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# EXTRUSION-BASED 3D BIOPRINTING WITH NATURAL-BASED BIOINK FOR THE PRODUCTION AND IN VITRO CHARACTERIZATION OF A HUMAN SKIN MODEL

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**A THESIS OF MASTER OF SCIENCE**
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**LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ**

# DOĞAL BİYOİNK KULLANILARAK EKSTRÜZYON TABANLI 3D BİYOBASKI İLE İNSAN DERİ MODELİNİN ÜRETİMİ VE İN VİTRO KARAKTERİZASYONU

**GAMZE EREN**

**YÜKSEK LİSANS TEZİ**
**BİYOMÜHENDİSLİK ANABİLİM DALI**

DANIŞMAN: DOÇ. DR. HAKAN OFLAZ

**ŞUBAT 2025**

**MASTER of SCIENCE JURY APPROVAL FORM**

A thesis submitted by Gamze EREN, defended on 28/02/2025 before the jury formed with the 17/02/2025 date and 2025/13 numbered decision of the GTU Graduate Administration Board, has been accepted as a MASTER of SCIENCE thesis in the Department of Bioengineering.

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**APPROVAL**

Gebze Technical University Graduate Administration Board

……/……./…… date and ……/…….. numbered decision.

SIGNATURE/SEAL

*To those who lit my path when the way was dark – this is for you.*

# ABSTRACT

Studies in the field of tissue engineering are increasing day by day. The most important reasons for these are skin injuries caused by burns, chronic wounds, and surgical interventions in the skin tissue. Many treatment methods are currently used. Autografts, allografts, and xenografts are some of them. However, since traditional treatment methods have inadequate mechanical properties and poor compatibility with tissue, new production technologies are needed in this field.

In this study, a human skin model was simulated using natural-based bioinks and a poli(ε-caprolactone) (PCL) mesh structure. Extrusion-based 3D bioprinting (EBB) was used to develop it. The focus of the skin structure is to successfully create dermal and epidermal structures. Since these skin structures consist of many different cells and layers, precise and layered skin models were produced using the Dr. INVIVO 4D2 brand bioprinter. Collagen-based bioink containing fibroblasts and keratinocytes was produced layer by layer on the PCL mesh structure to create the multi-layered structure of the skin structure. The PCL mesh structure improved the mechanical properties of the collagen structure and provided a porous architecture that facilitated efficient nutrient and oxygen exchange critical for cell viability and functionality.

A number of in vitro tests were performed to evaluate the performance of the artificial skin models. The dermal layer containing human dermal fibroblasts (HDFs) and the epidermal layer consisting of human epidermal keratinocytes (HEKs) were examined microscopically, and layers and cell viability like natural skin tissue were obtained. Fibroblasts actively synthesized ECM, and keratinocytes formed a barrier-like structure. The viscosity of the bioink was confirmed by rheological test. The model integrated into the PCL mesh structure obtained values like natural skin tissue as a result of mechanical testing.

When all these outputs were evaluated, it was determined that the artificial skin models produced were both more economical and more functional. By significantly reducing production time and costs with this approach, it provides a scalable platform for skin tissue engineering and offers promising solutions for the treatment of serious skin injuries and the advancement of wound healing therapies.



**Keywords: 3D Bioprinting, Extrusion-Based Bioprinting (EBB), Human Skin Model, Natural-Based Bioink, Skin Tissue Engineering, Wound Healing**

# ÖZET

Doku mühendisliği alanındaki çalışmalar her geçen gün artmaktadır. Bunun en önemli nedenleri, deri dokusunda meydana gelen yanıklar, kronik yaralar ve cerrahi müdahalelerle oluşan deri hasarlarıdır. Şu anda birçok tedavi yöntemi kullanılmaktadır. Otoğreftler, allogreftler ve xenogreftler bunlardan bazılarıdır. Ancak, geleneksel tedavi yöntemlerinin mekanik özellikleri yetersiz ve doku ile uyumsuz olduğu için bu alanda yeni üretim teknolojilerine ihtiyaç duyulmaktadır.

Bu çalışmada, doğal bazlı biyoyazılar ve poli(ε-kaprolakton) (PCL) ağ yapısı kullanılarak bir insan deri modeli simüle edilmiştir. Ekstrüzyon tabanlı 3D biyoprinting (EBB) tekniği ile bu model geliştirilmiştir. Deri yapısının odak noktası, dermal ve epidermal yapıların başarıyla oluşturulmasıdır. Bu deri yapıları birçok farklı hücre ve katmandan oluştuğundan, Dr. INVIVO 4D2 marka biyoprinter kullanılarak hassas ve katmanlı deri modelleri üretilmiştir. Fibroblastlar ve keratinositler içeren kollajen bazlı biyoyazı, deri yapısının çok katmanlı yapısını oluşturmak için PCL ağ yapısı üzerinde katman katman üretilmiştir. PCL ağ yapısı, kollajen yapısının mekanik özelliklerini iyileştirmiş ve hücrelerin canlılığı ve fonksiyonelliği için kritik olan besin ve oksijen değişimini kolaylaştıran poröz bir mimari sağlamıştır.

Yapay deri modellerinin performansını değerlendirmek için bir dizi in vitro test yapılmıştır. İnsan dermal fibroblastları (HDF'ler) içeren dermal katman ve insan epidermal keratinositlerden (HEK'ler) oluşan epidermal katman mikroskobik olarak incelenmiş ve doğal deri dokusuna benzer katmanlar ve hücre canlılığı elde edilmiştir. Fibroblastlar aktif olarak EKZ sentezlemiş, keratinositler ise bariyer benzeri bir yapı oluşturmuştur. Biyoyazının viskozitesi, reolojik test ile doğrulanmıştır. Model, mekanik testler sonucu PCL ağ yapısına entegre edilerek, doğal deri dokusuna benzer değerler elde etmiştir.

Tüm bu çıktılar değerlendirildiğinde, üretilen yapay deri modellerinin hem daha ekonomik hem de daha fonksiyonel olduğu belirlenmiştir. Bu yaklaşım ile üretim süresi ve maliyetleri önemli ölçüde azaltılarak, deri doku mühendisliği için ölçeklenebilir bir platform sunulmuş ve ciddi deri yaralarının tedavisi ile yara iyileşme terapilerinin ilerletilmesi için umut verici çözümler ortaya konulmuştur.

**Anahtar Kelimeler: 3D Biyoprinting, Ekstrüzyon Tabanlı Biyoprinting (EBB), İnsan Deri Modeli, Doğal Bazlı Biyoyazı, Deri Doku Mühendisliği, Yara İyileşmesi**

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Thank you all for being a part of this milestone in my academic life.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| % | : Percent |
| 3D | : Three-Dimensional |
| 3mPa/s | : Millipascal-second |
| μm | : Micrometer |
| ml | : Milliliter |
| kPa–MPa | : Kilopascal–Megapascal |
| Pa | : Pascal |
| °C | : Degrees Celsius |
| CSS | : Cultured Skin Substitutes |
| DPBS | : Dulbecco's Phosphate-Buffered Saline |
| EBB | : Extrusion-Based Bioprinting |
| ECM | : Extracellular Matrix |
| HDFs | : Primary Dermal Fibroblasts |
| HEKs | : Human Epidermal Keratinocytes |
| KCs | : Keratinocytes |
| MCs | : Melanocytes |
| PCL | : Poly(ε-caprolactone) |
| PGA | : Polyglycolic Acid |
| PLA | : Polylactic Acid |
| PLGA | : Poly (lactic-co-glycolic acid) |
| STL | : Stereolithography |
| UV | : Ultraviolet |
| VEGF | : Vascular Endothelial Growth Factor |

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# 1. INTRODUCTION

## 1.1. Introduction to Skin Injuries and Current Treatments

### 1.1.1. Statistics and Impact of Skin Injuries

The biggest organ in the body, the skin, makes up over 16% of an adult's total body weight [1]. It has many different functions. These include a range of functions such as protection, temperature regulation, and sensory perception. Due to the large surface area of our body, the skin is exposed to a wide variety of environmental factors and serves as the body's first line of defense. Exposure to these environmental factors can cause wounds to form on the skin. Wounds are injuries that damage the skin [2]. Depending on the healing processes, wounds are classified as acute or chronic. Chronic wounds can last longer than four weeks, while acute wounds can heal within a few days. Different degrees of skin injuries are shown in Figure 1.1[3].

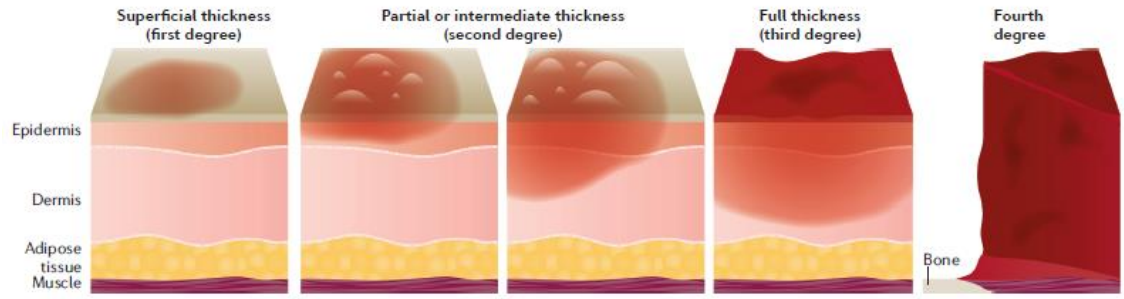


**Figure 1.1:** Different degrees of skin injuries affect.

Approximately 6.5 million people in the U.S. suffer from chronic wounds, and this condition drives annual healthcare costs above $25 billion. This issue, which has significant social and economic consequences, needs to be addressed with rapid and effective treatment techniques [4].

Skin tissue is a complex structure made up of many different layers [5]. The outermost layer is the epidermis. This structure is made up of keratinocytes (KCs).

Keratinocytes protect the skin against damage by heat and UV radiation and form the first protective barrier against harmful microorganisms [6]. It also contains melanocytes (MCs) and Merkel cells. The next layer in the middle is the dermis layer.

This structure contains blood vessels leading to the skin. In addition, the sebaceous gland, sweat gland and nerves are in this layer. The inner part of the skin tissue is hypodermis. Adipose tissue is in this layer and provides mechanical protection and temperature regulation for the body. Details of these structures are shown in Figure 1.2 [7].

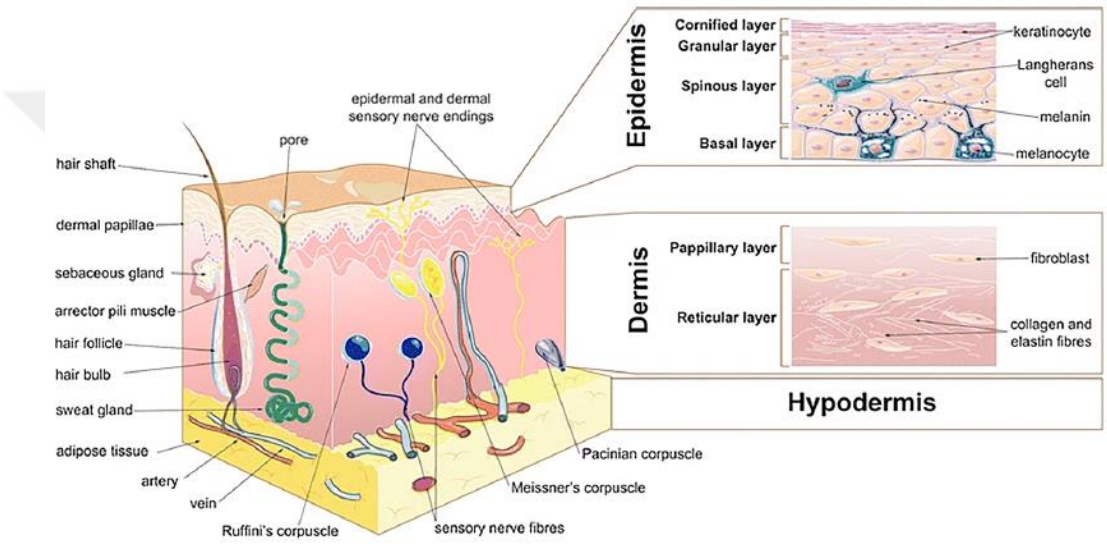


**Figure 1.2:** Schematic representation of the multilayered structure of human skin.

## 1.1.2. Overview of Current Treatment Options and Their Limits

Many different techniques are used in skin injuries. The main techniques include autografts, allografts, and xenografts. Autografts are the primary treatment method for healing skin defects. However, the technology is limited due to the inadequacy of donor sites. The same problem applies to allografts and xenografts as well. Due to the limited number of donors, they cannot repair large-scale skin damage [8].

