PROSPECT OF BIOPRINTED LUNGS FOR COVID-19 RECOVERED PATIENTS

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Declaration

It is hereby declared that

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

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Approval

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Abstract/ Executive Summary

Ever since its first case detected in December 2019 in Wuhan, the Covid-19 infection caused by the SARS-CoV-2 strain of the coronavirus, it has gone on to affect a total of 71,516,809 (last recorded on 12th December,2020) people all over the world. This resulted in the biggest pandemic since 1920 and caused over 16,03,054 deaths at a rate of approximately 3%.

One of the vital ORGANS of attack for the virus in humans is the lungs which is involved in arguably the most important function of the human body, respiration. Study showed that around 90% of the lungs get infected by the virus. While, majority of the population below the age group of 50 were successful in fighting off the infection, the virus tends to leave a lasting effect that leads to detrimental effects.

The purpose of this research is to integrate tissue engineering and bioprinting and look at Bioprinted lungs through transplantation as a viable alternative to treat lung infections. Furthermore, it underlines the functioning of the lungs and the parts which can be engineered to create artificial lungs, in order to initiate a successful transplantation. To add with it, the motive of this study is also to compare how a Bioprinted organ transplantation is much viable and provides less health complications than a normal transplantation.

Keywords: Bioprinting; tissue engineering; regenerative medicine; organ transplantation; COVID-19; animal tissue culture.

Dedicated To

Our Parents and Siblings.

Acknowledgement

First and Foremost, we would like to express the deepest gratitude towards the Almighty, who enabled us to successfully perform this thesis work. We are also eternally grateful to our respective parents and siblings who have showered unconditional blessings and love along the way.

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This thesis experience in laboratory will help us attain knowledge and polish our skills that we can use in the best possible way in the near future.

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List of Acronyms

3-D	Three Dimensional
ACE2	Angiotensin Converting Enzyme 2
ARDS	Acute Respiratory Distress Syndrome
CAM	Computer Aided Manufacturing
CAD	Computer Aided Design
COV	Coronavirus
CT	Computer Tomography
DLCO	Diffusing Capacity for Carbon monoxide
ECM	Extra Cellular Matrix
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
FDA	Food and Drug Administration
MSc	Mesenchymal Cells
MRI	Magnetic Resonance Imaging
SARS	Severe Acute Respiratory Syndrome
SLATE	Stereo Lithography Apparatus for Tissue Engineering
UV	Ultra Violet
WHO	World Health Organization

Chapter 1

Introduction

COVID-19 is an infectious disease which is caused by the SARS-CoV-2 virus. The symptoms of this disease include: fever, dry cough, fatigue, shortness of breathing, sore throat etc. Even though a lot of people are recovering from this disease, it can be fatal in case of many people according to the disease severity and health conditions [1]. While patients who survive COVID- 19 recover and get rid of the virus, however, this disease can leave a long lasting impact on different organs. The most severe damage that can be noticed in COVID recovered patients is lung damage which can be long lasting. Complications include pneumonia, acute respiratory distress syndrome (ARDS), sepsis [2]. Specialists are concerned a critical extent could be left with lung scarring, known as pulmonary fibrosis. The condition is irreversible and indications can incorporate severe shortness of breath, coughing and weakness. Research into the prevalence of lung harm brought about by Covid-19 is still at an early stage. [3]

It is believed that those with a mild case of the sickness are probably not going to endure lasting harm. In any case, those in the clinic, and especially those in intensive care or with a severe infection are more vulnerable. However, studies are still going on to find out if the lungs can recover from the damage over time. [4]

- **Pneumonia:** In pneumonia, the lungs become loaded up with fluid and inflamed, prompting breathing challenges. The pneumonia that COVID-19 causes will generally affect both lungs. Air sacs in the lungs load up with fluids, restricting their capacity to take in oxygen and causing shortness of breath, cough and other symptoms [5]. While the vast majority recuperate from pneumonia with no enduring lung harm, the pneumonia related with COVID-19 might be serious. Even after the sickness has passed, lung injury may bring about breathing challenges that may take a long time to improve.
- **Acute Respiratory Distress Syndrome (ARDS):** As COVID-19 pneumonia advances, a greater amount of the air sacs become loaded up with fluids spilling from the small veins in the lungs. In the long run, shortness of breath sets in, and can prompt acute respiratory distress syndrome (ARDS), a type of lung failure [7]. Patients with ARDS are frequently incapable to breath all alone and may require ventilator backing to help

circle oxygen in the body. Regardless of whether it happens at home or at the medical clinic, ARDS can be deadly. Individuals who endure ARDS and recuperate from COVID-19 may have enduring pulmonary scarring. [8]

