DLP 3D PRINTING IN THE DEVELOPMENT OF CARBOXYMETHYL CELLULOSE HYDROGEL FOR DERMAL DRUG DELIVERY

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by

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LIST OF SYMBOLS

cm	Centimetre
R^2	Coefficient of determination
cm^3	Cubic centimetre
°	Degree
°C	Degree Celsius
n	Exponent of release
g	Gram
h	Hour
kDa	Kilodalton
µg	Microgram
µm	Micrometre
mL	Millilitre
mm	Millimetre
min	Minute
nm	Nanometre
N	Newton
P	Partition coefficient
ppm	Parts per million
%	Percentage
rpm	Rotation per minute
s	Second
cm^2	Square centimetre
v/v	Volume/volume
w/v	Weight/volume

LIST OF ABBREVIATIONS

3D	Three-dimensional
AIC	Akaike information criterion
ANOVA	Analysis of variance
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
CLIP	Continuous liquid interface production
CMC	Carboxymethyl cellulose
DLP	Digital light processing
DM	Degree of methacrylation
DMD	Digital micromirror devices
DMEM	Dulbecco's Modified Eagle's Medium
DMI	Dimethyl isosorbide
DMSO	Dimethyl sulphoxide
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
FBS	Fetal bovine serum
GelMA	Gelatin methacryloyl
HPLC	High performance liquid chromatography
ICH	International Council for Harmonisation
IPM	Isopropyl myristate
ISO	International Organisation for Standardisation
LAP	Lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate
LCD	Liquid crystal display
LOD	Limit of detection
LOQ	Limit of quantification
MA	Methacrylic anhydride
M-CMC	Methacrylated carboxymethyl cellulose
MeOH	Methanol
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaOH	Sodium hydroxide

NIA	Niacinamide
NMR	Nuclear magnetic resonance
OA	Oleic acid
OECD	Organisation for Economic Co-operation and Development
PBS	Phosphate buffered saline
PG	Propylene glycol
R/F	Ratio of relaxational over Fickian contribution
SC	Stratum corneum
SD	Standard deviation
SLA	Stereolithography
TC	Transcutol® P
TZ	Tartrazine
UV	Ultraviolet

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PENCETAKAN 3D DLP DALAM PEMBANGUNAN HIDROGEL KARBOKSIMETIL SELULOSA UNTUK PENGHANTARAN DRUG DERMAL

ABSTRAK

Hidrogel adalah menarik untuk penghantaran ubat ke kulit kerana keupayaan takungan ubat yang besar dan biokompatibilitinya. Bagi membolehkan pembangunan hidrogel khusus dengan pantas, penghasilan hidrogel karboksimetil selulosa (CMC) melalui pencetakan 3D dengan pemprosessan cahaya digital (DLP) wajar diterokai. Walau bagaimanapun, ketepatan pencetakan hidrogel dipengaruhi oleh kesan penyebaran cahaya semasa pencetakan 3D DLP. Oleh sebab itu, tesis ini bertujuan untuk menghasilkan hidrogel CMC yang dicetak melalui 3D DLP dengan mengaji kesan tartrazina sebagai foto-penyerap atas sifat hidrogel serta menerokai potensinya dalam penghantaran ubat ke kulit. Hidrogel berbentuk silinder dibangunkan dengan menggunakan pencetak 3D DLP dengan resin khas yang terdiri daripada CMC metakrilat (M-CMC) yang disintesis daripada CMC, litium fenil(2,4,6-trimetilbenzoil)fosfinat sebagai fotoinisiator, dan tartrazina (0.005 – 0.025%w/v). Hidrogel yang dicetak dicirikan dari segi ketepatan dimensi dan sifat mekanikal. Formulasi terpilih ditambah dengan niasinamida (4%w/v) dan pelarut untuk pencirian lebih lanjut dan kajian pelepasan ubat dan penyerapan kulit *in vitro*. Kesitotoksikan *in vitro* tartrazina dan hidrogel yang dicetak dinilai melalui ujian 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromida. Hasilnya menunjukkan bahawa peningkatan kandungan tartrazina menurunkan peratusan penyimpangan dimensi cetakan dari 38% ke 1%. Hal ini disebabkan oleh pengurangan kesan penyebaran cahaya yang

meminimumkan pengerasan ultraungu yang berlebihan. Kepekatan tartrazina yang lebih tinggi mengurangkan kekuatan gel dari 0.54 ke 0.05 N dan pecahan gel dari 88% ke 74% kerana tahap pemautsilangan lebih rendah. Ini juga meningkatkan keliangan hidrogel dan nisbah pengembangan dari 216% ke 462%. Untuk membolehkan pengendalian hidrogel dengan senang dan fideliti bentuk, hidrogel dengan 0.01%w/v tartrazina dipilih untuk pemuatan ubat. Penambahan pelarut yang berbeza (Transcutol® P, propilena glikol dan dimetil isosorbida) mempengaruhi kelikatan resin yang dimuat dengan niasinamida. Semakin tinggi kelikatan resin, semakin tinggi peratusan penyimpangan dimensi cetakan hidrogel disebabkan oleh aliran resin yang kurang cekap. Oleh itu, fideliti bentuk yang lebih baik dapat diperolehi daripada hidrogel dengan Transcutol® P dan dimetil isosorbida kerana kelikatan resin mereka lebih rendah. Sekitar 4566 – 6019 μg (~2.3 – 2.8%w/v) niasinamida terdapat dalam hidrogel yang dicetak (diameter: 9 mm; tinggi: 3 mm). Hidrogel mencapai ~100% pelepasan niasinamida dalam masa 5 jam dan menunjukkan pengangkutan anomali niasinamida. Sekitar 87.7 – 1093.6 μg/cm^2 (1.5% – 18.4%) niasinamida meresap melalui kulit telinga khinzir dalam tempoh 24 jam dengan hidrogel tanpa pelarut menyampaikan jumlah niasinamida yang tertinggi (18.4%). Hidrogel dengan Transcutol® P menunjukkan retensi niasinamida dalam kulit yang tertinggi (1.8%). Semua kepekatan tartrazina yang diuji dan hidrogel yang dicetak dengan muatan niasinamida tidak menunjukkan kesitotoksikan (kebolehhidupan sel: 72.4 – 122.7%). Kesimpulannya, pencetakan 3D hidrogel M-CMC melalui DLP dengan kandungan tartrazina yang dioptimumkan dan dimuatkan dengan niasinamida adalah tidak sitotoksik dan mempunyai potensi penghantaran drug dermal.