At the same time, these technologies are also at risk of immune rejection [9]. In addition to repairing damaged skin, functional artificial skin is also used in other important areas such as drug development and screening, research on disease mechanisms, and testing cosmetic properties.

Although skin tissue engineering has revolutionized the production and application of artificial skin, there are still limitations such as simple tissue structure, the absence of skin functional units (glands, sensory neurons, hair follicles, etc.), and poor structural controllability. Therefore, there is a need to develop new methods for artificial skin.

## 1.2. Emerging Technologies in Skin Repair

### 1.2.1. Introduction to 3D Bioprinting Technologies

The main goal of tissue engineering is the reconstruction of damaged tissues, organs, and cells [10]. In recent years, various bioprinting technologies have developed due to the inadequacy of traditional manufacturing methods used in tissue repair. In Figure 1.3, the chronological development of bioprinting technologies is shown [11].

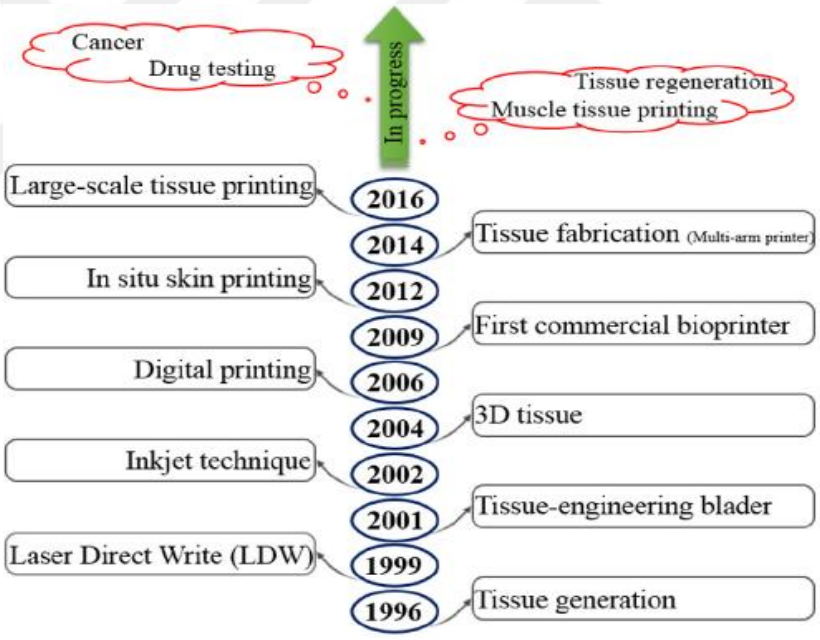


**Figure 1.3:** A schematic representing the progress of 3D bioprinting techniques.

Bioprinting technologies are fundamentally examined under three main headings [12]. These technologies include inkjet bioprinter, microextrusion bioprinter and laser-assisted bioprinter [13]. Complex tissue constructs are frequently created using the three primary bioprinting methods shown in Figure 1.4 [14].

These technologies are commonly used to create complex tissue constructs such as multilayered skin, circulatory networks, and organ-like structures. The most principal

elements in 3D bioprinting should be evaluated in terms of surface resolution, cell viability and biological materials used for printing.

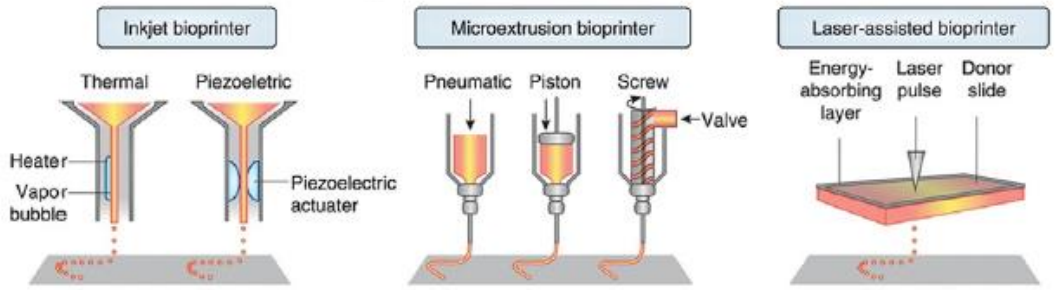


**Figure 1.4:** The three main bioprinting techniques.

Inkjet-based bioprinting is one of the oldest bioprinting techniques that uses bioinks, which are natural or synthetic substances that support cell adhesion, growth, and proliferation [12]. In this method, the bioink is passed through a nozzle to create droplets. Printers can have single or multiple printheads, each equipped with a chamber and a nozzle. Pressure pulses transmitted through piezoelectric, thermal, or electrostatic mechanisms set the bioprinter ink droplets in motion [15]. In piezoelectric inkjet systems, pressure pulses generated by an actuator deposit the bioink and sometimes require back pressure for droplet formation. Thermal inkjet printers create a vapor bubble that ejects droplets onto a scaffold by heating the bioink locally. Electrostatic bioprinters use voltage to manipulate fluid dynamics by creating and depositing bioprinter droplets as pressure plates are released [16]. These techniques enable the precise deposition of biological materials, including proteins and mammalian cells.

Extrusion-based bioprinting (EBB) is a technology that uses pressure on bioink to regenerate and repair tissues [1]. In this procedure, the bioink is deposited and extruded onto the substrate using mechanical pressure, often pneumatic pressure, a screw mechanism, or a piston [14]. The printhead can transfer bioink directly onto the substrate by moving in three directions: x, y and z.

Extrusion-based printers can handle bioinks with high cell density, hydrogels of varying viscosities, and biodegradable thermoplastics such polycaprolactone [17]. A detailed comparison of technologies is in the Table 1.1[14].

Laser-assisted bioprinting is another popular technique for printing living cells onto substrates [18]. This method is performed using a high-intensity light source or long-wavelength light. The main parts of a laser bioprinter consist of a laser pulse, a focusing lens, a donor slide, an energy absorption layer, a donor substrate and a collector slide [11]. The focusing lens directs the intense light to transfer the bioink onto the collector slide and then performs the printing process. Unlike inkjet printers, laser printers do not have nozzles. This allows the bioink to be deposited at high densities without the risk of clogging [14].

**Table 1.1:** Comparison of bioprinter types.

| | **Bioprinter Type** | | |
|---|---|---|---|
| | **Inkjet** | **Microextrusion** | **Laser-assisted** |
| **Material viscosities** | 3.5–12 mPa/s | 30 mPa/s to >6 × 107 mPa/s | 1–300 mPa/s |
| **Gelation methods** | Chemical, photo-crosslinking | Chemical, photo-crosslinking, sheer thinning, temp. | Chemical, photo-crosslinking |
| **Preparation time** | Low | Low to medium | Medium to high |
| **Print speed** | Fast (1–10,000 droplets per second) | Slow (10–50 μm/s) | Medium-fast (200–1,600 mm/s) |
| **Resolution or droplet size** | <1 pl to >300 pl droplets, 50 μm wide | 5 μm to millimeters wide | Microscale resolution |
| **Cell viability** | >85% | 40–80% | >95% |
| **Cell densities** | Low, <106 cells/ml | High, cell spheroids | Medium, 108 cells/ml |
| **Printer cost** | Low | Medium | High |

### 1.2.2. Advantages of 3D Bioprinting over Traditional Methods

3D bioprinting, a subset of additive manufacturing (AM), is the process of creating products out of living cells, biomaterials, and biological molecules. Unlike previous efforts, bioprinting allows for the creation of scaffolds with precise microarchitectures that give mechanical stability and promote cell development while avoiding undesirable repercussions such solvent cytotoxicity or pressure-induced apoptosis during material extrusion [16].

The capacity to integrate cells directly into scaffolds during fabrication is one of the main benefits of 3D bioprinting, eliminating the homogeneity issues associated with post-fabrication cell seeding. In vivo, scaffolds with homogeneous distribution loaded with cells have shown homogeneous tissue growth, less risk of rejection and faster integration with host tissues.

On the other hand, conventional static or dynamic cell seeding techniques often lead to reduced yields or unwanted morphological changes in cells [19]. The main difficulties of traditional tissue engineering methods are summarized in Table 1.2.

**Table 1.2:** Limitations of traditional tissue engineering methods.

| **The main challenges of traditional tissue engineering methods** |
|---|
| Difficulty in controlling cells, material composition, and pore sizes. |
| Adverse effects caused by long pre-processing times. |
| Data is inconsistent due to human error. |
| Dependence on animal-derived components. |
| Formation of necrotic cores. |

## 1.3. Objective of the Thesis

### 1.3.1. To Produce Human Skin Model Using Extrusion-Based 3D Bioprinting and Natural-Based Bioink

The main aim of this thesis is to examine the limitations of traditional skin grafts and to offer alternative solutions to skin injuries. The main parameters focused on are innovative production methods and the use of natural based materials.

As seen in the reviewed literature, extrusion based 3d bioprinter technique is an ideal method for this study. Using the Dr. INVIVO 4D2 bioprinter, this study aims to produce a multi-layered skin structure incorporating a polycaprolactone (PCL) scaffold and collagen-based bioink.

The primary goal is to achieve precise spatial placement of key skin cell types, including fibroblasts, keratinocytes, and melanocytes, to facilitate the formation of a layered epidermal structure and a robust dermal matrix.

### 1.3.2. To Conduct in-vitro Characterization Studies of the Produced Human Skin Model

The study also aims to evaluate the mechanical properties, porosity, and biological functionality of the created skin substitutes. Through in-vitro characterization, the thesis aims to demonstrate the applicability of this approach in supporting epidermal stratification, cellular integrity, and tissue functionality.

Determining the structural and functional integrity of the designated skin model requires in vitro characterization. To confirm that the skin model can withstand physiological stress and mimic the biomechanical behavior of human skin, the mechanical characteristics of the constructions such as tensile strength, elasticity, and durability will be examined.

The characterization process includes:

1. Rheological Testing: Measures the viscosity and shear behavior of collagen bioink to ensure its suitability for bioprinting.
2. Mechanical Testing: Assesses tensile strength, elasticity, and durability of the skin model using an Instron Universal Testing System, comparing it to human skin.

3. Porosity and Water Absorption: Evaluates the moisture retention and porosity to ensure proper cell infiltration and nutrient exchange.
4. Cell Viability Testing: Assesses the health and metabolic activity of incorporated human keratinocytes (HEKs) and dermal fibroblasts (HDFs) using the LIVE/DEAD assay.

# 2. LITERATURE REVIEW

## 2.1. Overview of 3D Bioprinting Technologies

### 2.1.1. Different Types of 3D Bioprinting Technologies

Skin tissue studies are sought in literature as part of the development of bioprinting technology. Researchers have turned to 3D bioprinting due to limitations with existing approaches. When we examine the literature, the investigations undertaken by Lee et al. in 2009 are groundbreaking. For the first time, they used 3D bioprinting to print keratinocytes and fibroblasts in a layered configuration on flat and curved surfaces. They employed collagen-based bioink and demonstrated that the cells maintained their shape. However, the print resolution (~300 μm) impacted cell proliferation [20]. The pore size of the produced skin models should allow for cell proliferation. Otherwise, there will be no nutrient exchange in the produced tissue, and the cells cannot proliferate.

Another study, Binder et al., used a pig model to print a skin substitute including collagen, fibrinogen, keratinocytes, and fibroblasts over skin lesions assessed with a laser scanner. Epithelialization began within two weeks, and complete re-epithelialization occurred eight weeks later [21].

As technology has advanced over time, bioprinting techniques have also evolved. If we examine the research done in this area, Koch et al. They used the Lazer-assisted bioprinting technique in 2012 to print cells at high resolution on matriderm collagen and elastin-based framework. Consequently, basal lamina development and cell interactions with Cx43 protein were verified [22].

Numerous investigations have been made possible by this. In the following work, Michael et al. (2013) used a laser-assisted approach to imprint fibroblasts and keratinocytes into mouse skin lesions. Ki67 staining verified cell proliferation, however there was little vascularization or keratinocyte differentiation [23]. The researchers were led by the reasons for this.