- **Sepsis:** Another possible intricacy of a serious instance of COVID-19 is sepsis. Sepsis happens when a disease reaches, and spreads through the circulatory system, causing tissue harm wherever it goes. Even if one survives sepsis, it will leave the individual with long lasting damage to the lungs and other organs. [9]
- **Superinfection:** When a person fights with COVID- 19, the immune system works hard to fight off the invader. This results in more vulnerability towards different infections. This eventually causes additional damages to the lungs. [10]

Three factors play a major role in COVID- 19 lung damage [6,2]:

1. Disease severity
2. Health condition
3. Treatment

Chapter 2

Lungs

2.1 Anatomy

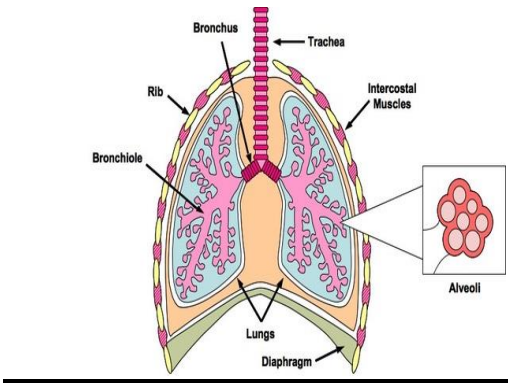

Fig 1: Anatomy of lungs [11]

The lungs are a pair of spongy, air-filled organs which are located on either side of the chest, the thorax. They are sacks of tissue located just below the rib cage and above the diaphragm. They are an important part of the respiratory system and waste management system of the body [12].

One of the largest organs in the human body is lungs. The surface area of the lungs and the subsequent length of the airways running through them are approximately 1500miles. A person's lungs are not the same size. Even though the right lung is wider than the left lung, it is shorter in length. Each of the lungs is divided into different lobes. The left lung has two lobes and the right lung has three; these are similar to sponge-like tissues in a balloon. Although each lobe receives air from its own branch of the bronchial tree, they all have the same function which is to bring oxygen into the bloodstream and remove carbon dioxide. As a result, it is possible to live with just one lung. Generally, a woman's lungs can hold less air than a man's. When at rest, the lungs of a man can hold around 750 cubic centimeters of air, while a woman's lung can hold around 285 to 393 cc of air. [13]

The lungs are important for various functions related to the human respiratory system. The trachea, also known as the windpipe, conducts inhaled air into the lungs through its tubular branches, called bronchi. The bronchi then divide into smaller and smaller branches called bronchioles, thus finally becoming microscopic. These bronchioles then further leads towards the alveoli duct which houses the responsibility for exchanging gases with the blood. [14] Furthermore, the bronchioles lack hyaline cartilage, which surrounds the bronchi and prevents them from collapsing. Smooth muscle tissues, composed of sheets or strands of smooth muscle cells, surround the bronchioles, thus reducing their size. In addition, the lungs are covered by a thin tissue layer called the pleura. The pleura is a vital part of the respiratory tract which cushion’s the lungs and reduces the friction developed between the lungs, rib cage and chest cavity. This also consists of a two-layered membrane that covers each lungs and are separated by small amounts of viscous fluids known as pleural fluids. [15]

2.2 Functions

Without oxygen, no cell in the human body can survive. The air breathed in by the humans contains oxygen and many other gases. One the oxygen moves into the lungs, it is moved into the bloodstream and carried throughout the body. At every cell point of the body, the oxygen are exchanged with carbon dioxide gas. As a result, these gases are then carried back to the lungs through the bloodstream and finally removed via exhalation. This is just one of the many important functions carried out by the lungs [16].

After breathing in, the air containing oxygen enters the windpipe, passes through the bronchi and finally reaches the air sacs. These air sacs, known as alveoli (as mentioned above) are responsible for the gas exchange. The oxygen passes through the thin lining of the air sacs and into the blood vessel via diffusion. Diffusion is the movement of gases from its higher concentration to its lower concentration down a concentration gradient. [17] Oxygen and carbon-di-oxide move in the opposite direction. The oxygen moves from the atmosphere to the lungs and then into the bloodstream, where it is carried into cells whereas carbon-di-oxide is transported from the bloodstream across the lining of the air sacs, into the lungs and finally into the atmosphere. It is essentially a waste product of cellular metabolism [18].