DLP 3D PRINTING IN THE DEVELOPMENT OF CARBOXYMETHYL CELLULOSE HYDROGEL FOR DERMAL DRUG DELIVERY

ABSTRACT

Hydrogel is attractive for dermal drug delivery due to its huge drug reservoir and biocompatibility. To allow rapid fabrication of customisable hydrogels, the development of carboxymethyl cellulose (CMC) hydrogel using digital light processing (DLP) 3D printing is worth to be explored. Yet, the printing accuracy of hydrogel is affected by the light scattering effect in DLP 3D printing. Hence, this thesis aims to develop 3D printed CMC hydrogels using DLP by studying the effect of tartrazine as the photoabsorber on hydrogel properties and exploring its dermal drug delivery potential. Cylindrical hydrogels were fabricated using DLP 3D printer with customised resin consisting of methacrylated CMC (M-CMC) synthesised from CMC, lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate as photoinitiator and tartrazine (0.005 – 0.025%w/v). The printed hydrogels were characterised for dimension accuracy and mechanical properties. Selected formulations were added with niacinamide (4%w/v) and solvents for further characterisation and *in vitro* drug release and skin permeation studies. The *in vitro* cytotoxicity of tartrazine and printed hydrogels were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. It was found that an increased tartrazine content reduced printed dimension deviation from 38% to 1% due to a reduced light scattering effect which minimised overcuring. Higher tartrazine concentration lowered the gel strength from 0.54 to 0.05 N and gel fraction from 88% to 74% due to a lower degree of photocrosslinking. This also

increased the hydrogel porosity and swelling ratio from 216% to 462%. For ease of handling and shape fidelity, hydrogel with 0.01%w/v of tartrazine was selected for drug loading. The addition of different solvents (Transcutol® P, propylene glycol and dimethyl isosorbide) affected the viscosity of niacinamide-loaded resin. The higher the viscosity of resin, the higher the percentage deviation of printed dimension of hydrogels due to the inefficient flow of resin. Hence, better shape fidelity was obtained from the hydrogels with Transcutol® P and dimethyl isosorbide owing to the lower viscosities of their resins. Around 4566 – 6019 µg (~2.3 – 2.8%w/v) of niacinamide was recovered in the printed hydrogels (diameter: 9 mm; height: 3 mm). The hydrogels reached ~100% of niacinamide released within 5 h and showed anomalous transport of niacinamide. Around 87.7 – 1093.6 µg/cm^2 (1.5% – 18.4%) of niacinamide permeated across the porcine ear skin over 24 h with the solvent-free hydrogel delivered the highest amount of niacinamide (18.4%). The hydrogel with Transcutol® P showed the highest skin retention of niacinamide (1.8%). All tested tartrazine concentrations and the printed niacinamide-loaded hydrogels exhibited no cytotoxicity (cell viability: 72.4 – 122.7%). In conclusion, DLP 3D printed niacinamide-loaded M-CMC hydrogels with optimised tartrazine content formed were non-cytotoxic and possessed dermal delivery potential.

CHAPTER 1 INTRODUCTION

1.1 Overview

The development of advanced drug delivery systems has gained significant attention in recent years, with three-dimensional (3D) printed hydrogels emerging as a promising platform for dermal delivery. Dermal drug delivery refers to administration of drugs across the skin for localised therapeutic effects (Pünnel and Lunter, 2021). The presence of the stratum corneum (SC) which is the skin natural barrier poses challenges for a more effective dermal delivery of drugs.

Hydrogel is a 3D, crosslinked polymer network with a high water-retaining capacity. Dermal delivery using hydrogel can provide sustained release of drug as the hydrogel acts as a drug reservoir and improve hydration of the SC owing to the occlusive property of hydrogels (Almoshari, 2022; Cao et al., 2024; Kamenova et al., 2024). Among the different sources of polymers, cellulose and its derivatives show great potential for hydrogel fabrication (Ciolacu et al., 2020). 3D printing, also known as additive manufacturing, has become an attractive method to fabricate hydrogels as 3D printing offers personalisation, printing of models with high complexity and allows on-demand manufacturing to save cost (Zhang and Wang, 2022).

In short, this chapter provides a fundamental understanding of dermal drug delivery, the applications of carboxymethyl cellulose (CMC) hydrogels for dermal delivery and 3D printing of hydrogels via digital light processing (DLP). To gain a deep understanding of dermal delivery, the structure of the skin and the permeation pathways of drug are summarised. The use of skin penetration

enhancers or solvents to promote drug delivery across the skin is reviewed. Besides, the exploitation of CMC in biomedical applications, particularly for dermal delivery, is discussed. This chapter also discusses vat photopolymerisation, which involves the light curing of liquid resin to form 3D structure with focus on the DLP technology for CMC hydrogel fabrication.

1.2 Dermal drug delivery

Dermal delivery provides a non-invasive method for drug administration through intact skin for local effects such as therapies for skin diseases and infections (Pünnel and Lunter, 2021). Advantages of this system include avoiding first-pass metabolism, improving patient compliance and allowing easy therapy termination (Kováčik et al., 2020; Phatale et al., 2022). Formulations for dermal delivery are designed to allow penetration into the skin while avoiding systemic absorption (Brown et al., 2006). Due to the complex skin barrier, attention has been drawn not only to have a deeper understanding of the skin anatomy but also to the formulation strategies to improve the efficacy of dermal therapies.

1.2.1 Human skin

The skin, covering an average surface area of around 2 m², is the largest organ of the human body (Benson, 2011; Hadgraft, 2001). The skin is composed of four distinct layers: the SC (non-viable epidermis), the viable epidermis, the dermis, and the subcutaneous tissue (hypodermis), along with associated

structures such as hair follicles and sweat glands (Figure 1.1). Functionally, the skin acts as a crucial barrier, preventing water loss while protecting against toxins, ultraviolet (UV) radiation, and foreign organisms (Hadgraft, 2001). Moreover, the skin plays a central role in homeostasis by regulating body temperature and blood pressure (Benson, 2011). Additionally, the skin serves as a sensory organ, transmitting signals related to pressure, pain and temperature to the central nervous system (Benson, 2011). Despite being an attractive site for drug administration, the dermal route also presents obstacles to the penetration of most substances due to the complex structure of the skin.

