupta, G., Singh, M., & Dua, K. (2019). Microneedles: A smart approach and increasing potential for transdermal drug delivery system. *Biomedicine and Pharmacotherapy*, *109*(October 2018), 1249–1258. https://doi.org/10.1016/j.biopha.2018.10.078

Wright, N. C., Looker, A. C., Saag, K. G., Curtis, J. R., Delzell, E. S., Randall, S., & Dawson-Hughes, B. (2014). The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. *Journal of Bone and Mineral Research*, *29*(11), 2520–2526. https://doi.org/10.1002/jbmr.2269

Xu, J., Danehy, R., Cai, H., Ao, Z., Pu, M., Nusawardhana, A., … Guo, F. (2019). Microneedle Patch-Mediated Treatment of Bacterial Biofilms [Research-article]. *ACS Applied Materials and Interfaces*, *11*(16), 14640–14646. https://doi.org/10.1021/acsami.9b02578

Yang, G., Chen, Q., Wen, D., Chen, Z., Wang, J., Chen, G., … Gu, Z. (2019). A Therapeutic Microneedle Patch Made from Hair-Derived Keratin for Promoting Hair Regrowth. *ACS Nano*, *13*(4), 4354–4360. https://doi.org/10.1021/acsnano.8b09573

Yu, L. M., Tay, F. E. H., Guo, D. G., Xu, L., & Yap, K. L. (2009). A microfabricated electrode with hollow microneedles for ECG measurement. *Sensors and Actuators, A:*

Physical, *151*(1), 17–22. https://doi.org/10.1016/j.sna.2009.01.020

Yun, J., Kim, J., & Lee, J. H. (2017). Fabrication of fine electrodes on the tip of hypodermic needle using photoresist spray coating and flexible photomask for biomedical applications. *Journal of Visualized Experiments*, *2017*(129), 1–8. https://doi.org/10.3791/56622

Zhu, D. D., Wang, Q. L., Liu, X. B., & Guo, X. D. (2016). Rapidly separating microneedles for transdermal drug delivery. *Acta Biomaterialia*, *41*, 312–319. https://doi.org/10.1016/j.actbio.2016.06.005