To add to gas exchange, lungs also play a vital role in protecting our body from harmful chemicals and substances. Since lungs are constantly exposed to the air, it plays a vital role in protecting our body and is thus linked to the immune system. [19] When people breathes in, pollutants and other chemical/gaseous substances enter our body along with oxygen. As a result, mucus is produced in the walls of the airways to keep the lung clean. Mucus is a slippery, long and thin fluid-like substance produced by many lining tissues in the body. It functions in keeping our lungs clean and well lubricated. It is moved by tiny hairs called cilia that lines the airways. They move back and forth sweeping a thin layer of mucus out of the lungs and into your throat. Unwanted materials/pollutants then stick to the mucus, until it reaches the throat where it gets swallowed unconsciously [20].

Finally, lungs can play a role in our sense of smell. The sense of smell involves various parts of the olfactory system. These olfactory systems inside the nose contain olfactory bulbs which have the ability to detect molecules in the air which are known as volatiles and finally send a signal to our brain. In the brain, the responses are processed via the sensory neuron before signals are sent out for any movements or memory, through the actions of the motor neurons.

Further studies suggest lungs are able to identify volatiles which are made by microbial organisms and invaders. [21]

Hence, this time, the target of the signal is not the brain but rather the immune system. As a result, the lungs have been identified as playing a major role in protecting our body from various infections [22].

2.3 How the virus infects the lungs:

The virus infects cells along the airways by attaching to ACE2 and other molecules on those cells. It uses ACE2 as a pathway to get into the cells and multiplies. This results in an immune response which includes inflammation, damages the air sacs, causes them to scar and stiffen and fill with fluid. This blocks the movement of oxygen from lungs to the bloodstream. As a result, shortage of breathing occurs due to the fall of oxygen levels. ACE2 helps reduce inflammation, but this effect is lost when viruses invade them. In mild cases of COVID-19, when the disease Is contracted, the lining of the bronchioles gets damaged, which irritates the lining of the airways causing dry coughs. It has been found that when such infected lungs go through a CT scan, white hazy patches are noticed inside them. This phenomenon is known as Ground- glass opacity. ARDS sets in a few days later. [23]

When a SARS-COV-2 virus enters alveoli, our immune system tries to fight it. Immune cells such as macrophages attack the virus. Sometimes, it defeats the virus and sometimes it recruits more immune cells like neutrophils. In the process of attacking the virus, they can injure the alveolus as well, by breaking down its walls. Fluid rushes into the alveolus from the blood vessels, which blocks the exchange of oxygen. [24]

Angiotensin-converting enzyme 2 (ACE2) is a receptor molecule which connects the inside and outside of our cells with the help of cell membranes. The SARS-COV-2 virus enters the cells of our airway through this receptor. This results in a phenomenon known as cytokine storm where an intense immune response is produced when the virus is encountered. Blood clotting also takes place at this stage. All of these leave the lungs damaged. To replace the damaged cells of the lungs, the body replaces them with scar tissues which are thick and stiff in nature. This often results in a condition called pulmonary fibrosis. [25] According to Dr. Brittany Bankhead-Kendall, an assistant professor of surgery with Texas Tech University, in Lubbock, the condition of a post- COVID infected lungs is usually worse

than that of the lungs of smokers. Furthermore, it was also stated that the lungs gradually collapse and breathing difficulties continue to persist. It was seen that the lungs of the patients who have had symptoms of COIVD- 19 showed a severe chest X-ray every time. Whereas, the people who were asymptomatic showed a severe chest X-ray almost 70-80% of the time.