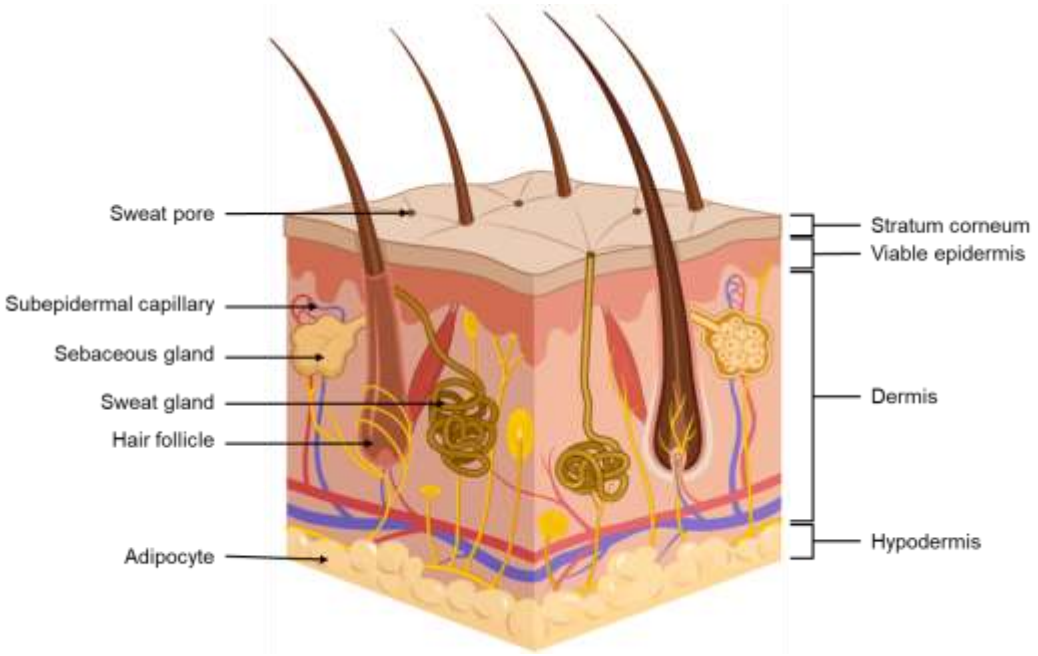


Figure 1.1 Cross-section of human skin (adapted from Benson (2011); created using Biorender.com)

1.2.1(a) Epidermis

The epidermis, consisting of five layers of interconnected keratinocytes that act as its structural units. The epidermis acts as a barrier against dehydration and protection from toxins and pathogens (Wertz, 2013). The thickness of the

epidermis varies, measuring as little as 60 μm on the eyelids and reaching up to 800 μm on the palms and soles, where enhanced durability is necessary (Benson, 2011). New layer of keratinocytes formed at the stratum basale undergo structural changes and lose their organelles as they differentiate towards the other three sublayers of viable epidermis, which are stratum spinosum, stratum granulosum, and stratum lucidum. The keratinocytes then finally turn into proteinaceous corneocytes, forming the outermost SC.

1.2.1(a)(i) Stratum corneum (SC)

The SC, also known as the horny layer, is 10 – 20 μm thick with low hydration level at approximately 10 – 20%, compared to around 70% in other body tissues (Benson, 2011). The SC functions as the skin's primary barrier despite being only 10 – 15 cells deep. Structurally, the SC resembles a brick wall, where the bricks and mortar represent the corneocytes and intercellular lipids, respectively, with desmosomes serving as molecular rivets between the corneocytes (Benson, 2011).

The elongated-shaped corneocytes are anucleated cells packed with 70 – 80% of keratin and 20% of lipid, enclosed by cornified cell envelope (Benson, 2011). This envelope contains a protein component made of structural proteins such as involucrin, loricrin small proline-rich proteins, desmosomal proteins, elafin, cystatin A, filaggrin and keratin intermediate filaments, cross-linked by disulfide bonds and N-(γ-glutamyl) lysine isopeptide bonds (Benson, 2011; Tong et al., 2006). Whereas the lipid components, comprised of ω-acylated-hydroxy-ceramides, and omega-hydroxy-fatty acids, are bound covalently to the

involucrin of the protein layer to form the intercellular lipid lamellae of the SC (Benson, 2011; Elias et al., 2014). Moreover, the intercellular lipid domains in the SC such as free fatty acids, ceramides, cholesterol, cholesterol esters and cholesterol sulphate also contribute to the skin's barrier function (Benson, 2011).

Approximately 15 – 20% of water is contained in the SC which binds primarily to the keratin within corneocytes, with minimal amounts located in the intercellular polar head group regions (Benson, 2011; Marjukka Suhonen et al., 1999). Natural Moisturising Factor in the SC possess water-binding ability to support corneocyte swelling which can enhance skin permeability without directly altering the lipid ordering (Benson, 2011; Gunnarsson et al., 2021).

1.2.1(b) Dermis

The thickness of the dermis ranges from 2 – 6 mm, depending on its location (Oltulu et al., 2018). The dermis consists of two sub-layers. The stratum papillare, located closest to the epidermis, consists of loosely woven collagen fibres, extracellular matrix proteins and connective tissue (Hofmann et al., 2023). This layer is highly vascularised for exchange of nutrients and waste products, body temperature regulation, supporting wound repair and immune response (Benson, 2011). Nerve endings in this layer are responsible for sensory detection of touch, vibration, and heat. Under this layer is the stratum reticulare, where the sweat glands, sebaceous glands, nerve fibres, hair roots, lymphatic and blood vessels are located (Hofmann et al., 2023). The sparse cell population of the dermis and the spongy matrix of the stratum papillare

lead to a better permeability of most drugs but the permeation of highly lipophilic actives to deeper layers may be limited (Benson, 2011; Hofmann et al., 2023).

Dermal fibroblasts, the predominant cell type in the dermis, secrete extracellular matrix proteins including collagen, elastin and ground substances (Barbieri et al., 2014; Hofmann et al., 2023). The collagen and elastin provide strength and flexibility to the skin, while ground substances such as glycosaminoglycans and proteoglycans resist compression, maintain tissue hydration, regulate proteins secreted and act as the membrane coreceptors (Barbieri et al., 2014). Various immune-competent cells, including dermal dendritic cells, macrophages, mast cells, eosinophils, neutrophils, B-lymphocytes, and T-lymphocytes are found in the dermis and responsible for the immune response and tissue reconstruction (Hofmann et al., 2023).