In a later work, Lee et al. used a high-resolution robotic system (15 nl sensitivity) to print fibroblasts and keratinocytes. Over 95% cell viability was attained. For this investigation, cell viability was a significant accomplishment [24].

Cubo et al. then used a modified extrusion bioprinter to print a combination of human plasma, fibroblasts, calcium chloride, and keratinocytes in 35 minutes. The development of the stratum corneum and basal lamina was noted in a mouse model aged eight weeks [25]. This demonstrated imprinted skin's complete distinction and possible medical uses.

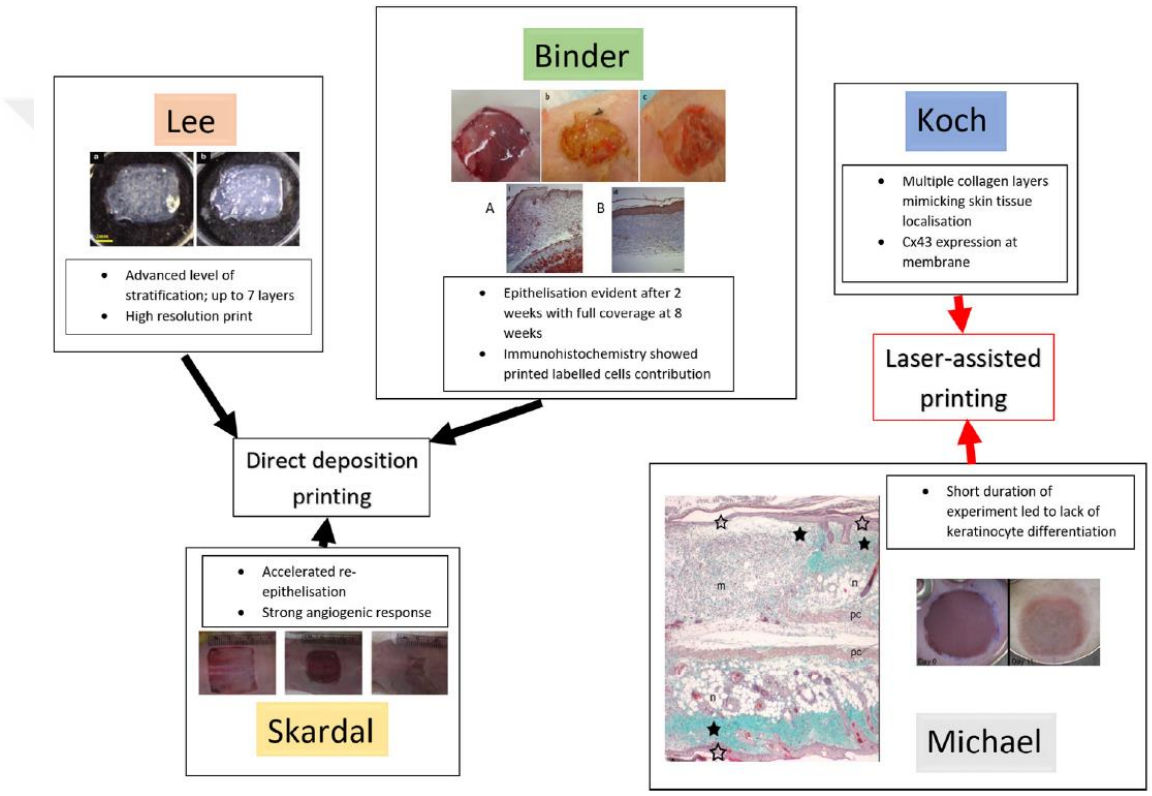


**Figure 2.1:** A selection of histology images from existing literature that provide an overview of bioprinted skin replacements [26].

### 2.1.2. Comparison and Selection Rationale for Extrusion-Based Bioprinting

Three primary topics were covered in the discussion of 3D bioprinting techniques. These three types of bioprinting are extrusion-based, inkjet-based, and laser-assisted [27]. Each technique deposits materials and cells using a distinct process. Because of its affordability, ease of usage, and ability to replicate tissue complexity, extrusion-based bioprinting (EBB) is the most popular approach in both research and commercial settings when we analyze the papers in the literature [28][29]. I therefore decided to use this approach for my thesis research.

When compared to alternative techniques, extrusion-based bioprinting (EBB) technology has numerous benefits [30]. The most significant benefit, if we list all of these, is that it supports a wide variety of materials and cell kinds. The regulated deposition of biomaterials with physiological cell density and superior interlayer accuracy is an additional benefit [31]. It results in comparatively less process-induced cell damage than other approaches. It permits the use of stem cells for a variety of purposes [32]. Figure 2.2 schematic representation of the stages of tissue engineering method with extrusion-based bioprinting [27].

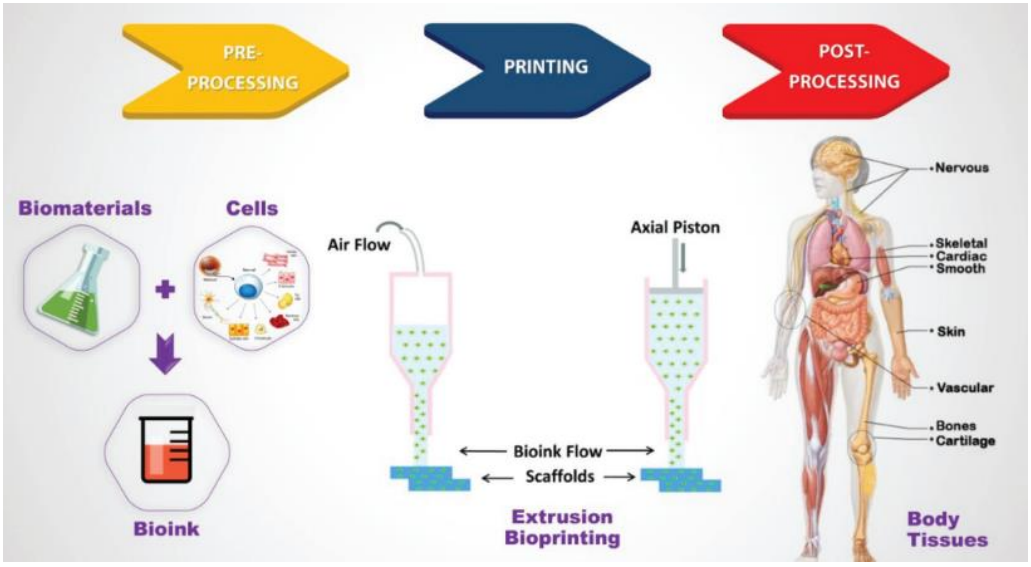


**Figure 2.2:** Schematic representation of the extrusion-based bioprinting tissue engineering method steps.

## 2.2. Natural-Based Bioinks

### 2.2.1. Types of Natural-Based Bioinks

Material selection is one of the most crucial steps in the 3D bioprinting procedure. The structures utilized in the bioprinting process are known as bioinks, and they are made from a mixture of different polymers, physiologically active chemicals, and living cells. Supporting tissue architecture and giving cells an appropriate habitat are the primary goals of the bioink. Bioinks fundamental building blocks are polymers [33]. Typically, bioinks take the shape of hydrogels made of either synthetic or natural polymers. Bioinks fall into three categories: composite, synthetic, and natural [34].

Bioinks derived from natural sources play a significant role in tissue engineering. Their capacity to replicate the extracellular matrix (ECM) of natural tissues, biocompatibility, and biodegradability are their most crucial properties. The list of natural bioinks and their attributes that have been often used in research is provided below Table 2.1 [34].

**Table 2.1:** Natural based bioink types and properties.

| Natural-Based Bioinks | Source | Advantages | Limitations |
|---|---|---|---|
| Collagen | Mammals | Biocompatibility, cell support [35] | Temperature sensitivity, mechanical weakness [35] |
| Gelatin | Hydrolyzed collagen | Low cost, high mechanical strength, cell adhesion [34] | - |
| Chitosan | Crustacean shells | Antibacterial, biodegradable [36] | Poor mechanical properties [36]. |
| Fibrin | Plasma proteins | Biocompatibility, cell adhesion [34] | Low viscosity, weak mechanical properties [34] |
| Alginate | Brown algae | Low cost, short gelation time, biocompatible [37] | No cell adhesion [37] |
| Hyaluronic Acid | Natural ECM | Biocompatibility, regenerative properties [38] | Structural instability [38] |

In my thesis research, I favored collagen-based bioink. This is because of its capacity to replicate the extracellular matrix (ECM) seen in nature, as well as its biocompatibility, biodegradability, and capacity to promote cell adhesion [34]. In tissue engineering applications, it promotes wound healing and offers a physiologically active environment. Its poor mechanical qualities, however, are a drawback. To get rid of this, synthetic polymers are utilized.

Collagen is the most abundant structural protein in mammals. It maintains the integrity of the external matrix (ECM) in various tissues and organs. It has many disadvantages. Cells can easily adhere and proliferate. Therefore, it is an ideal biomaterial for 3D printing. Accordingly, it also has some disadvantages.

It is highly sensitive to temperature and requires controlled conditions during preparation and handling to maintain its structural integrity. In addition, sterilization

processes can compromise its functional properties, making it difficult to ensure sterility without affecting its bioactivity [15].

Materials used for 3D bioprinting should have various properties [15].

Criteria for the best biomaterials for bioprinting:

- Tissue biocompatibility.
- Biodegradability at a pace that corresponds to the production of new tissue.
- Non-immunogenicity and non-toxicity.
- Ideal mechanical characteristics, such as strength, stiffness, and elasticity.
- Sufficient porosity and shape for signaling, nutrition exchange, and cell transport.
- Printability, which includes cross-linking ability, viscosity, and shear-thinning behavior.

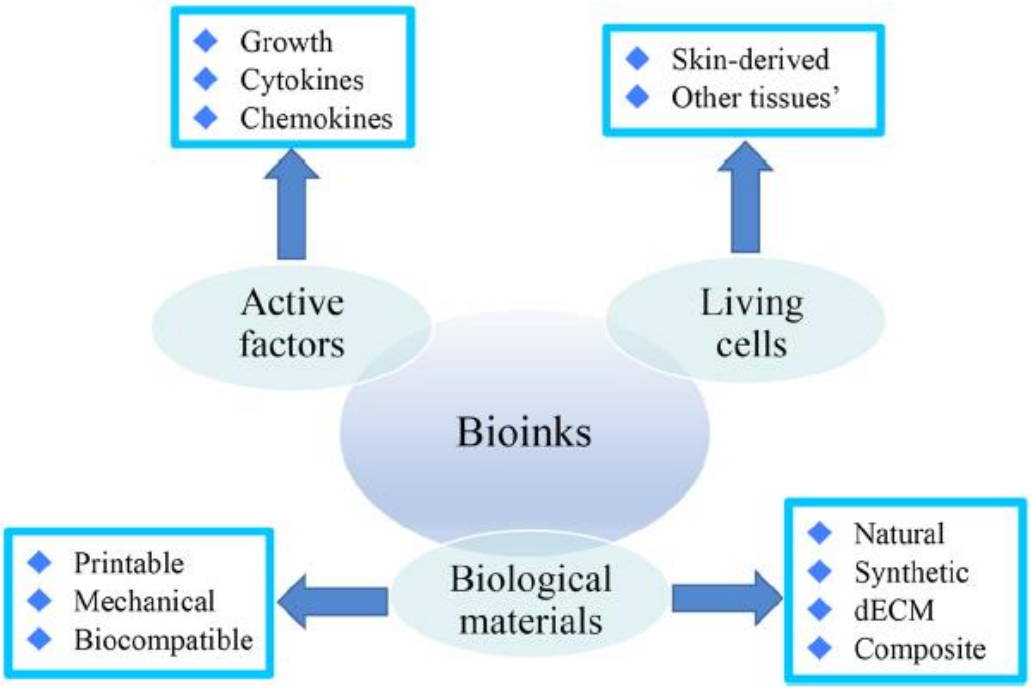


**Figure 2.3:** Bioink components for 3D bioprinting, as well as their formulations.

Another type employed in tissue engineering is synthetic biopolymers. Polymers include polylactic acid (PLA), polyglycolic acid (PGA), poly(ε-caprolactone) (PCL), poly (lactic-co-glycolic acid) (PLGA), polyanhydrides, polycarbonates, polyorthoesters, and polyurethane [34]. They are mostly employed as scaffolding

materials in tissue engineering. This is because they have excellent mechanical qualities.