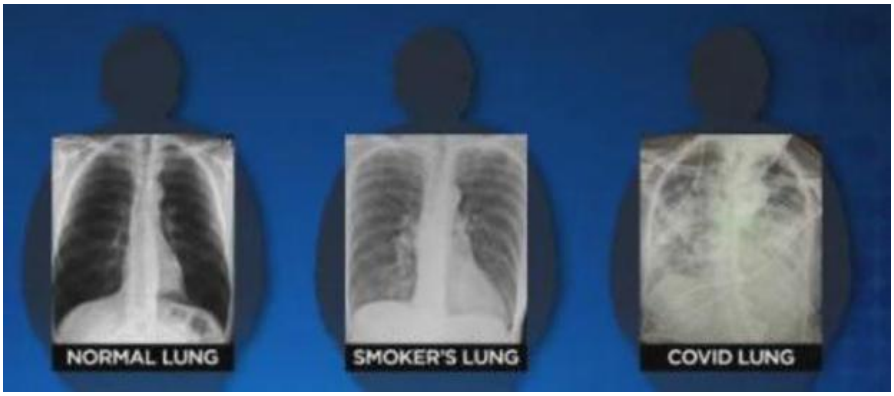

Fig 2: X-rays of a normal lung, a smoker's lung and a COVID patient's lung [26, 1]

In the X-ray of a normal lung, the portion which is back mainly indicates clean air. In case of a smoker's lung, the white parts indicate congestion and scarring. It is seen that in COVID-19 infected lungs, mostly white arts are seen which specifies that the condition gets worse that a smoker's lungs. [26,2]

Lung fibrosis cannot be cured on the grounds that scarring in the lung tissue is permanent. However, new medications can hinder the severity of the infection and even stop it totally if it is detected in time. [27]

Chapter 3

Statistical Analysis of Covid-19 patients recovering from lung damage:

Covid-19 patients suffer from long term lung damage and this tends to improve over time for many. Researchers in Austria recruited coronavirus patients from the covid-19 hotspot regions to their study, who were hospitalized at the University Clinic of Internal Medicine in Innsbruck, the St Vinzenz hospital in Munster, Austria. Their research consisted of a total of 150 participating patients, between 29th April to 9 June. The patients were evaluated 12 and 24

weeks after their discharge from the hospital. With the help of these evaluations, laboratory tests, clinical examinations, analysis of the oxygen amounts and carbon dioxide in the arterial blood, CT scans and echocardiograms lung function tests, were all supervised. [28,1]

During the course of the first visit in week 1, almost over 50% (>75 out of 150) of the patients had at least one persistent symptom, the most common being breathlessness and coughing and the CT scans showed persistent lung damage in 88% (132 out of 150) of the patients [28,2].

During their next visit after discharge in week 12, the symptoms had drastically improved and the lung damage was improved in 56% (84 out of 150) of the patients. Another finding was that most COVID patients showed lung impairment after discharge. However, it did become considerably better over time because lungs have built-in mechanisms to repair themselves. [28,3]

The average age of the patients were 61 years and about 65% of them were male. Of the 65% that were male, nearly half (50%) of them were current or former smokers and 65% of the hospitalized patients were suffering from obesity or being overweight. 32 (21%) patients had been in ICU, 29 (19%) of them required ventilation and the average length of stay in the hospital were 13 days [28,4].

A total of 98 patients (65%) showed persistent infections in the first 6 weeks. Breathlessness was the most common symptom in about 47% of the patients (73 out of 150), followed by coughing in 15% (23 out of 150) patients. By the 12 weeks visit, breathlessness was persistent in about 31% of the patients but 13% of the patients were still coughing, continuously. [28,5]

Tests of lung function included FEV1 which is the amount of air that can be expelled forcibly per second. The total volume of air which was expelled forcibly is FVC whereas to measure how smoothly oxygen passes from the lungs to the blood, DLCO was used. These measurements in patients improved between week 6 to week 12 of visits. At week six, 23% of the patients showed FVC at less than 80% of the normal which further improved to 19% by the end of week 12. Moreover, the DLCO improved from 23% to 19% at 12 weeks. [28,6]

Dr. Sahanic concluded that the findings from this study illustrated the importance of implementing a structured follow-up care for patients, suffering from severe Covid-19 infections. Patients infected by coronavirus may have long term lung damage and require an early treatment. [28,7]

Chapter 4

Tissue Engineering:

4.1 Introduction

Tissue engineering integrates the field of biology with engineering and is a part of biomedical engineering, which focuses on creating tissues or cellular products outside the body or utilizes the knowledge of biology to repair damaged tissues within the body [29,1]. Furthermore, it combines cells, scaffolds and other biologically active molecules into functional tissues [29,2]. The main goal of tissue engineering is to assemble functional constructs that improve, restore and maintain the damaged tissues or the entire organ inside the human body [30,1]. To construct new and improved tissues or organs, FDA approval is mandatory and as of now they have approved the use of artificial skin and cartilages but currently they have very limited uses in human patients. [30,2]

To improve the concept of tissue engineering, many new cellular therapies are being developed which will enhance a better functioning of the damaged tissues. [31] This implementation includes grafting, tissue harvest, cell processing and isolation, safety testing, cell activation or differentiation, assay and medium development, storage and stability, quality assurance and quality control issues that need to be addressed. Devices such as bioreactors are under observation and their functioning, choice, manufacturing and how they treat biomaterials for cell growth and device construction are all important for them to be taken under consideration [32].