1.2.1(c) Hypodermis

The hypodermis, also called the subcutaneous layer, attaches the dermis to the muscles and bones beneath it. This layer consists of larger nerves, blood vessels, loose connective tissue, white adipose tissue and rich in glycosaminoglycans and proteoglycan which can absorb excess interstitial fluids (Wong et al., 2016). The hypodermis not only serves as a heat insulation, but also provides an energy reserve and protection from physical trauma (Benson, 2011; Hofmann et al., 2023). This layer also functions as the stem cell reservoir, an endocrine organ to control skin homeostasis and regulates skin aging (Liu et al., 2024).

1.2.2 Permeation pathways

Drug molecules can permeate through the SC via different routes, namely the transcellular, intercellular and transappendageal routes (Figure 1.2).

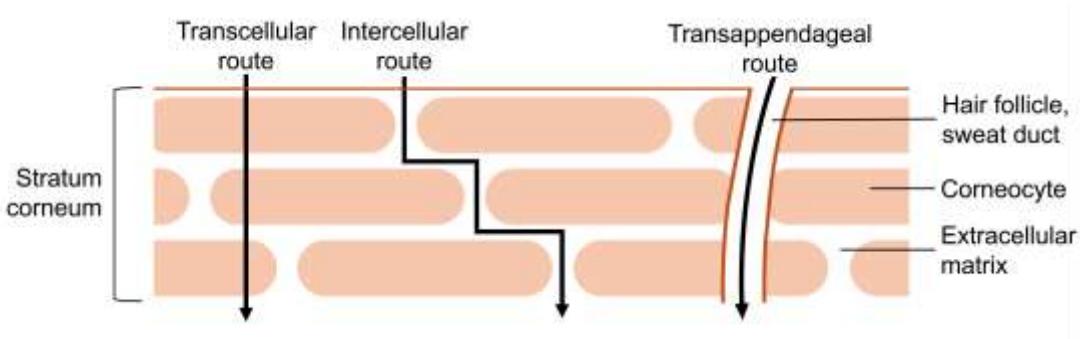


Figure 1.2 Routes of drug permeation across SC

1.2.2(a) Transcellular route

This route presents significant challenges to drug molecules as they must pass through numerous hydrophilic and hydrophobic structures including free intercellular lipid, covalent lipid monolayer, protein cell envelope and hydrated keratin (Hmingthansanga et al., 2022; Vaseem et al., 2024). As a result, the drug molecules need to undergo several times of partitioning and diffusion across the SC via this route. Highly lipophilic drugs face difficulties permeating the hydrophilic domains when following this pathway (Hmingthansanga et al., 2022). On the other hand, the partitioning of hydrophilic drugs in the cell membrane of corneocytes is limited unless the drugs are small or facilitated by membrane transporters (Vaseem et al., 2024).

1.2.2(b) Intercellular route

This route, also known as the paracellular route, provides a pathway through the lipid matrix surrounding the corneocytes for lipophilic or non-polar molecules (Alkilani et al., 2015). Despite having a longer penetration path, the intercellular route is more preferred by most small drug molecules because the extracellular matrix is the only continuous phase in the SC (Kováčik et al., 2020; Vaseem et al., 2024). The small hydrophilic drugs travel along the lipid lamellae by interacting with the polar head of lipids (Neupane et al., 2020).

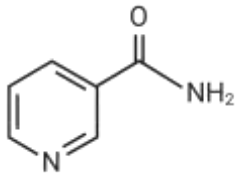
1.2.2(c) Transappendageal route

The transppendageal or shunt route allows drug molecules to permeate across the SC through the appendages including hair follicles and sweat ducts which only occupy around 0.1% of the skin surface (Kováčik et al., 2020). The drug penetration rate via this route is limited by the density, opening width and volume of the appendages (Sivadasan and Madkhali, 2024; Vaseem et al., 2024).

1.2.3 Niacinamide (NIA) as model drug

NIA or otherwise called nicotinamide or 3-pyridinecarboxamide, is a heterocyclic aromatic amide derived from vitamin B3 (niacin). Its physiochemical properties are summarised in Table 1.1.

Table 1.1 Physiochemical properties of NIA

Chemical structure	
Empirical formula	$C_6H_6N_2O$
Molecular weight (g/mol)	122.14 (Cosmetic Ingredient Review Expert Panel (CIREP), 2005)
Density	1.40 (CIREP, 2005)
Melting point (°C)	129 (CIREP, 2005)
Boiling range (°C)	150 – 160 (CIREP, 2005)
pK_a	3.3 (20°C) (Zhang et al., 2019b)
Solubility	Soluble in water, ether and glycerol (CIREP, 2005)
Solubility parameter	13.9 $(cal/cm^3)^{1/2}$ (Zhang et al., 2019b)
log *P*	−0.37 (CIREP, 2005)

Topical application of NIA has shown various dermal benefits including skin hydration, skin brightening, skin anti-aging and alleviating skin conditions such as dermatitis and eczema (Castanedo-Cazares et al., 2013; Greatens et al., 2005; Hakozaki et al., 2002; Jerajani et al., 2010; Kawada et al., 2008; Khodaeiani et al., 2013; Lee et al., 2014; Nisbet et al., 2019; Ong and Goh, 2024; Shariff et al., 2022; Zhu et al., 2023). Owing to its dermal benefits, NIA is widely used in cosmeceuticals and skin care products.

For dermal drug delivery, drug molecules must pass through the SC which acts as a strong barrier, especially for drugs with high molecular weights (> 500 Da) (Neupane et al., 2020). Other physiochemical properties of drugs such as degree of ionisation, log partition coefficient (log *P*) and melting point also influence its skin permeability (Hmingthansanga et al., 2022). It is accepted that the criteria of a drug to be delivered into the skin includes log *P* value of 1

– 3, molecular weight of less than 500 Da, melting point of less than 200°C (Iliopoulos et al., 2022; Richard et al., 2021).

Although the molecular weight and melting point of NIA meet the requirements as a dermal drug candidate, its low log *P* value of −0.37 presents challenges for effective skin delivery. Different strategies have been studied to enhance NIA permeation across the skin, such as incorporating solvents or surfactants, delivering in the form of emulsions, gels or vesicle systems and utilising ultrasound or microneedles (Basto et al., 2021; Bhattaccharjee et al., 2020; Hakozaki et al., 2006; Iliopoulos et al., 2020; Offerta et al., 2016; Sohn and Choi, 2023; Zhang et al., 2019a). Hence, in this study, NIA was used as the model drug to develop an innovative delivery platform using hydrogel to improve the skin permeation of NIA.