The key advantage is the ability to customize mechanical properties and degradation kinetics by modifying the polymer structure to suit various biomedical applications, such as bone or skin grafting. In my thesis, I used PCL biopolymer to support the mechanical properties of collagen. Among the most crucial grounds for my decision are that it is biodegradable and has mechanical qualities that are appropriate for my research.

### 2.2.2. Design-Driven in Situ Constructive Modeling for a SuitableSkin Substitute

The extracellular matrix (ECM) material of an ideal skin substitute aims to mimic a microenvironment that facilitates cellular renewal processes (adhesion, proliferation, and differentiation). Biomaterials used in tissue engineering should have qualities comparable to skin tissue. Table 2.2 displays the features, composition, and functions of the skin tissue layers [39]. The skin models developed should have similar qualities. For this, the qualities of the biomaterials used are critical. Biopolymers have a strong ability to mimic ECM and stimulate cellular activity, making them ideal for building dermal and epidermal layers. Collagen and gelatin are examples [39].

**Table 2.2:** Properties, composition, and functions of several layers of skin tissue.

| Layer | Thickness | Composition |
|---|---|---|
| Epidermis | ~95 μm [40] | Keratinized, epithelium |
| Dermis | 1.4 mm [40] / 1.094–1.033mm [41] | Blood vessels, nerves, hair follicle |
| Hypodermis | 0.8 mm [40] / 1.1–3.16 mm [42] | Blood vessels, nerves, hair follicle |

Synthetic polymers have high mechanical strength and configurable breakdown rates. As an example, consider PCL and PLGA. When these polymers are mixed with natural polymers, mechanical and biological constraints are reduced. Table 2.3 displays the skin's mechanical, viscoelastic, and structural properties [39]. When creating skin models, these criteria should be used as a guide.

**Table 2.3:** Mechanical, viscoelastic, and structural characteristics of native human skin tissue.

| Name | Skin | Ref |
|---|---|---|
| Young's Modulus | 250 kPa-140 MPa subject to anatomical location | [43] |
| Tensile Strength | 15.9 MPa 0.06 is (quasistatic)<br>25.8 MPa 167/s (intermediate) | [44] |
| Strain at Break | 1.17–3.07 | [44] |
| Stress Relaxation | 4.74 MPa (forearm) 7.8 MPa (forehead) | [45] |
| Final Absolute Relaxation | 0.01–0.04 MPa, forearm and submandibular | [45] |
| Storage Modulus G′ | 325 + 93.7 Pa to 1227.9 + 498.8 Pa | [46] |
| Loss Modulus G | 68.5 + 21.2 Pa to 189.9 + 56.0 Pa | [46] |
| Porosity | 200-400 μm porosity 68.53 + 5.8% mean pore | [47] |

Several factors need to be taken into consideration when developing the ideal skin model. They are as follows:

1. Porosity: The size of pores promotes tissue development, nutrition exchange, and cellular adhesion [48]. A pore size of 200–400 μm is ideal. Large pores enable proteins of any size to bind, whereas small pores restrict the area where proteins can bind [49]. The tissue's high surface area/volume ratio promotes cellular attachment and migration.
2. Biodegradability: When the artificial skin tissue produced starts to regenerate in the tissue destroyed in-situ productions, the artificial skin tissue begins to disintegrate. The most important point here is that the inward growth of blood vessels, fibroblasts, and epithelial cell proliferation requires at least three weeks for complete regeneration [49]. For this, the biodegradability of the structure should be adjusted accordingly. Another important point is that biomaterials should not cause unwanted foreign body reactions after disintegration [50].
3. Crosslinking: The chemical linking of two or more molecules together by covalent bonds is called crosslinking. 3D structures are formed by crosslinking

polymeric chains [51]. These structures promote cellular behaviors (e.g., adhesion, proliferation, and migration). Cross-linking reactions can be physically, chemically, or enzymatically driven. Figure 2.4 shows cross-linking methods [51]. Cross-linking methods directly affect mechanical and biochemical properties.

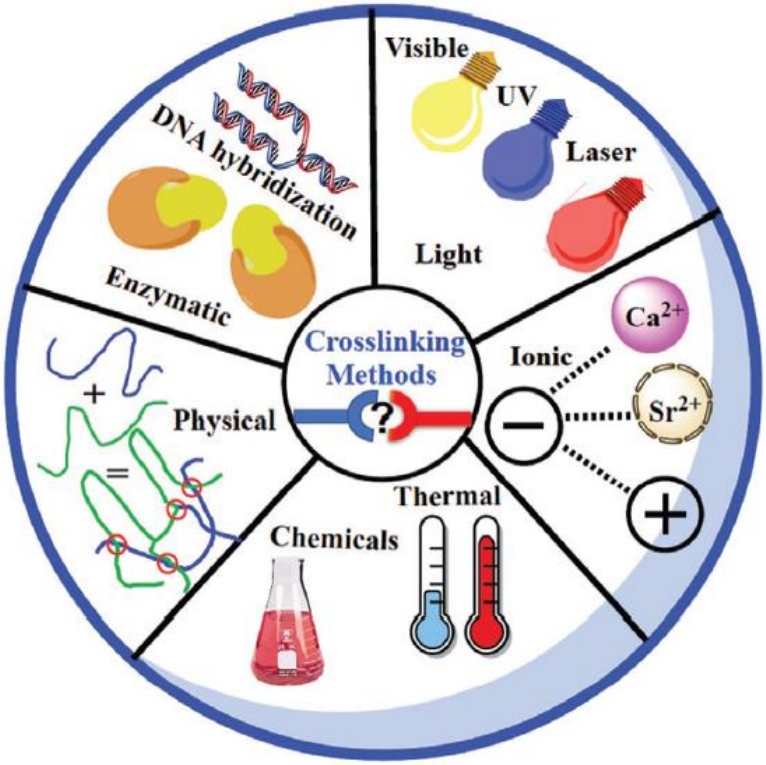


**Figure 2.4:** Several crosslinking methods have been employed for 3D bioprinting bioinks [51].

4. Surface topography: The surface characteristics of the generated skin tissue play a significant role in cell contact. The surface profile of the tissue influences blood protein adsorption and the healing process [52]. Different surface roughnesses have varying impacts on protein adsorption and cell responsiveness. The optimal surface topography may promote cellular adhesion and proliferation while inhibiting foreign body reaction [53].
5. Stiffness and stability: Cell differentiation is determined by ECM rigidity. Reducing micro-movements lowers the chance of implant rejection and avoids irritation [54].
6. Biocompatibility: The type of biomaterial implanted, and the subsequent healing response determines biocompatibility. Maximum tissue integration and

low inflammatory reaction are provided by biocompatible materials. The capacity of inert and biodegradable materials to create a local milieu for tissue integration, wound healing, and reconstruction would be considered biocompatibility in the context of in situ bioprinting [55].

## 2.3. Previous Studies on 3D Bioprinted Skin Models

### 2.3.1. Review of Past Research and Development

Numerous businesses have made investments in the creation of artificial skin using 3D bioprinting to cure a range of ailments and injuries, such as diabetic ulcers, chronic wounds, and burns.

Big businesses like Organovo, Procter & Gamble, and L'Oréal are leaders in this area. In this sense, both industry and academics are very interested in the investigation of artificial skin creation [56].

Rokit, the Korean market leader in this field, which develops many innovative devices for bioprinting, is an organization that leads this field and has a voice in the field of bioprinting thanks to its collaborations with many organizations around the world [57]. Dr. INVIVO 4D2 printer developed by this company was used in the thesis study.

There are many artificial skin substitutes commercially produced and under development [58]. Despite significant advances, the complexity of human skin is still difficult to mimic.

Although no engineered skin substitute mimics natural structure and function, many substitutes have proven to be effective in wound healing. Commercially produced skin models and details are listed in Table 2.4 [59].

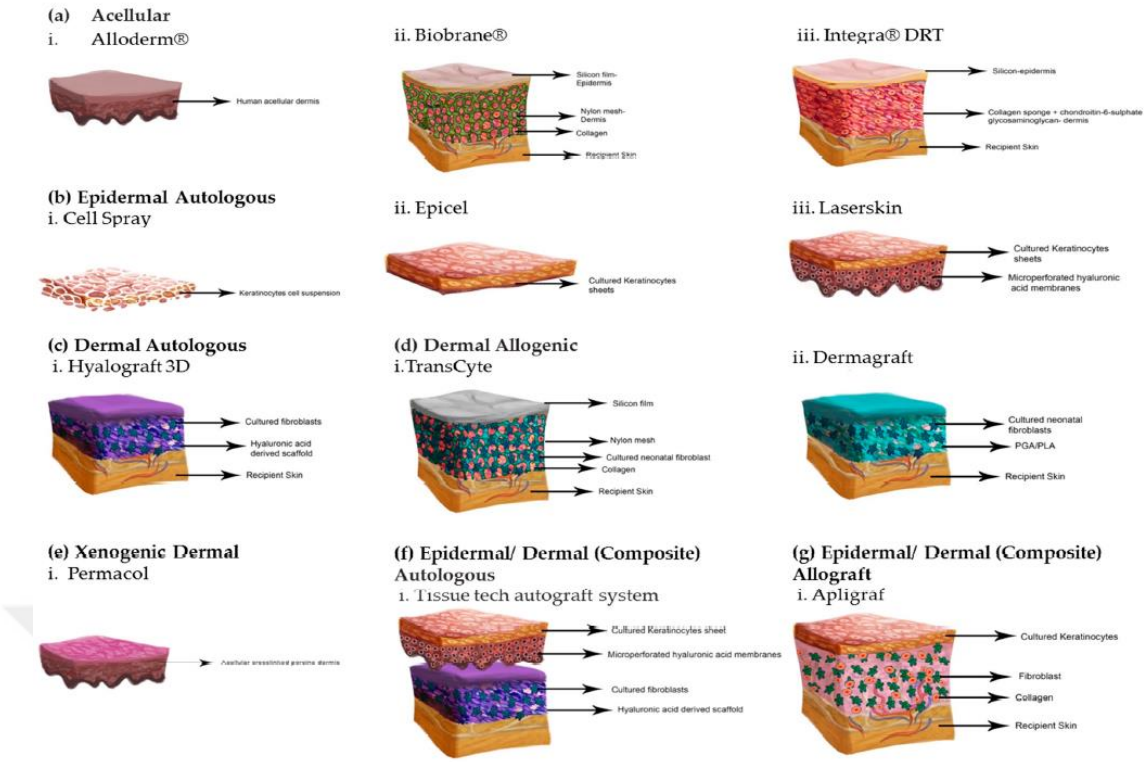


**Figure 2.5:** Tissue engineered skin substitutes[58].

**Table 2.4:** Engineered Skin substitutes.

| Product | Company | Description | Application |
|---|---|---|---|
| (a) Alloderm | LifeCell Corporation, NJ | Acellular freeze-dried allogeneic dermis | Treat full- and partial thickness wounds |
| (g) Apligraf | Organogenesis Inc., MA | Allogeneic keratinocytes on a bovine collagen sponge containing allogeneic fibroblasts | Venous and diabetic foot ulcers |
| (a) Biobrane | UDL Laboratories Inc., IL | Porcine collagen bound to silicone/nylon membrane | Temporary covering of burns and wounds |
| (c) Dermagraft | Advanced Biohealing Inc., CA | Cryopreserved fibroblast-derived dermal matrix | Diabetic foot ulcers |
| Epicel (b) | Genzyme Tissue Repair Corp., MA | Autologous keratinocytes on petrolatum gauze backing | Burns and chronic ulcers |
| Hyalograft 3D (c) | Fidia Advanced Biopolymers, Italy | Esterified hyaluronic acid matrix seeded with autologous fibroblasts | Wounds and ulcers |
| Integra (a) | Integra Life Sciences Corp., NJ | Silicone epidermal substitute over collagen scaffold | Burns and chronic wounds |
| Suprathel | Institute of Textile Eng., Germany | Synthetic epidermal substitute made of DL-lactatide monolayer | Partial-thickness burns |

If we categorize the commercial models produced, Biobrane has produced cell-free and synthetic skin substitutes, which stand out for wound dressings. The product consists of a nylon/silicone matrix embedded with collagen peptides that form a semi-permeable barrier against fluid loss and microbial invasion. It is often used as temporary dressing for burns [60].

The model developed by Integra is a two-layer structure consisting of a bovine collagen-chondroitin-6-sulfate dermal matrix and a synthetic silicone epidermal layer. The dermal matrix facilitates fibroblast infiltration and ECM regeneration, while the silicone layer provides protection until autografting. Its use is usually for large burn areas [56].