4.2 Tissue Engineering Mechanism:

As mentioned previously, tissue engineering combines scaffolds, cells and biologically active molecules into functional tissues and helps in tissue repairments or organ replacement. Each cell has their own matrix, a supporting structure which is also known as scaffold [33]. This scaffold other than supporting cells, also acts as a relay station for various signaling molecules. Upon receiving messages from the local environment, the cells become available and induce a chain of response which determines the fate of the cell [34]. Each individual cell responds to signals, interacts with the environment and organizes into tissues and organisms in their own

unique way and by studying these variations, researchers have been able to manipulate these processes and fix or repair and even create new tissues [35].

Tissue engineering combines the principle of cell transplantation to develop substitute tissue or even regenerate them. It is initiated by building a scaffold from various sources, starting from proteins to plastics [36,1] After creating/developing scaffolds, cells with or without any growth factors can be introduced and under the right condition or environment, tissue develops. Sometimes, the cells, scaffold and the growth factors are all mixed together rat once which allows them to self-assemble [36,2].

An alternative is to create new tissue using existing scaffolds. The cells from the donor organ are stripped and the remaining collagen scaffold is used to grow new tissue [37]. This has been fundamentally used to engineer organs such as heart, liver, lung and kidney tissues. This process can be taken under consideration to replace lungs that are being infected by the coronavirus as long as the grafting dies not get rejected by the recipient's immune system [38].

4.3 Animal Tissue Culture:

It is an in-vitro culture where cell tissues and organs are isolated in an artificial environment. A wide range of cells and tissues are now being grown in labs.

Three methods are used to obtain cultures from animals:

1. Organ Culture: It is an in-vitro method where the whole organs from the embryo or partial adult organs are used to initiate the process. These cells in the organ culture often maintain their differentiated characteristics, their functional activity and also their in-vivo architecture or outlook. The growth is not very rapid and the cell proliferation is limited to the explant. The cultures cannot be propagated for long periods and as a result, fresh culture is required before every experiment. This fresh explanation before every experiment leads to inter experimental variation in terms of reproducibility and homogeneity. Organ culture enables the studying of the cell properties and experimenting drugs. [39]

2. Primary explant culture: The fragments separated from the animal tissues are maintained in a number of ways. The tissue adheres to the surface via the help of extracellular matrix (ECM) constituents such as collagen or plasma clot. [39] This in turn gives rise to cells migrating from

the periphery of the explant. This culture is often known as the primary explant and the migrating cells are called outgrowth. This has been used to analyze the growth and characteristics of cancer cells compared to their normal counterparts, in terms of cell morphology and growth pattern [40].

3. Cell culture: It is the most commonly used method of tissue culture and is conducted by collecting the cells that grow from the explants or that disperse from cell suspensions. Cells can either be obtained by mechanical means or by enzymatic treatments [41].

4.4 Organ Reconstruction:

Using tissue engineering and animal culture methods, damaged organs can also be replaced. Cells that are propagated as cell suspension or monolayer offer many advantages but they often lack the potential for cell-to-cell interaction and also cell-matrix interaction, as seen in organ cultures. As a result, many culture methods which start off with a dispersed population of cells, encourage the arrangement of these cells into organ-like structure [42].

There are two types:

1. Histotypic Culture: By using the appropriate ECM, soluble factors and by growing cell cultures at high cell densities, cell-cell interactions that are similar to the tissue densities can be obtained. This can be done by:

- Growing cells at high concentration on agarose mediums.
- By Growing the cells in a comparatively larger reservoir with an adequate medium containing a filter where the cells can be crowded.
- Growing the cells on the outer surface of hollow fibers where the cells are seeded on the outer surface and medium is pumped through the fibers from a reservoir [43].