1.2.4 Skin penetration enhancers (solvents)

There are different methods to enhance the skin permeation of drugs which can be classified into the active and passive strategies. Active strategies, such as electroporation, sonophoresis, iontophoresis and microneedling, employ external energy to weaken the SC barrier, while the passive strategies utilise skin penetration enhancers, ionic liquids and nanocarriers (Gupta et al., 2019; Yuan et al., 2022).

The current study used a simple passive strategy to enhance NIA delivery across the skin, which is the incorporation of skin penetration enhancers, also known as solvents. Examples of different classes of solvents are alcohols, ether alcohols, esters, amides, glycols, fatty acids, surfactants, sulphoxides,

pyrrolidones, urea, terpenes and terpenoids (Lane, 2013; Sim et al., 2025). The solvents can be used individually or in combination with other solvents. Solvent released from the formulation not only can increase the thermodynamic activity of actives in the vehicles but also can improve the partitioning of actives into the skin (Hmingthansanga et al., 2022; Thong et al., 2007). In addition, solvents can be used to reduce the resistance of the SC to the actives by disrupting the packing of SC lipids (Hmingthansanga et al., 2022; Musakhanian et al., 2024; Virani et al., 2023). It is also believed that some solvents may extract the SC lipids, allowing the passage of actives through the SC (Mistry and Notman, 2024; Thong et al., 2007; Virani et al., 2023). Besides, solvents can cause modification to the intercellular keratin confirmation, leading to swelling and promoting hydration of the SC (Kováčik et al., 2020). Some solvents have the ability to distort protein and reduce the cohesion between corneocytes by targeting corneodesmosomes (Hmingthansanga et al., 2022; Kováčik et al., 2020; Sim et al., 2025).

Some of the solvents that were reported for topical delivery of NIA in the form of neat, binary and ternary solvent systems were being investigated in the current study, including propylene glycol (PG), Transcutol® P (TC), dimethyl isosorbide (DMI), oleic acid (OA), isopropyl myristate (IPM) and glycerol (Iliopoulos et al., 2020; Sohn and Choi, 2023; Zhang et al., 2019a; Zhang et al., 2019b; Zhang et al., 2020). These solvents are generally recognised as safe (GRAS) by the US Food and Drug Administration or pose the least risk of acute dermal toxicity as classified by the Globally Harmonised System (Becker et al., 2019; Kim et al., 2024; Liston et al., 2022; Yu and Goh, 2024; Zhang et

al., 2021; Zhang et al., 2020). The mechanism of actions of these solvents to enhance dermal delivery are summarised in Table 1.2.

Table 1.2 Chemical classification of solvents used in the current study with their respective proposed mechanism of action in dermal delivery

Solvent studied	Chemical classification	Mechanisms of action	References
PG	Glycol	• Increase drug solubility in the skin • Enhance the thermodynamic activity of active in the vehicle • Induce the SC protein dehydration • Increase fluidity of skin lipids • Induce the SC lipid extraction	Iliopoulos et al. (2020); Yu and Goh (2024)
TC	Ether	• Increase drug solubility in the skin • Promote swelling of the SC to act as drug reservoir • Enhance the thermodynamic activity of active in the vehicle • Increase fluidity of skin lipids	Iliopoulos et al. (2020); Musakhanian et al. (2024); Sim et al. (2025)
DMI	Ether	• Promote drug partitioning in the SC by increasing the SC polarity	Iliopoulos et al. (2020)
OA	Fatty acid	• Increase fluidity of skin lipids • Form separated fluid domains in the SC lipids	Kováčik et al. (2023); Saitoh et al. (2023)
IPM	Ester	• Increase fluidity of skin lipids • Induce phase separation as IPM can be incorporated completely in the SC lipid bilayers • Promote drug partitioning into the SC	Engelbrecht et al. (2012); Lane (2013); Santos et al. (2012)
Glycerol	Triol	• Increase fluidity of skin lipids by enhancing the hydration of the SC	Iliopoulos et al. (2020); Yamada et al. (2021)

1.3 Hydrogels

Hydrogel is a 3D crosslinked polymer network that can absorb and retain a large amount of fluids. Owing to their exceptional water-holding capacity, biocompatibility and adaptability, hydrogels have emerged as a versatile material with applications across various fields (Almoshari, 2022). Hydrogels have been utilised extensively in biomedical applications including contact lenses, tissue engineering, biosensors, 3D bioprinting, drug delivery and cell therapy (Almoshari, 2022; Birman and Seliktar, 2021; Radulescu et al., 2022; Völlmecke et al., 2022). Hydrogels can be prepared with synthetic or natural polymers and can be loaded with hydrophilic or hydrophobic drugs (Zöller et al., 2025). Examples of synthetic polymers include poly(vinyl alcohol), polyethylene glycol, poly(acrylic acid), poly(meth)acrylates and poloxamers (Cao et al., 2024; Zöller et al., 2025). Whereas natural polymers can be classified into polysaccharides such as chitosan, cellulose and alginate and proteins such as collagen, elastin and albumin (Cao et al., 2024; Zöller et al., 2025).

1.3.1 Cellulose-based hydrogels

As a natural polymer, cellulose offers several attractive characteristics such as biocompatibility, biodegradability, good mechanical and thermal properties while also being low cost and abundant (Ciolacu et al., 2020; Ribeiro et al., 2018). Cellulose-based hydrogels can be formed via physical gelation or chemical gelation. Physical gelation arises from the interaction of cellulose chains through van der Waals interaction, hydrogen bonds, chain

entanglements and hydrophobic or ionic associations (Ciolacu et al., 2020). On the other hand, chemical gelation involves crosslinking of cellulose chains via radical copolymerisation, graft copolymerisation or free radical polymerisation (Fu et al., 2018). The process interferes with the packing and self-association of cellulose chains, producing hydrogels with a greater porosity, reduced crystalline regions and improved swelling properties (Ciolacu et al., 2020).

Owing to their advantages, cellulose-based hydrogels have been developed for various applications including wound dressings, smart sensors, treatment for infection, tissue engineering and drug delivery (Fu et al., 2018; Zou et al., 2022). Besides being biocompatible, cellulose-based hydrogels have been extensively studied as drug delivery systems due to the high porosity which favours drug loading and can maintain a controlled release of drug over a longer period, especially when the drug release is dependent on the specific environmental trigger such as changes in temperature and pH (Ribeiro et al., 2018). By controlling the amounts of cellulose and crosslinker, cellulose-based hydrogels with different porosity and morphology can be fabricated which may influence the drug loading capacities and releasing patterns of hydrogels (Ciolacu et al., 2020). The stable structures, adhesive and degradable properties of cellulose-based hydrogels also make them a promising biomaterial as an efficient drug delivery system (Ribeiro et al., 2018).