Another alternative is allogeneic substitutes. Alloderm, obtained from human allograft skin, is processed to remove cellular components and leaves behind an ECM scaffold that supports dermal remodeling without immune rejection. It shows potential in repairing soft tissue defects and burn wounds. The Alloderm company has developed it [61].

Some companies have developed composite skin substitutes. Basically, these structures integrate allogeneic keratinocytes and fibroblasts into collagen-based scaffolds. It has been shown that Apligraf is effective in treating venous leg ulcers and diabetic foot ulcers by providing faster and less painful healing compared to traditional dressings [56].

Autologous cells are used to cover permanent wounds and prevent immune responses. Autologous skin substitutes developed by Epicel are commonly used in burn injuries. It provides long-term coverage but is mechanically fragile. Cultured skin substitutes (CSS) overcome this limitation by incorporating autologous keratinocytes and fibroblasts into a collagen-glycosaminoglycan substrate. the cosmetic results that will occur afterwards will also be improved [62].

### 2.3.2. Identification of Research Gaps and Opportunities

There are many limitations and problems with the commercially available artificial leather substitutes on the market. These are mentioned in the literature. These deficiencies are anatomical and functional limitations such as the inadequacy of cell

types other than fibroblasts and keratinocytes, lack of vascularization, and irregular pigmentation [34].

Limitations in anatomy and cell composition often result from the fact that the models contain only fibroblasts and keratinocytes. This is insufficient to reproduce the function of natural skin tissue. Including additional cell types, such as endothelial cells, in these models can overcome these limitations. For example, the addition of endothelial cells to existing products may reduce skin graft failure caused by poor vascularization by initiating angiogenesis [59].

Another problem is vascularization difficulties. Cultured skin substitutes lack vascular plexus, which causes delayed vascularization after grafting. Investigating techniques to increase vascularization, such as the use of endothelial cells or genetically modifying keratinocytes to overexpress angiogenic factors such as VEGF (vascular endothelial growth factor), may significantly improve the implant life and integration of the artificial skin graft [59].

Another problem is the pigmentation problems of the artificial skin grafts produced. The grafts lack pigmentation due to the absence or irregular distribution of melanocytes. Melanocytes were added to solve this problem; regular pigmentation could be obtained, but the problem of controlling the pigment intensity continues. It is a suitable field of study for researchers [59]. Table 2.5 summarizes the areas to be addressed in future study, categorizing them by field and focus [14]. These developments reveal the potential to offer an innovative and versatile platform for systemic treatments.

**Table 2.5:** The summary of the topics to be addressed in future research.

| Area | Focus for Future Research |
|---|---|
| Bioprinter Technology | *Compatibility with physiologically relevant materials and cells<br>* Increased resolution and speed<br>*Scaling up for commercial applications<br>*Combining bioprinter technologies to overcome technical challenges |
| Biomaterials | *Developing complex material combinations<br>*Designing biomaterials for printability and stability<br>*Using tissue-specific ECM scaffolds |
| Cell Sources | *Identifying reliable and functional cell sources<br>*Controlling proliferation and differentiation |
| Vascularization | *Developing vascular networks for tissue perfusion<br>*Ensuring mechanical stability for surgical use |
| Innervation | *Achieving proper innervation for tissue function<br>*Simulating innervation pre-transplant in bioreactors |
| Maturation | *Simulating innervation pre-transplant in bioreactors<br>*Using bioreactors for pre-implantation testing |

# 3. MATERIALS AND METHODS

## 3.1. Bioink Preparations

### 3.1.1. Selection of Natural-Based Bioinks

In this thesis, natural bioinks and synthetic polymer materials were used together. The choice of bioink was made considering the need to mimic the natural skin microenvironment and, at the same time, to provide structural integrity with printing ability. The bioink used is collagen. Bioink preparation stages:

Concentration of Collagen Solution: The highly concentrated collagen solution is placed in the centrifuge and centrifuged until the desired concentration is reached. Hettich EBA 20 model was used as centrifuge. This stage is shown in Figure 3.1.

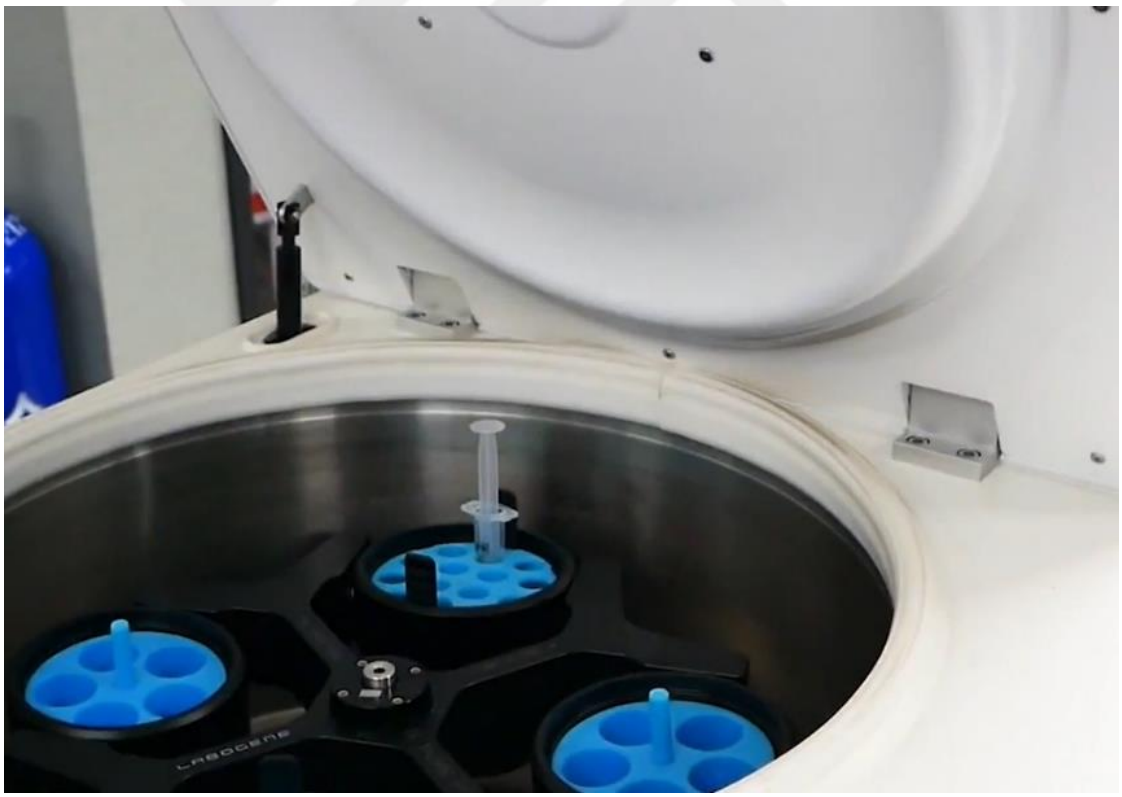

**Figure 3.1**: Centrifugation of collagen bioink.

One of the most abundant structural proteins in the body is Collagen Type I. This structure is abundant in the body, especially in skin, tendon, bone, and connective tissues. Therefore, it is frequently used in tissue engineering and 3D bioprinting studies [63].

### 3.1.2. Preparation and Optimization Bioink Formulation

Collagen hydrogel precursor (Rat tail, type I) was used as the skeleton material of the hydrogels. The stock collagen precursor was diluted to 3.0 mg/mL with 1-Dulbecco's phosphate buffered saline solution without calcium and magnesium and kept on ice until ready for printing. This solution was loaded into a syringe (which served as a printing cartridge), and printing was started [63]. Figure 3.2 and Figure 3.3 show the preparation stages.


**Figure 3.2**: Placing the collagen bioink in a syringe.

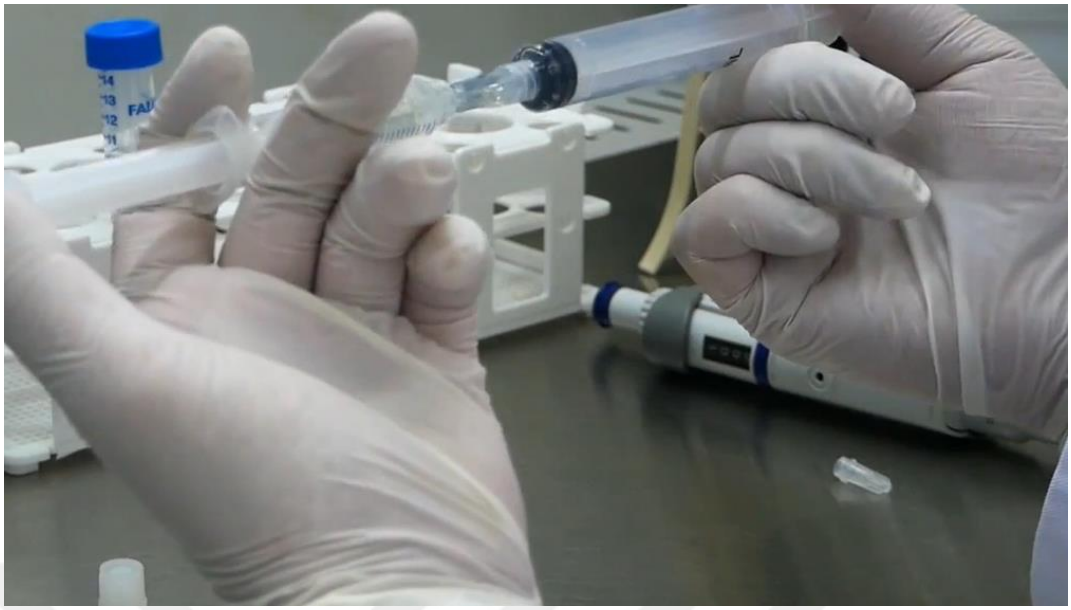

**Figure 3.3**: Mixing the collagen solution.

## 3.2. 3D Bioprinting Process

### 3.2.1. Equipment and Software Setup for Extrusion-Based 3D Bioprinting

In this work, the Dr. INVIVO 4D2 bioprinter was utilized, and the extrusion-based 3D bioprinting approach was chosen. The device and its auxiliary equipment are depicted in Figure 3.4. The temperature controller, air compressor, and air dispenser controller are other components that are utilized. Figure 3.4 illustrates the device and its auxiliary equipment.


**Figure 3.4:** Dr. INVIVO 4D2 bioprinter and its auxiliary equipment.

The Dr. INVIVO 4D2 bioprinter used is a closed system, thus creating a sterile environment. The device has side equipment. The temperature controller controls the temperature of the printhead and ensures the melting of the materials. The air compressor meets the pressure requirement of the device, and the air dispenser controller controls the use of pressure during printing. Figure 3.5 shows the details of the Dr. INVIVO 4D2 bioprinter [64].

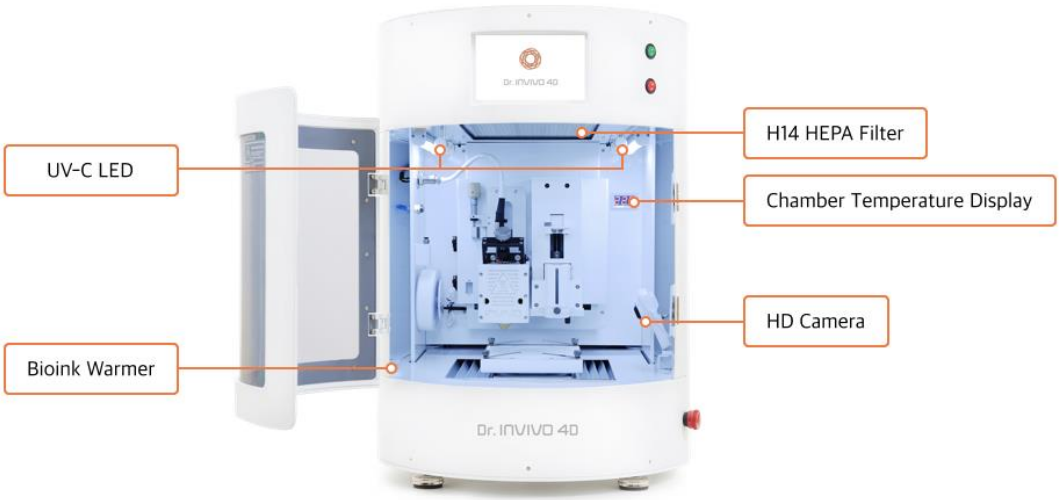


**Figure 3.5:** Dr. INVIVO 4D2 bioprinter detailed demonstration.


**Figure 3.6:** Preparing to print with the filament extruder.

While the bioprinter is being prepared for use, the materials to be used are loaded. Figure 3.6 shows the PCL filament loading stage. Figure 3.7 shows the loading stage of Collagen bioink.