3. Organotypic culture: cells of different lineages are recombined to induce a heterotypic cell-cell interaction. Co-culturing of epithelial and fibroblast cell clones from the mammary gland allows the cells to differentiate functionality under the correct hormonal environment [44]

4.4 Tissue Engineering of Covid-infected lungs:

The recent outbreak of coronavirus has left people helpless. The world is in need of a new vaccine or a medical procedure that can enable them to tackle the effects left behind by the virus or totally avoid getting infected. Some of the vaccines are still under trial while others such as the Pfizer vaccine are available in the market. Before usage, the clinical trials of all these vaccines must show that they are safe and effective [45].

It has been noted that the SARS-Cov-2 caused severe lung damage, which includes damage to the respiratory system and causes acute respiratory diseases such as bronchitis, pneumonia and fibrosis [46]. Thus, tissue engineering approaches offer a viable alternative to drugs, to those that are already suffering from chronic or acute lung-failure or damages [47].

Due to the overexpression of cytokines, the lung faces serious damage after covid-19. But, due to its regenerative properties and anti-inflammatory capability, the mesenchymal cells (MSc) can repair damaged lung tissues and also further stabilize endothelial fluid leakage and reduce the alveolar capillary barrier function. Thus, this reduces the probability of the lung developing edema [48]. The exogenous mesenchymal cells that are transplanted, provide the necessary signals to stimulate endogenous lung progenitors and create a partnership between exogenous and endogenous cell activities [49].

Furthermore, the lining of the bronchioles got damaged which irritates the lining of the airways, causing dry coughs. The walls are also broken, which damages the alveoli. To resolve these issues, TE mediated tissue regeneration strategies could be obtained [50]. By isolating the cell/tissue culture and growing them at high cell-densities with the use of appropriate ECM, the tissues can be replaced. Afterwards, using organotype cultures, cells from different sources can be co-cultured and their functionality could be restored [51].

According to studies, stem cell therapy can reduce lung tissue damage and improve the chances of survival. An implantable airway was produced by using tissue engineering strategies. The human bone marrow derived mesenchymal stem cells were prepared and cultured on a cell free scaffold under favorable conditions [52]. The results obtained show that these cells were suitable for cell-free lung regeneration and treatment. Hence, according to studies, it was

established that damaged lung tissues could be regenerated after being infected by Covid-19 [53].

Bioprinting, along with the manufacturing process with correct biomaterials can then be utilized to create lungs and replace them in patients that are suffering serious health complications in their respiratory pathway, due to covid-19 [54].

Chapter 5

Transplantation:

5.1 Introduction

Transplantation is such a treatment which is widely used for replacing non-functioning organs and tissues with healthy tissues and organs. It is also known as graft where cells, tissues and organs from one individual are taken and placed into a different individual.[55] A donor is the person who provides the graft, and the person receiving it is the recipient or host. Ever since the first transplantation in 1954, several organs have successfully been clinically transplanted such as kidney, heart, liver, stem cells etc. Lungs, however, are yet to be successfully transplanted from one body to another [56]. The replacement of a Covid-19 infected lungs by 3-D bio-printed lungs revolves around the transplantation process. [57]

5.2 Cases of Lung transplants due to COVID-19:

Recently, a 20-year-old COVID-19 survivor in Chicago went through a lung transplant to treat pulmonary fibrosis. Two other lung transplants were also performed on COVID-19 survivors with post-COVID fibrosis: one was in China and the other in Vienna. [58]

According to WHO, COVID- 19 may increase the risk of long- term health problems which include damage to lung tissue and restrictive lung failure. [59]

Chapter 6

3-D Bioprinting

6.1 What is 3D Bioprinting?

Bioprinting is a multidisciplinary technique that covers 3D manufacturing innovation which includes tissues, organs and cells for clinical and biotechnology applications. [60] Scaffolds, nanomaterials, biomaterials, 3D printing technology, imaging and CAD/CAM software and hardware, post-printing bioreactor maturation, cell and biological factor patterning, bio-fabrication, tissue engineering and other applications of 3D bioprinting technology are also part of it. The process uses a material which is known as bioink to make these structures in different layers. The strategy is broadly material to the fields of medication and bioengineering. [61,1]

The innovation bioink to make these structures in different layers. The strategy is broadly material to the fields of medication and bioengineering. The whole process requires a sterilized condition and goes through several cycles before coming into reality. [62]