1.3.1(a) Carboxymethyl cellulose (CMC) hydrogels

Cellulose is a potential polymer for hydrogel fabrication but it is known that cellulose is limited by its poor water solubility which is not suitable for hydrogel formation (Zou et al., 2022). Hence, the cellulose needs to be chemically modified to produce water-soluble cellulose derivatives such as methylcellulose, ethyl cellulose, CMC, hydroxyethyl cellulose and hydroxypropyl methylcellulose. The presence of the hydroxyl groups of cellulose favours the chemical modification such as esterification, acetylation, etherification and nitration (Ciolacu et al., 2020; Ribeiro et al., 2018). One of the commonly used cellulose derivatives is CMC (Figure 1.3).

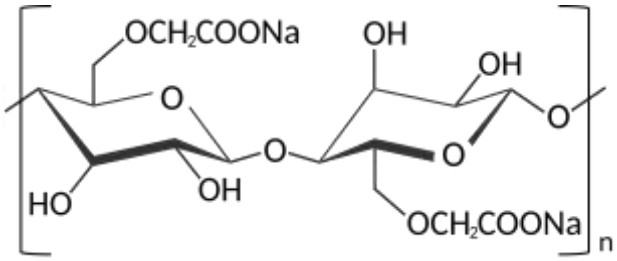


Figure 1.3 Chemical structure of CMC

CMC is a water-soluble, anionic polysaccharide where hydrogen atoms in some hydroxyl groups of cellulose are being substituted with carboxymethyl (CH_2COO^-) groups (Rahman et al., 2021). CMC is often used in its sodium salt form, sodium CMC, in pharmaceutical preparations as compared to the calcium form of CMC (Lavinia et al., 2016). The hydrophilicity of CMC is contributed by the presence of the hydrophilic CH_2COO^- groups which contribute to the hydrogen bonding and water absorption ability (Javanbakht and Shaabani, 2019). This results in the gel-forming property as CMC can absorb a large amount of fluids. Besides, the presence of carboxyl groups in

CMC allows the CMC-based hydrogel to be pH sensitive and eventually affecting its swelling behaviour (Javanbakht and Shaabani, 2019). As CMC hydrogels are highly biocompatible, biodegradable, low toxicity, possess intelligent responsiveness and good mechanical properties, their uses in biomedical fields are greatly explored, especially in drug delivery (Chen et al., 2020; Li et al., 2020). CMC hydrogels have been studied for their uses in ocular, nasal, oral, gastric, colon, dermal and transdermal drug delivery as well as cancer therapy (Ciolacu et al., 2020).

1.3.1(a)(i) CMC hydrogels for dermal delivery

Apart from the benefits of hydrogels such as a high water content, good adhesiveness, non-greasy texture, CMC hydrogels also offer several attractive characteristics for dermal applications. For example, biocompatibility, adjustable mechanical strength related to the degree of crosslinking, reversible viscosity and the ability to incorporate drugs (Ciolacu et al., 2020). Dermal delivery using hydrogels also offers a non-invasive method of drug delivery which can improve the patient's compliance and their quality of life (Kondiah et al., 2022).

Previously, Salerno et al. (2010) showed the ability of CMC hydrogel to deliver microemulsion containing fluconazole, an antifungal drug, across porcine skin *in vitro* to treat topical mycosis. CMC hydrogel was also being utilised to deliver nanoparticles loaded with hydrocortisone which was shown to interact with the steroid receptors in the skin and could potentially treat inflammatory skin condition such as psoriasis vulgaris (Kondiah et al., 2022). Dermal delivery of

quercetin, a plant-derived flavonoid, using CMC-based hydrogel was proven through *ex vivo* porcine skin permeation study by Szulc-Musioł et al. (2023). Besides, *in vivo* studies using Sprague Dawley rates by Kharwade et al. (2023) proved the ability of CMC-based hydrogel loaded with tioconazole transferosomes to treat atopic dermatitis when applied topically. In addition, CMC-based hydrogels have been developed for wound healing purposes as demonstrated by Shah et al. (2023) where an injectable CMC-chitosan hydrogel containing curcumin micelles successfully promoted cell migration, tissue regeneration and angiogenesis. Sadeghi et al. (2020) reported a sustained release of water-soluble clindamycin over seven days from CMC-keratin hydrogel. The hydrogel also showed potential for wound treatment as the hydrogel exhibited antibacterial properties, allowed cellular attachment and spreading. Whereas Aldaghi et al. (2024) showed enhanced pressure ulcer healing through *in vivo* studies on rat model using doxycycline-loaded CMC-based hydrogel when applied topically. Dermal delivery using hydrogels that contain active ingredients are also found in the current market with CMC as one of the polymers including Luxe Organix® Hydrocolloid Acne Patch, HRFZ RAIN Acne Removal Patches and Tiger Balm® Pain Relieving Patch. CMC is also used in some semi-solid skin care products such as Pacifica® Rose Jelly Beauty Sleep Undereye Gel, Nelly De Vuyst® Bioacne Purifying Gel and Oxygen Botanicals™ Hydrating Mask. With these studies and the commercialised products, CMC hydrogel has shown to be a promising platform to be incorporated with different drugs or active ingredients for dermal delivery.

1.4 Three-dimensional (3D) printing of hydrogels

3D printing is a type of manufacturing technique where the product is formed as the materials are added together, hence also known as additive manufacturing. 3D printing is a computer-controlled process based on the digital model data to produce 3D construct through layer-by-layer deposition of materials (Polamaplly et al., 2019; Wang et al., 2017). This technology is in contrast to the conventional manufacturing processes such as computer numerical control machining where the product is formed through removal of unwanted material and moulding which forms product by re-forming a material (Jayawardane et al., 2022; Kong, 2023). 3D printing has shown greater capabilities and promising results in fabricating detailed and complex structures that could not be done via the conventional ways.