**Figure 3.7:** Preparing to print with the syringe bio-dispenser.

The NewCreatorK slicing program is the data preparation stage of 3D bioprinting by converting 3D model designs into printer-readable G-code. It is used to set precise

parameters such as nozzle size, layer height and extrusion speed. The program supports the use of multiple heads (such as syringe head and extrusion head) and multiple material, layer customization. The simulation feature helps to optimize settings before printing, improving structural integrity and cell viability. Once the parameters are set, the generated G-code is transferred to a bioprinter such as Dr INVIVO 4D2 to produce in vitro models. Figure 3.8 shows the stages of creating G-code [64].


**Figure 3.8:** Slicing software steps to convert STL file to G-code file.

PCL mesh design was made in Fusion 360 program. The design visual is shown in Figure 3.7. Precise control over scaffold dimensions including parametric modelling, pore size, layer thickness and geometry was achieved.

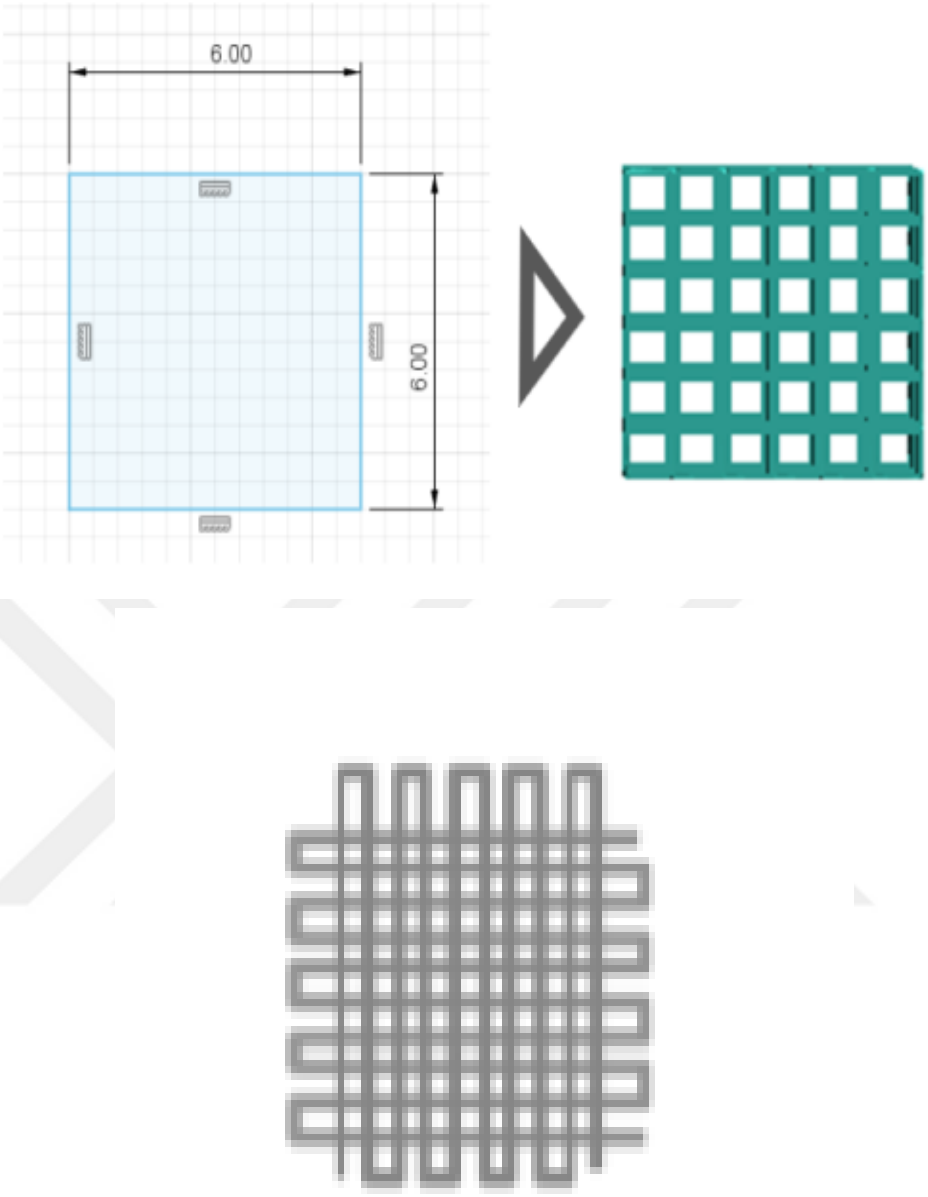


**Figure 3.9:** PCL mesh design.

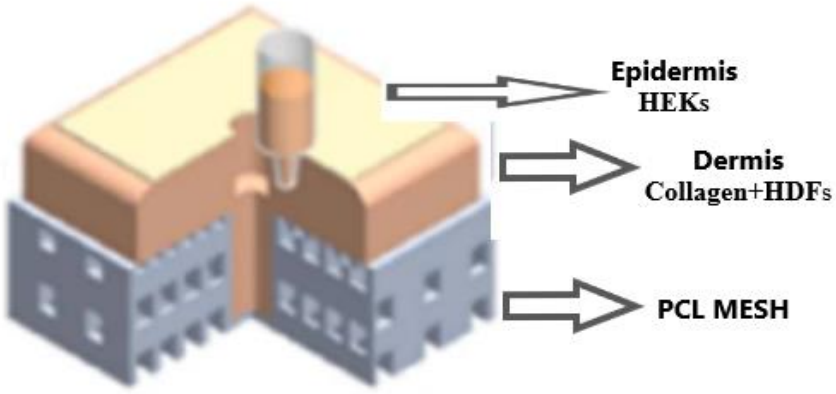


**Figure 3.10:** Artificial skin model.

### 3.2.2. Parameters and Protocols for Printing Human Skin Model

During the production of the artificial skin model, production was carried out by creating many parameter combinations. The main ones are 3D bioprinting parameters. They are shown in detail in Table 3.1. These parameters and features were adjusted from the user interface in Figure 3.11.

**Table 3.1:** Technical specifications of Dr. INVIVO 4D2 bioprinter.

| Dr. INVIVO 4D2 Features | Details |
|---|---|
| Nozzle Diameter | 100~400 μm (Optional) |
| Material Compatibility | Bioinks, biopolymers, ceramics, PCL, PLGA, PLLA, hydroxyapatite, chitosan, etc. |
| Dispenser Types | Syringe dispenser, filament extruder, hot melt dispenser, pneumatic dispenser |
| Temperature Control | (-4°C ~ 60°C) (Temperature control for dispenser and bed) |
| UV Curing | 365 nm / 405 nm wavelength (Optional) |
| Sterilization | H14 HEPA filter, UV lamp (12W/254nm) |
| Software | NewCreatorK |
| Build Volume | 10 cm x 10 cm x 8 cm |
| Speed | Maximum 20 mm/s |
| Pressure Control | Maximum 900 kPa |
| Material Types | Hydrogels (collagen, gelatin, fibrin, hyaluronic acid, alginate), thermoplastics, etc. |
| Additional Features | Photopolymerization, chemical cross-linking |


**Figure 3.11:** Dr. INVIVO 4D2 bioprinter user interface.

The next parameter study was performed for the materials used. Collagen bioink was prepared using Collagen Type I hydrogel precursor diluted to a concentration of 3.0 mg/mL in 1× Dulbecco's calcium and magnesium-free phosphate buffered saline (DPBS). Collagen was supplied by the Rokit company. Collagen was kept at ~0°C until the printing stage to prevent destabilization. Cross-linking of the collagen bioink took place at 37°C. It reached structural stability in approximately 20-30 minutes. Collagen production parameters are shown in Table 3.2.

**Table 3.2:** Collagen printing parameters.

| Printing Parameters | Value/Details |
|---|---|
| Bioink Composition | Collagen Type I hydrogel precursor (Rat tail), diluted to 3.0 mg/mL |
| Dilution Medium | 1× Dulbecco's phosphate-buffered saline (DPBS) without calcium and magnesium |
| Temperature | Solution kept on ice (~0°C) until printing |
| Crosslinking Method | Thermal gelation at 37°C (inherent collagen property) |
| Nozzle Diameter | 200 μm |
| Dispenser Types | Syringe dispenser |
| Temperature Control | 10°C |
| Gelation Time | ~20 minutes at 37°C |
| Syringe Preparation | Bioink loaded into syringe serving as a printing cartridge |

Another parameter is the values used for the PCL mesh structure. These parameters are parameters applied and optimized by Rokit for PCL filament. It is included as a default parameter in the NewCreatorK slicer program.

The PCL mesh structure is designed to provide mechanical stability and a supporting framework for collagen-based bioink. PCL mesh acted as a scaffold since collagen cannot maintain its form during the production process.

PCL filament printing parameters are detailed in Table 3.3. PCL filaments were melted layer by layer by heating the temperature up to 120°C with the filament extruding head. The PCL filament used is shown in Figure 3.12. After the PCL mesh production was completed, collagen printing was performed on it. This double printing pattern is shown in Figure 3.13.

**Table 3.3:** PCL filament printing parameters.

| Printing Parameters | Value |
|---|---|
| Nozzle Size (mm) | 0.4 |
| Dispenser Type | Filament Extruder |
| Layer Height (mm) | 0.2 |
| Fill Density (%) | 100 |
| Print Speed (mm/s) | 10 |
| Travel Speed (mm/s) | 12 |
| Bottom Layer Speed (mm/s) | 8 |
| Retraction Speed (mm/s) | 6 |
| Retraction Distance (mm) | 3 |
| Filament Diameter (mm) | 1.75 |
| Infill Flow (%) | 100 |
| Bed Temperature (°C) | RT (Room Temperature) |
| Dispenser Temperature (°C) | 120 |

**Figure 3.12:** PCL filament.

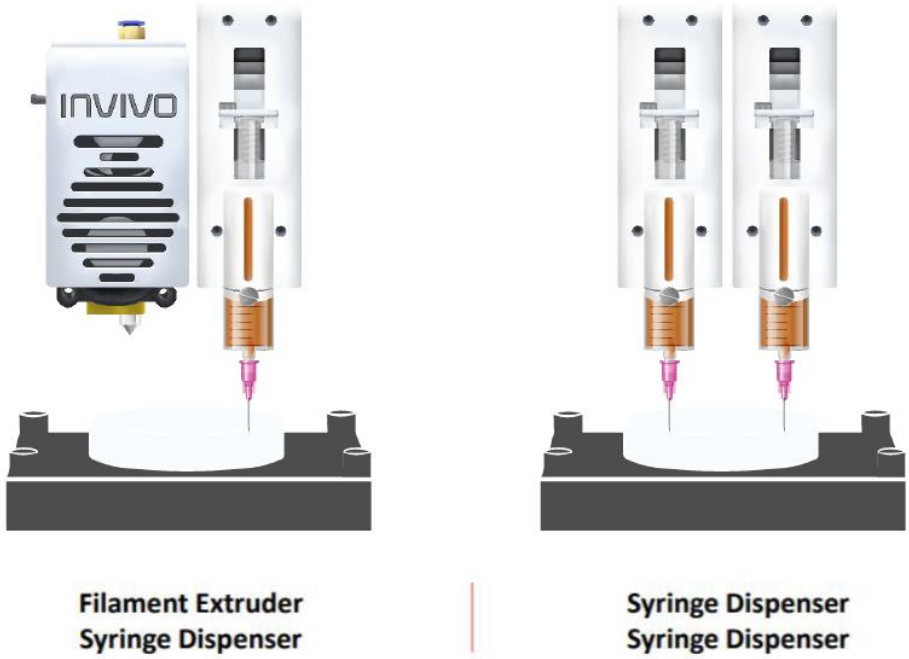


**Figure 3.13:** Dual printing head systems.

## 3.3. Cell Culture and Incorporation

### 3.3.1. Types of Cells Used

In the thesis study, 2 types of human skin cells, human epidermal keratinocytes (HEKs) and human dermal fibroblasts (HDFs), were used. The intended use of these two main cells is important for the study. HDFs play an active role in the formation of the human dermal layer. These cells synthesize the extracellular matrix (ECM) and form the dermal layer.