The cycle chiefly includes planning, printing, development, and application. This can be summed up in the three key advances:

- Pre-bioprinting includes making the advanced model that the printer will deliver. The advancements utilized are Computed Tomography (CT) and magnetic resonance imaging (MRI) scans.
- Bioprinting is the real printing measure, where bioink is put in a printer cartridge and deposition happens dependent on the digital model.
- Post-bioprinting is the mechanical and chemical incitement of printed parts to make stable structures for the organic material. [63]

6.2 Mechanism of 3D Bioprinting:

There are different techniques which are generally based on inkjet, acoustic, extrusion, or laser technologies. There are a few fixed steps that every 3D bioprinting method follows:

- **3D Imaging:** To get the specific components of the tissue, a standard CT or MRI check is utilized. 3D imaging ought to give an ideal fit of the tissue with next to zero change needed with respect to the specialist.
- **3D Modeling:** A diagram is created utilizing AutoCAD programming. The diagram additionally incorporates layer-by-layer guidance in high detail. Fine changes might be made at this stage to stay away from the exchange of imperfections.
- **Bioink Preparation:** Bioink is a blend of living cells and a viable base, similar to collagen, gelatin, hyaluronan, silk, alginate or nanocellulose. The latter furnishes cells with a framework to develop on and nutriment to make due on. The total substance depends on the patient and has a specific function.
- **Printing:** The 3D printing measure includes keeping the bioink layer-by-layer, where each layer has a thickness of 0.5 mm or less. The conveyance of more modest or bigger stores exceptionally relies upon the quantity of nozzles and the sort of tissue being printed. The combination emerges from the nozzle as an exceptionally viscous fluid.
- **Solidification:** As deposition happens, the layer begins as a viscous fluid and sets to hold its shape. This occurs as more layers are constantly kept. The way toward mixing and solidification is known as crosslinking and might be helped by UV light, explicit synthetics, or warmth (additionally regularly conveyed by means of an UV light source). [61,2]

6.3 Applications of 3D Bioprinting:

3D bioprinting has shown promising results and prospects in terms of drug research, cell scaffolds which help to repair damaged joints and ligaments, repairing or replacing damaged tissues and organs, prosthetics etc. [64]

6.4 Prospects of 3D Bioprinting for COVID- 19 recovered patients:

The SARS-COV-2 virus attacks several organs including the heart, lungs and liver. However, the most dominant and common impacts are seen in the lungs of COVID-19 patients as it is a serious respiratory disease. To minimize the damages of terminal lung diseases, 3D bioprinting can be considered to replace or repair damaged lungs. In case of COVID-19, the lungs are harmed mainly due to the damage occurred to the lining of the bronchioles and the breaking of

the alveolar walls. Targeting these specific defacement, 3D bioprinting can be a propitious treatment option for COVID-19 recovered patients.

In the year 2019, a group of researchers developed a lung-mimicking air sac using 3D printing technology. Jordan Miller, assistant professor of bioengineering at Rice's Brown School of Engineering and his team developed a bioprinting technology for the first time, that addresses the challenge of multi-vascularization in a direct and comprehensive way. They have sorted out some way to print artificial renditions of the body's intricate vascular networks, which copy our regular pathways for blood, air, lymph, and other fundamental fluids. [65]

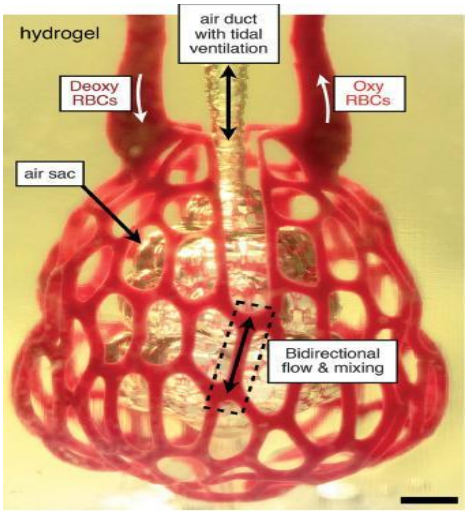

Fig 3: 3D printed lung-mimicking 'air sac' [66]

The specialists made an open-source bioprinting innovation they call "Stereolithography Apparatus for Tissue Engineering," or SLATE [67]. The method includes layer-by-layer printing of a structure utilizing a fluid pre-hydrogel arrangement that gets strong when presented to blue light. Utilizing this innovation, the scientists built up a counterfeit lung-impersonating structure total with aviation routes and veins. The scientists are presently investigating the advancement of considerably more perplexing structures utilizing the innovation. [68]