Advancements in high-precision manufacturing and biotechnology have significantly expanded the use of 3D printing technology for biomedical and pharmaceutical applications. Compared to the traditional manufacturing, 3D printing of hydrogels offers several advantages, especially as pharmaceutical products. Firstly, 3D printing allows personalisation of drug delivery platform based on the patients' needs such as their required dose, disease severity, targeted area and anatomical characteristics (de Oliveira et al., 2021; Zhang and Wang, 2022). For instance, an anti-acne mask loaded with salicylic acid was 3D printed based on the shape of the nose of an individual, showing the potential to 3D print various structures of mask with adjustable dose of salicylic acid for different patients with acne vulgaris (Goyanes et al., 2016). Through 3D printing, small-scale manufacturing of customised drug delivery platform can be realised with a lower production cost. Moreover, rapid and on-demand

manufacturing of hydrogels can be achieved via 3D printing (Okwuosa et al., 2018; Zhang and Wang, 2022). This feature is important when need to prepare a delivery platform with a specific drug formulation within a short period of time. Furthermore, complex shape and structure of hydrogels can be fabricated via 3D printing. This is particularly advantageous for wound healing as 3D printed wound dressings can be customised for different wound types including diabetic, burn, infectious, tumour, pressure and trauma wounds with good applicability (Zhang and Wang, 2022; Zhou et al., 2023).

A variety of 3D printing technologies are used for the hydrogel fabrication such as inkjet-based, extrusion-based and photopolymerisation-based 3D printing methods (Kaliaraj et al., 2023). Inkjet-based 3D printing works by depositing polymer ink droplets either continuously or dropwise at the set locations (Cheng et al., 2024a). While extrusion-based 3D printing involves the use of external force either air pressure or mechanical pressure to extrude the polymer ink out of a nozzle to build the 3D model (Kaliaraj et al., 2023). On the other hand, photopolymerisation-based technique uses a light source to cure a photosensitive liquid polymer resin (Kaliaraj et al., 2023).

1.4.1 Vat photopolymerisation

One of the 3D printing technologies is the vat photopolymerisation method, such as stereolithography (SLA) and digital light processing (DLP) and continuous liquid interface production (CLIP) (Figure 1.4). SLA utilises a movable photon source to cure resin through point-by-point exposure, printing objects layer by layer. On the other hand, DLP uses a projected light source to

cure an entire layer at once, resulting in a faster printing than SLA. While CLIP is a process that employs an oxygen permeable window to create a thin, oxygen-rich layer, known as the dead zone, that inhibits free radical photopolymerisation, allowing a layerless printing of monolithic objects (Bagheri and Jin, 2019).

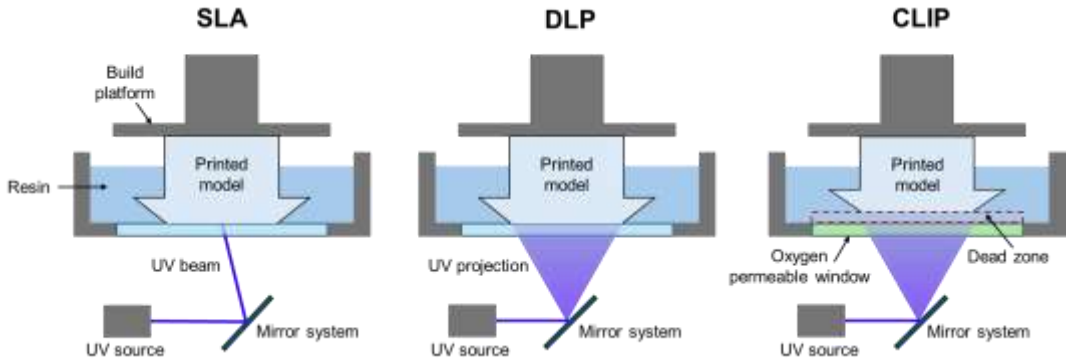


Figure 1.4 3D printing technologies based on vat photopolymerisation method including stereolithography (SLA) and digital light processing (DLP) and continuous liquid interface production (CLIP) (adapted from Lee (2024); Shebeeb et al. (2023))

Vat photopolymerisation involves curing and polymerising of a vat or tank of liquid monomers or oligomers on a platform when exposed to light with a certain wavelength (Bagheri and Jin, 2019; Layani et al., 2018). In this process, chain growth is driven by the reactive species which is converted from photolytic energy by photoinitiator or photoinitiator system under light irradiation (Bagheri and Jin, 2019). The light source used in 3D printing can be either UV, visible or near-infrared light. Some UV light-sensitive photoinitiators such as diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate (LAP) and (2,2,6,6-tetramethyl 1-piperidinyloxy) showing light absorption at more than 400 nm have also been used in 3D printing under visible light (Palaganas et al., 2017; Park et al., 2018).

Although extrusion-based techniques are more widely used in 3D printing for hydrogel, these techniques are limited by their low printing speed, low resolution and they only work on resin with high viscosity (Malda et al., 2013; Mandrycky et al., 2016). Based on the resin's rheology and object orientation, extrusion printing tends to result in structural warping or deformation (Caprioli et al., 2021; Jungst et al., 2016; Schwab et al., 2020). On the other hand, vat photopolymerisation allows 3D printing of geometrically complex hydrogels in a shorter amount of time with higher resolution (Caprioli et al., 2021; Mandrycky et al., 2016). Investigations have been carried out to enable extrusion-based 3D printing at a lower temperature for formulations containing heat-sensitive drugs such as diflunisal, rifampicin, dipyridamole and ramipril (Abdella et al., 2021; Guo et al., 2014; Kollamaram et al., 2018; Lee et al., 2020; Okwuosa et al., 2016). However, vat photopolymerisation may be a better alternative for 3D printing of drug delivery platforms as the photopolymerisation method occurs at room temperature, reducing the risk of heat-induced drug degradation (Ansari and Kamil, 2021; Zhang et al., 2022a). Moreover, fine tuning of resin physiochemical properties such as volatility, surface tension and rheology is not required for vat photopolymerisation (Jungst et al., 2016; Layani et al., 2018; Schwab et al., 2020). This can eventually save time and cost for the development and optimisation of new material for 3D printing.

1.4.1(a) Methacrylation of polymers

The photopolymerisation occurs using either the radical system or the cationic system. In the radical photopolymerisation, (meth)acrylate-based

photocurable system is commonly utilised. When exposed to light, the photoinitiator converts photolytic energy into reactive species which leads to initiation of photopolymerisation of (meth)acrylate-based resin. The free radicals react with carbon-carbon double bonds, resulting in crosslinking between the monomers (Mishbak et al., 2019) (Figure 1.5). To develop a photocurable resin, the polymer used can be chemically modified through methacrylation which incorporates double bonds that can react to form crosslinking network upon light irradiation (Wang et al., 2023a). Some of the commonly used (meth)acrylate-based resins are 2-hydroxyethyl methacrylate, methyl methacrylate and triethylene glycol dimethacrylate (Jin et al., 2022a). Nonetheless, these resins have led to allergic, genotoxic and cytotoxic effects along with concentration-dependent cellular necrosis and apoptosis (Jin et al., 2022a).