Human epidermal keratinocytes (HEKs) are densely located in the epidermal part of the skin layer and provide barrier properties to the skin layer. Since it forms the outer layer, it plays a key role in wound healing and cellular communication. It has been used to form the epidermis layer.

Figure 3.14 shows microscope images of human epidermal keratinocytes and human dermal fibroblasts [65].

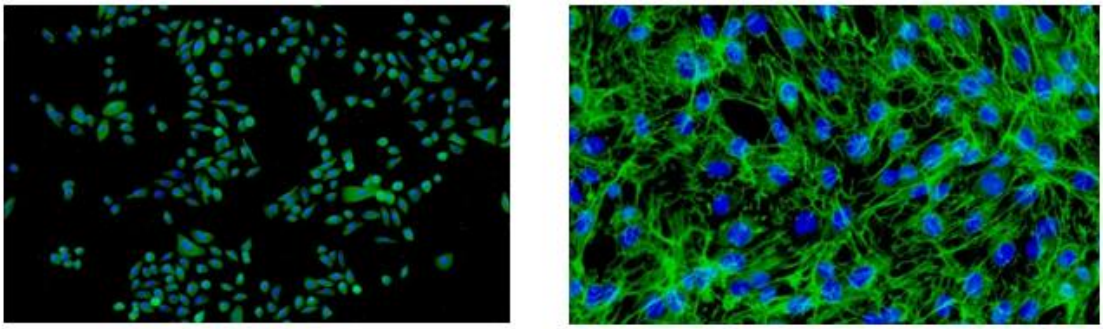

**Figure 3.14:** Human epidermal keratinocytes and human dermal fibroblasts**.**

### 3.3.2. Cell Culture Conditions and Methods for Integrating Cell into Bioinks

Primary dermal fibroblasts (HDFs) and human epidermal keratinocytes (HEKs) were used in this study. Dermal fibroblasts and keratinocytes were obtained from ROKIT Healthcare in South Korea.

The epidermal layer, which is in charge of the skin's barrier function and cellular differentiation, was created by HEKs, and the dermal layer was formed by HDFs through the production of extracellular matrix components.

Fibroblasts were cultured in a special medium called Dulbecco's Modified Eagle Medium (DMEM). This medium contains essential nutrients necessary for the growth and survival of the cells. The medium was refreshed every two days so that the cells could continue to grow healthily. This allows waste to be removed and fresh nutrients to be provided. The fibroblast cells for printing were optimized for use in printing by adding them to the collagen bioink at a ratio of $2.5 \times 10^5$ cells/mL, i.e., 250,000 cells per milliliter of solution.

Keratinocytes are not directly added to the bioink; instead, they are prepared in growth media and placed on the dermis during printing to form the epidermis layer. This is because keratinocytes prefer to grow at the air-liquid interface and are usually placed on the upper surface to form the epidermis layer rather than being completely embedded in the ECM. Dual syringe heads were used for this purpose. Collagen and fibroblast were formed in one syringe and keratinocytes in the other syringe and loaded into the device. The cell culture stage is shown in Figure 3.15.

**Figure 3.15:** Cell culture stage.

## 3.4. In Vitro Characterization

### 3.4.1. Method for Assessing the Structural and Functional Properties of Printed Skin Model

A series of in vitro characterization studies were carried out to evaluate the bioprinted artificial skin models produced in this study. These studies were based on the methods used in literature in this field.

1. Rheological Testing

Rheological tests are used to evaluate the viscosity, shear thinning performance, and storage modulus (G') of materials. Measurements were made to evaluate the suitability of the yield of collagen-based bioink for bioprinting. The rheometer used is shown in Figure 3.16.

**Figure 3.16:** TA Instruments Discovery HR-2 Rheometer.

2. Mechanical Testing

Mechanical tests measure the tensile strength, elongation and overall durability of materials. In this study, the mechanical strength and elongation properties of printed collagen layers and PCL scaffold were used to compare the skin model with the mechanical behavior of human skin. Mechanical test devices used are shown in Figure 3.17.

**Figure 3.17:** Instron Universal Testing Systems.

3. Water Absorption Capacity

This test was used to evaluate the hydrophilic properties of the models produced. The aim here is to analyze the moisture retention ability of the artificial skin models produced.

4. Cell Viability Testing

It is a test used to evaluate the cell viability rate of artificial skin models produced in the study under experimental conditions. The main purpose of the test is to measure the biocompatibility and toxicity effect of the materials used on the cells. LIVE/DEAD assay method was generally used in the study. In this method, live cells stained with Calcein-AM emit green fluorescence and dead cells stained with Ethidium Homodimer-1 emit red fluorescence. The results observed with a fluorescence microscope provide the opportunity to quantitatively evaluate the healthy growth and metabolic activity of the cells. The fluorescence microscope used is shown in Figure 3.18.

**Figure 3.18:** Olympus fluorescence microscope.

# 4. RESULTS

## 4.1. Structural Properties of the Printed Skin Model

In the study, artificial skin models produced with the extrusion-based 3D bioprinting method simulated 2 different skin layers. These are dermal and epidermal layers.

The dermal structure was created using collagen bioink combined with human dermal fibroblasts (HDFs). The aim here is the formation of the ECM structure by fibroblasts. Fibroblasts are responsible for ECM synthesis. After cross-linking at 37°C, the collagen matrix exhibited a stable structure that supported the encapsulation of fibroblasts and promoted the formation of the cellular environment of the dermal layer. When the dermal structure was examined by fluorescence microscopy, well-organized collagen fibrils and a dense cellular structure were observed.

The epidermal structure is formed by placing epidermal keratinocytes (HEKs) on the collagen print produced. This layer is the outermost layer of the artificial skin model. Compared to the skin structure, it basically acts as a barrier. This structure formed a layered, epithelial-like layer that mimics the barrier function of natural skin. Microscopic imaging revealed a cell-rich surface.

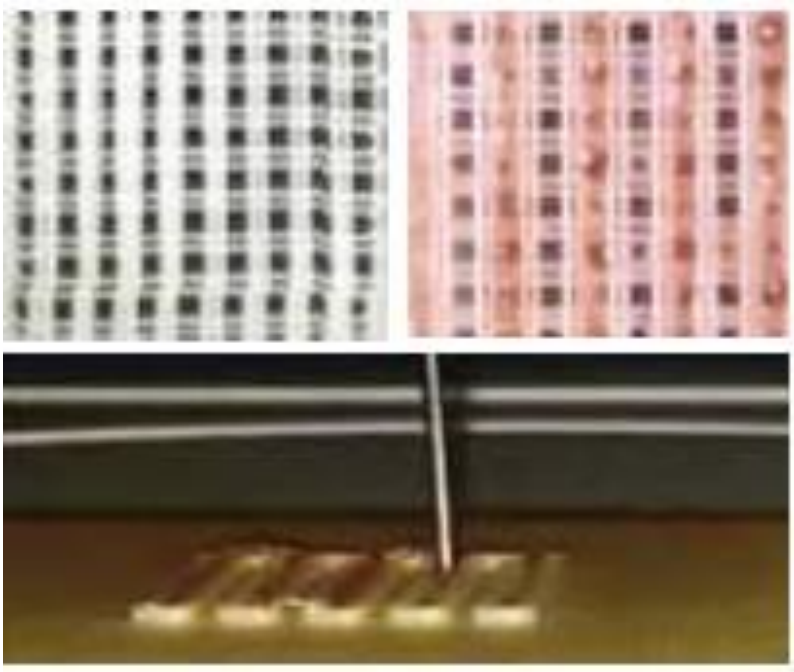

**Figure 4.1:** View of the PCL mesh, along with the dermal and epidermal layers.

Figure 4.1 shows the dermal and epidermal layers printed on the PCL mesh structure. After production, the dermal and epidermal structures were placed inside the PCL network structure, preserving the collagen structure form and being mechanically supported. This stage is simulated in Figure 4.2.

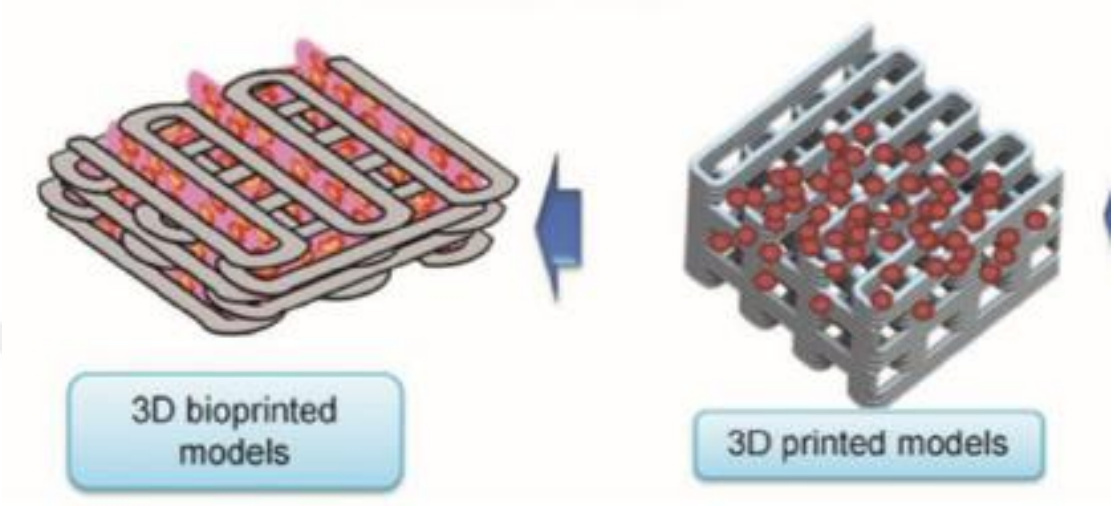


**Figure 4.2:** The dermal and epidermal structures were integrated into PCL network architecture.

When the produced artificial skin model was examined by fluorescence microscopy, a separation between the dermal layer and the epidermal layer was observed. Figure 4.3 shows this distinction.

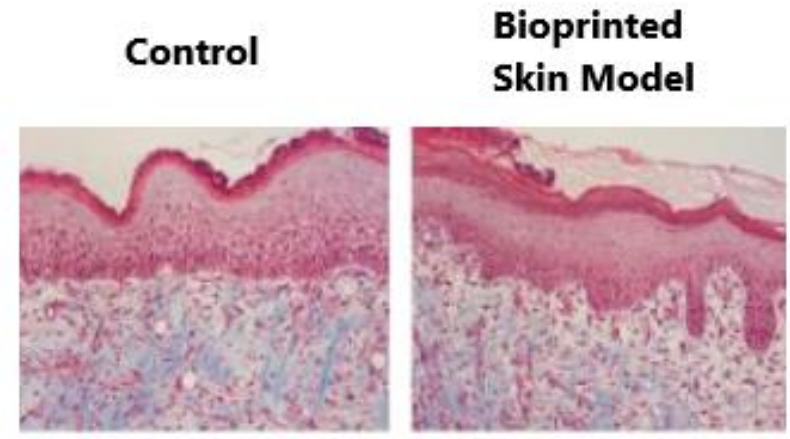


**Figure 4.3:** Significant distinction between the dermal and epidermal layers.

## 4.2. Cell Viability Testing

The cell viability of the produced artificial skin models was evaluated using the LIVE/DEAD assay. The results of the assay showed that both dermal fibroblasts and epidermal keratinocytes of the produced models showed high viability after printing. Viable cells giving green fluorescence when stained with Calcein-AM were predominant throughout the model, with minimal dead cells (red fluorescence) present. Figure 4.4 shows fluorescence microscope images of the LIVE/DEAD experiment.