They have recognized promptly accessible food colors that can fill in as powerful photo absorbers for biocompatible and cytocompatible creation of hydrogels containing useful vascular geographies for investigations of fluid mixers, valves, intervascular transport, supplement conveyance, and host engraftment. With the help of their stereolithographic cycle,

there is potential for concurrent and symmetrical authority over tissue engineering and biomaterials for the plan of regenerative tissues. [69]

With proper research into this field, a plausible replacement can be brought out for COVID-19 infected lungs with the help of tissue engineering and 3D bioprinting.

6.5 Cost Efficiency of 3D Bioprinting:

3D bio-printed organs have the prospect to become a great competitor of organ transplantation. The cost effectiveness of 3D bio-printed organs compared to that of the original one is very significant. For instance, a typical kidney transplant costs an average of $330,000, according to the National Foundation for Transplants. On the contrary, a conventional 3D bioprinter costs only $10,000 [70]. Moreover, the average cost of a typical lung transplantation is around $135,622. [71] If lungs can be replaced and repaired through 3D bio-printed, it can cut a noticeable amount of sum and can be feasible for a larger population. In the near future, 3D bioprinted organs have ample plausibility to replace traditional organ transplantation due to its low cost and simple treatment procedures. Additionally, in terms of 3D bio-printed organs, the materials that are used are biocompatible and consist of the patient's cells. Thus, the risk of graft rejection is prevented. This results in an additional cost cut off compared to traditional organ transplantation where immunosuppressed drugs are used to prevent graft rejection.

6.6 Strengths and Limitations of 3D Bio-printed Lungs:

Strengths:

3D bio-printed organs are patient specific which reduces the chances of graft rejection. Reproducibility of tissue is ensured through tight control of both composition and geometry. The process is faster and more precise than traditional organ transplant and additionally, waiting times for organ donors is reduced. Effects of disease states or drugs may be more accurately observed without the need for human subjects. Furthermore, as it is created entirely in the laboratory through 3D printers and tissue engineering, the risk of organ trafficking and animal testing is reduced. Compared to typical organ transplantation, 3D bio-printed organs are more cost friendly and can target a larger population. [72]

Limitations:

In the development of organ printing technology and adaptation of rapid prototyping technologies for bioprinting and bio-fabrications involves challenges such as design and fabrication of the bioprinter and a biologically friendly rapid prototyping machine. [73] The viability of the printed tissues includes cell survival during loading of bioprinting cartridges, processing and post processing. [74] One of the major challenges of this process is the complex vascularization of organs. More research work is needed in this field to overcome these challenges. However, in under- developed countries, funding is not sufficient enough to work on such projects. In addition to this, even though 3D bioprinting is much more cost efficient than typical organ transplantation, however, it will not be feasible for all classes of people to afford it. Also, it raises various ethical concerns.

Chapter 7

Discussion & Conclusion:

Lungs is a vital organ which carries out the most important function in providing oxygen to every cell and/or organs in the body. It also helps in disposing off carbon dioxide (waste gas), thus performing successful gas exchange and aiding respiration.

COVID-19 however disrupts the flow of the respiratory system, causing inflammation and making it difficult for humans to breathe. This leads to life threatening infections and respiratory failures resulting in deaths.

Tissue engineering (TE), integrated with bioprinting offers a possible alternative. TE allows construction, restoration or even an entire organ. When coupled with quick development speed and high precision of 3D bioprinting, a successful lung construction and transplantation is possible. Furthermore, 3D bioprinting meets the prerequisite on individual clinical treatment and provides favorable circumstances such as low dismissal response. Yet, it is additionally gone up against with generally enormous difficulties in biomechanics, determination of stent material, assurance of microbe free climate, trim of printed structure, blood supply for printed structure and long-haul endurance of printed structure simultaneously.

However, 3D bioprinting is not yet a completely mature technology and it is as yet needing researchers' constant endeavors and forward leaps. As of now, the technology has not been widely applied in different countries or laboratories.

To conclude, 3D bio-printed lungs provide a much more feasible root for treating long term lung complications/ infections caused due to COVID-19 as it provides a more cost efficient, safer means than a typical organ transplantation.

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