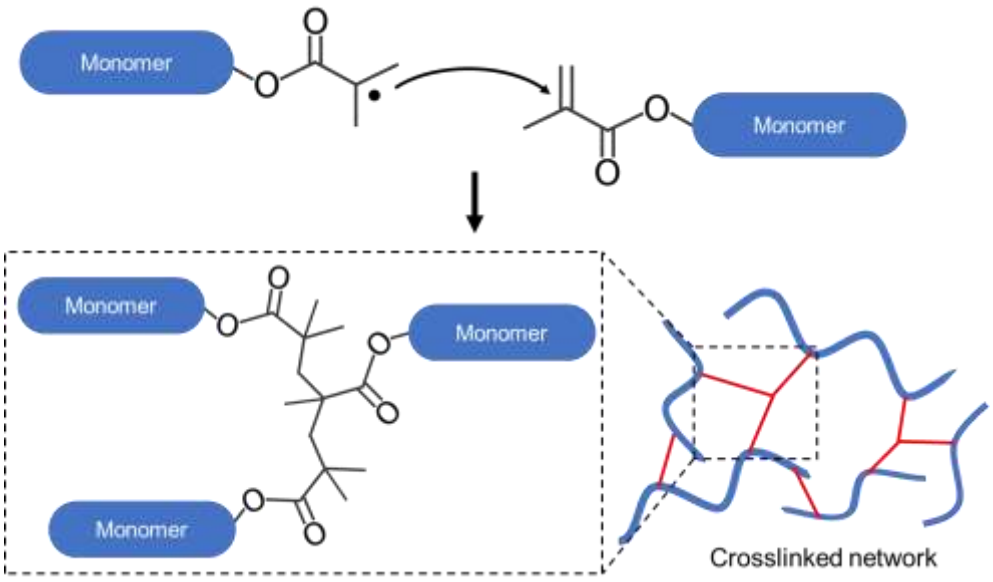


Figure 1.5 Schematic diagram representing the photopolymerisation process of methacrylate-based monomer (adapted from Mishbak et al. (2019))

In order to overcome the toxicity issues, biopolymers have been developed for the hydrogel fabrication due to their biocompatibility, biodegradability and biofunctionality (Luca et al., 2023; Muir and Burdick, 2021). Biopolymers such as gelatin, hyaluronic acid, chitosan, cellulose, collagen, xanthan and dextran were shown to be successfully modified using methacrylic anhydride (MA) or glycidyl methacrylate (Ali et al., 2022; Klak et al., 2024; Luca et al., 2023; Nichol et al., 2010; Spearman et al., 2020; Van Den Bulcke et al., 2000; Wang et al., 2023a; Wu et al., 2017; Zanon et al., 2022).

Several studies have reported methacrylation of cellulose and its derivatives for 3D printing. For example, Cafiso et al. (2022) carried out methacrylation on both CMC and cellulose nanocrystals for 3D printing of hydrogels. Stalling et al. (2009) showed the development of photocrosslinkable methacrylated methylcellulose to fabricate hydrogels for soft tissue reconstruction. Besides, methacrylation of cellulose nanofibrils and cellulose nanocrystals was conducted by Ma et al. (2020) to develop UV-curable resin.

1.4.1(b) Digital light processing (DLP)

In DLP, the light source illuminates each layer of resin all at once from the bottom of the resin, unlike SLA which is based on point-by-point curing (Figure 1.4). Instead of oxygen-permeable window used in CLIP, the fluorinated ethylene propylene film in DLP prevents the curing layer to be in direct contact with oxygen which can inhibit the photopolymerisation (Bagheri and Jin, 2019). By using 3D modelling software, 3D models in STL file, a commonly used file format for 3D printing, can be generated which is then processed in slicing

software before being 3D printed. The resin solidifies layer by layer as each layer conforms to the mask pattern projected from the bottom of resin vat. Once the photopolymerisation of a layer is complete, the motorised stage moves the printing build platform along the Z-axis. This process repeats, with new image for each layer initiating further photopolymerisation, until the entire 3D structure is fully formed (Figure 1.6). This technique consumes less resin as the building platform is dipped into the resin and move upwards as each layer is cured (Bagheri and Jin, 2019).

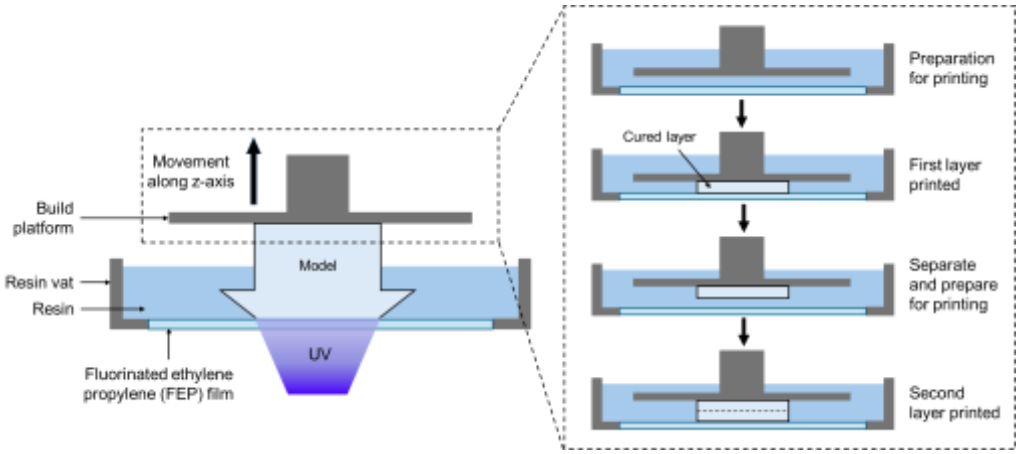


Figure 1.6 Schematic diagram of the working procedure of DLP

DLP technology utilises either digital micromirror devices (DMD) or liquid crystal display (LCD) in its light engine. In DMD-based DLP, the light generated passes through the DMD, which is made up of arranged cells of microscopic mirrors. With each mirror representing one pixel in the dynamic bitmap image, the light is then projected precisely according to the image onto the resin (Nam and Kim, 2024). While LCD-based DLP uses LCD screen to generate images of each layer which allows the light to pass through following the image and project directly onto the resin instead of redirected light projection (Nam and Kim, 2024; Wang et al., 2023a). Despite having a lower contrast and limited