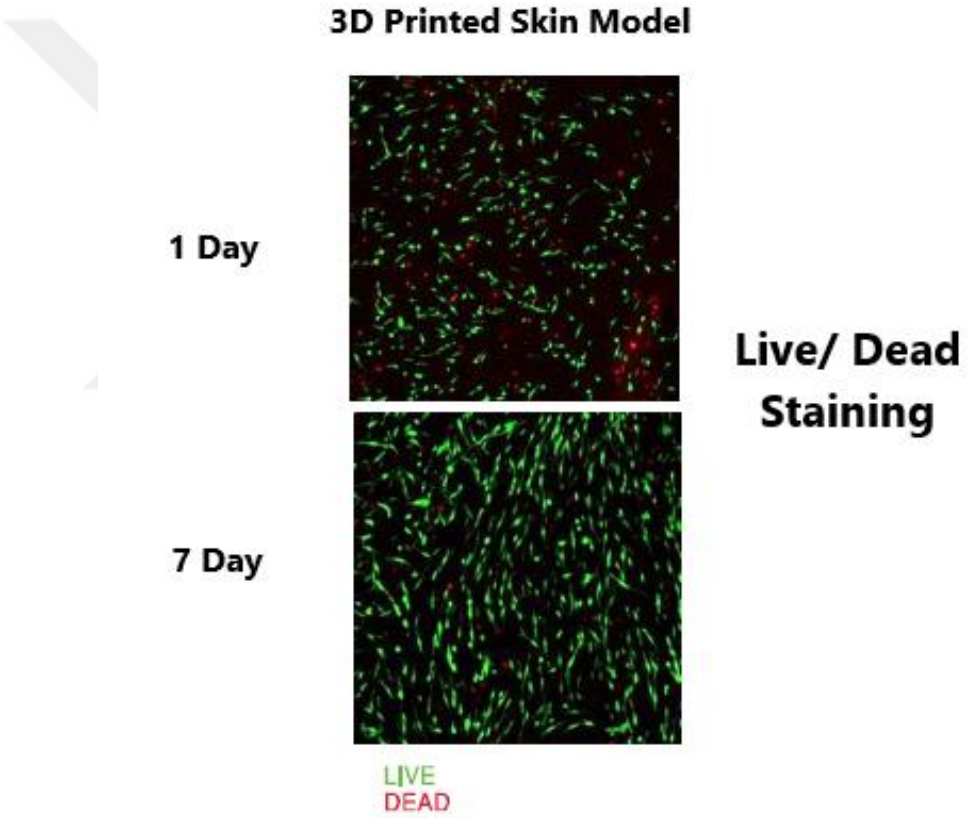


**Figure 4.4:** The fluorescence microscope images of the LIVE/DEAD assay.

## 4.3. Rheological Analysis

The rheological properties of the collagen bioink were analyzed to evaluate its suitability for extrusion-based 3D bioprinting. In the test, Collagen bioink showed the expected shear thinning behavior. During extrusion, at higher shear rates, viscosity decreased and a uniform flow through the syringe dispenser was achieved.

The storage modulus (G') obtained because of the test is a value indicating the ability of the collagen bioink to form a stable gel structure at room temperature and during the crosslinking process.

The rheological data presented in Figure 4.5 showed that the collagen bioink has a storage modulus (G') of approximately 300-500 Pa, which is suitable for maintaining its structural stability while allowing controlled extrusion.

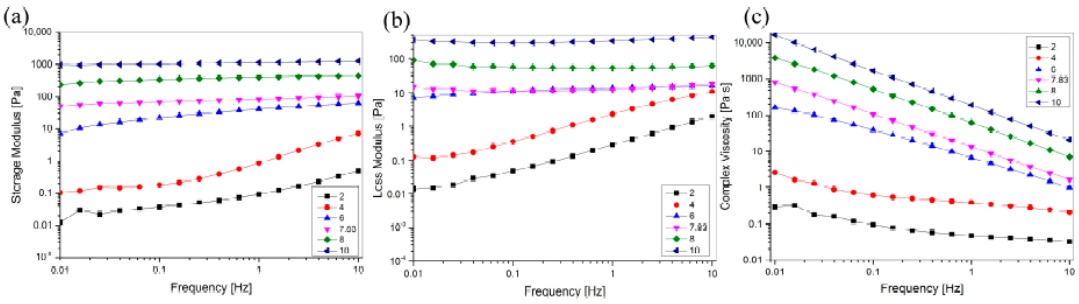


**Figure 4.5:** Rheological behavior of collagen bioink.

## 4.4. Mechanical Properties

The mechanical properties of the produced leather models were evaluated using tensile tests to compare the behavior of the artificial leather with that of natural human skin. The tensile strength of the artificial skin model was found to be in the range of 0.1-0.2 MPa, like that of human skin, which generally shows tensile strength values in this range.

The dermal layer supported by the PCL scaffold supported the mechanical properties of the artificial skin model. The mechanical test result is shown in figure 4.6.

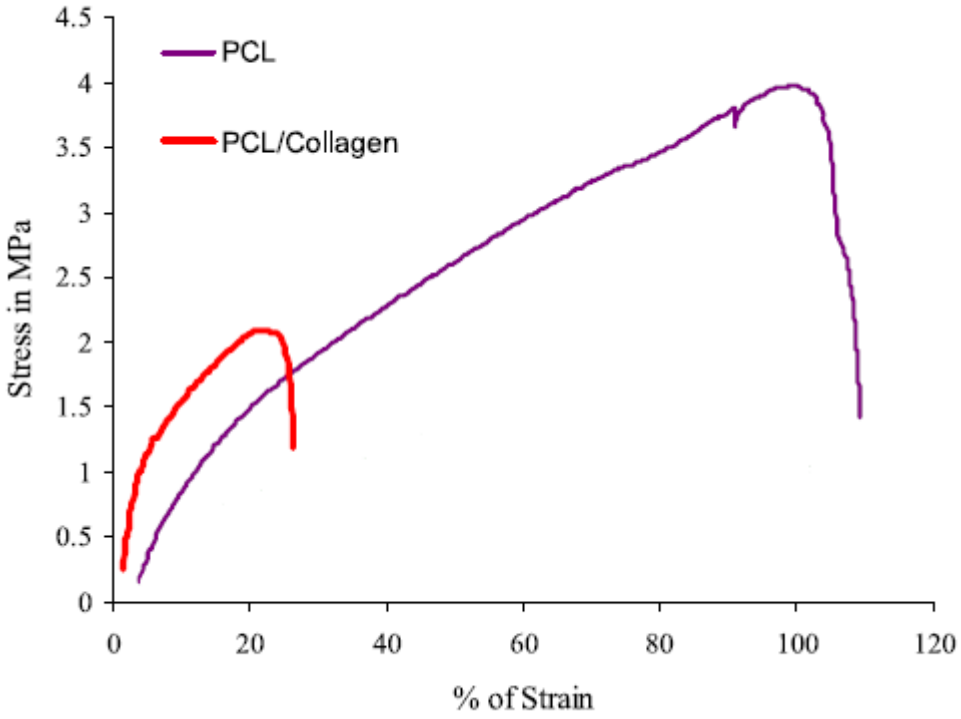


**Figure 4.6:** PCL / PCL Collagen stress-strain curve.

The elongation coefficient of the artificial skin models showed good elongation (stretchability) with an elongation at break of about 25-30%, which is typical for human skin. This property is critical for the skin model to simulate the mechanical behavior of natural tissue, which can stretch and deform under stress without tearing.

## 4.5. Water Absorption Capacity

The hydrophilic properties of the produced artificial skin models were evaluated by measuring the water absorption capacity of the model. The water absorption test is important to assess the potential of the model to mimic the moisture retention and barrier properties of human skin.

The test data were consistent with the skin properties, indicating that the model may be suitable for applications in wound healing or skin injury treatment. Figure 4.7 shows the water absorption capacity of PCL.

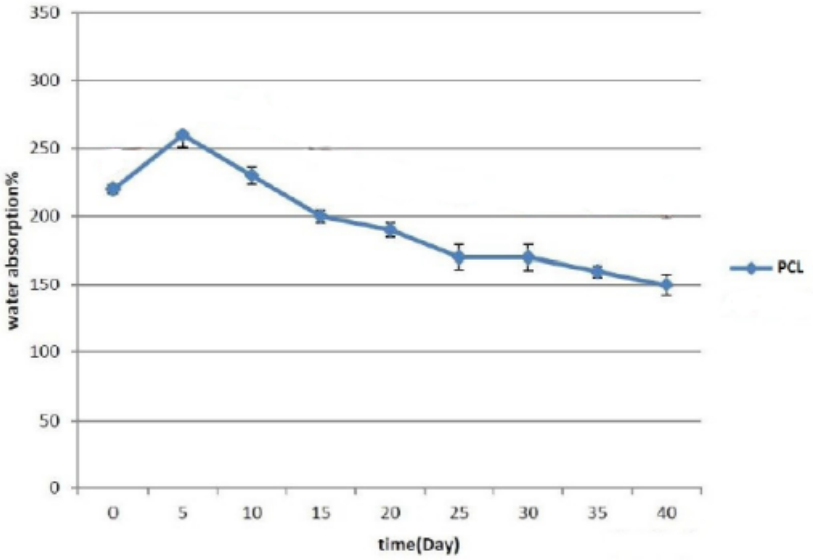


**Figure 4.7:** Water absorption capacity of the PCL.

# 5. CONCLUSIONS

The aim of this study is to determine the deficiencies of artificial skin grafts used in the field of tissue engineering, to produce models that mimic human skin with 3D bioprinting tools, and to improve existing models. The results obtained have provided important data to produce multilayer skin structures in both clinical and research fields.

If we compare the findings obtained in the study with the literature, data to support the literature were obtained. When the rheological properties of the collagen-based bioink mixture used were examined, the shear thinning required for the formation of the 3D scaffold was compatible with the literature, and accordingly, a smooth printing process was achieved. Fibroblast integration of materials such as GelMA or alginate, other bioinks used other than collagen studied in the literature, is weak. Since collagen bioink simulates ECM, cellular attachment and proliferation are higher than in other studies.

In the study, a skin model with dermal and epidermal layers was created. The layers were formed distinctly. This approach supports studies on multilayer skin formation in literature. Here, the addition of PCL network structure to provide mechanical stability to the collagen form differentiates this study from other studies. The hybrid scaffold approach has contributed to the studies carried out only with soft hydrogels in the literature.

Extrusion-based bioprinting method was used in this study. Compared to artificial skin models obtained by traditional production methods, it provided superior spatial and stylistic control. Pore structures were controlled to create ideal cavities for cell growth. The complexity of the skin structure could be simulated in this way. Live/Dead experiment results emphasized the importance of printing parameters in maintaining cell viability. Temperature-dependent parameters were found to be directly related to cell viability.

In the study, the mechanical test results confirmed that the PCL mesh structure increased the mechanical properties of the collagen structure. It was determined that the tensile strength of the artificial skin model produced approached the mechanical properties of the natural skin structure with the increase in tensile strength. This result also confirmed the studies stating that synthetic scaffolds increase structural strength.

In the study, layered skin structure was successfully produced, but the produced tissue contains various limitations. These limitations require further research areas.

**Limitations and Future Perspectives**

- Limitations and Future Perspectives: Skin tissue is surrounded by vascular structures and provides vascularization. This system is a very new and sensitive system. The resolution of current production technologies is insufficient to create this structure. In addition, the absence of a vascular network in the artificial skin produced limits nutrient and oxygen diffusion, which is critical for long-term viability, and causes cell death.
- Long-Term Stability: Although the mechanical and biological properties of the produced artificial skin model are promising, evaluation studies under dynamic in vitro or in vivo conditions are needed. Dynamic culture systems such as bioreactors can be integrated to mimic physiological mechanical stresses and enhance tissue maturation.
- Inclusion of Other Skin Components: When systems such as sebaceous glands, hair follicles and a complex sensory neural network are added to the artificial skin model to mimic natural skin tissue, it can further increase the realism of the model and shed light on new study topics.

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# BIOGRAPHY

Gamze EREN graduated from Erciyes University in 2017 with a bachelor’s degree in biomedical engineering, where she gained a solid foundation in the principles of engineering and their applications in healthcare and medical technologies. Driven by her passion for advancing biomedical innovation, she pursued a master's degree in Bioengineering at the Graduate School of Gebze Technical University, starting in 2022. Her research focuses on cutting-edge topics in tissue engineering and 3D bioprinting.

In 2021, she began her professional career at Form Additive Manufacturing and Robotics Technologies Inc., where she has been working as an Additive Medical Engineer. In this role, she specializes in designing and developing innovative solutions using additive manufacturing technologies, particularly for medical applications. Her work includes contributing to the development of advanced medical devices and personalized healthcare solutions, bridging the gap between engineering and medicine. Gamze’s combined academic and professional experiences reflect her dedication to driving technological advancements in the biomedical field and her commitment to contributing to transformative solutions in healthcare.

# PUBLICATIONS AND PRESENTATIONS FROM THESIS

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