زانکۆی سەلاحەدین-هەولێر

Salahaddin University-Erbil

3Dprinting technology in regenerative Medicine

Research project

Submitted to the department of (chemistry) in partial fulfillment of the requirements for the degree of B.A or BSc in (chemistry)

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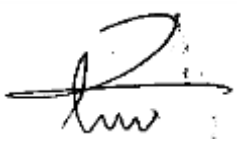
CERTIFICATE

This project has been written under my supervision and has been submitted for the award of the degree of B.Sc in **chemistry** with my approval as supervisor

Signature  Name: ***Lec. Hemn J. Majeed***

Date: 4 / 4 /2024

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Date: 4 / 4 /2024

Dedication

To all my family members and friends.

To all my teachers, especially my supervisor.

To all who helped me to learn

Acknowledgment

First and foremost to god almighty, for his immeasurable blessings and guidance throughout the study and for the wisdom he bestowed upon the researchers to complete's the research successfully

I would like to acknowledgment and give my warmest thanks to my supervisor (Hemn J. Majeed)

Advice carried me through all the stages of writing my project. I would also like to give special thanks to my family as a whole for their continuous support and understanding when undertaking my research and writing my project.

Finally, I would like to thank god for letting me through all the difficulties. I have experienced your guidance day by day.you are the one who let me finish my degree. I will keep on trusting you for my future.

Abstract

Regenerative medicine is an emerging field that centers on the restoration and regeneration of functional components of damaged tissue. Tissue engineering is an application of regenerative medicine and seeks to create functional tissue components and whole organs. Using 3D printing technologies, native tissue mimics can be created utilizing biomaterials and living cells. Recently, regenerative medicine has begun to employ 3D bioprinting methods to create highly specialized tissue models to improve upon conventional tissue engineering methods(Saini, Segaran et al. 2021).

In the review discusses how recent advances in the field of regenerative medicine and improve 3D bio printing and the concept of tissue engineering techniques, as well as selecting biomaterials for tissue engineering and regenerative medicine. In addition, Scaffold fabrication techniques and 3Dpriting technology also described. Finally, relationship between Three-dimensional (3D) bioprinting and regenerative medicine deliberated.

Contents:

Title	Page Number
CERTIFICATE	I
Dedication	II
Acknowledgment	III
Abstract	IV
1.Introduction	1
2.Tissue engineering 2.1 Biomaterials for Tissue Engineering and Regenerative Medicine 2.1.1 Natural Polymers 2.1.2 Synthetic polymers 2.1.3 Inorganic biomaterials 2.1.4 Organic-inorganic hybrid biomaterials 2.2 Types of cells 2.2.1 Stem cell classification based on origin 2.2.1.1 Embryonic stem cell 2.2.1.2 Adult stem cell 2.2.1.3 Tissue-resident stem cell 2.3 Scaffolds fabrication techniques	3 4 4 5 5 7 7 8 8 9 9 9
3.3D printing 3.1 Type of 3D printing 3.1.1 Inkjet bioprinting 3.1.2 Laser-assisted printing 3.1.3 Extrusion printing 3.1.4 Stereolithography printing 3.1.5 Fused deposition modeling 3.1.6 Direct Ink writing 3.2 Applications of 3D printing	10 10 10 11 12 13 14 15 16
4.Discussion	18
5.Conclusion	20
6.References	21

Table of figures

Figures	Page Number
Figure 1	3
Figure 2	4
Figure 3	5
Figure 4	6
Figure 5	7
Figure 6	11
Figure 7	12
Figure 8	13
Figure 9	14
Figure 10	15
Figure 11	16
Figure 12	17

1. introduction

3D printing also known as additive manufacturing technology has been dubbed the next big thing and be as equally wide spread as cellular telephone industry. 3D printers print objects from a digital template to a physical 3-dimensional physical object. The printing is done layer by layer (Additive manufacturing) using plastic, metal, nylon, and over a hundred other materials. 3D printing has been found to be useful in sectors such as manufacturing, industrial design, jewellery, footwear, architecture, engineering and construction, automotive, aerospace, dental and medical industries, education, geographic information systems, civil engineering, and many others(Mpofu, Mawere and Mukosera 2014).

The use of additive manufacturing techniques, also known as “3D printing”, has steadily increased in a variety of scientific fields(Gu, Choi et al. 2018). There are a number of inherent advantages to these fabrication methods over conventional manufacturing due to the way that they work, which is based on the layer-by-layer material-deposition principle. These benefits include the accurate attribution of complex, pre-designed shapes, as well as the use of a variety of innovative raw materials. Its main advantage is the ability to fabricate custom shapes with an interior lattice network connecting them and a porous surface that traditional manufacturing techniques cannot adequately attribute. Such structures are being used for direct implantation into the human body in the biomedical field in areas such as bio-printing (Unal and Arora 2021).

Were this potential is being heavily utilized. The fabricated items must be made of biomaterials with the proper mechanical properties, as well as biomaterials that exhibit characteristics such as biocompatibility, bioresorbability, and biodegradability, in order to meet the strict requirements that such procedures impose. 3D objects by adding successive layers of materials at a regulated rate and thickness. These materials could be made of concrete, metals, ceramics, polymers, resins, biomaterials, or other substances. The dearth of variety in 3D-printable materials continues even though printing time, processing speed, and printing resolution have all increased over the past few years. The compatibility and flowability of printing ink with the current printing procedures are crucial for developing fields such as the 3D printing of biomaterials, tissues, and high-viability cells (Kantaros 2022) .

The earliest 3D printing manufacturing equipment was developed by Hideo Kodama of the Nagoya Municipal Industrial Research Institute, when he invented two additive methods for fabricating 3D models. According to the World Health Organization, only 10% of the world's need for tissue and organs is being fulfilled, making it a severe public health issue. Additionally, the long-term success of organ transplantation is unknown as recipients must take lifelong immunosuppressive treatment regimens, increasing the risk of fatal infections, and because half of the transplants fail after ten years Other difficulties with tissue/organ transplantation include ethical permission, persuading family members to give tissue/organs, and the fact that many hospitals, particularly in middle- and low-income countries, lack the resources to maintain the organs/tissue of brain-dead patients In addition to tissue/organ transplantation, a significant problem faced by biomedical research organizations is figuring out the cellular and molecular causes of human disease to provide novel methods for therapeutic intervention, prevention, or diagnosis(Shabbirahmed, Sekar et al. 2023).

The artificial generation of tissues, organs, or even more complex living organisms was throughout the history of mankind a matter of myth and dream. During the last decades this vision became feasible and has been recently introduced in clinical medicine. Tissue engineering and regenerative medicine are terms for the field in biomedicine that deal with the transformation of these fundamental ideas to practical approaches. Several aspects of generating new tissues and organs out of small pieces of living specimens are now scientifically solved, but at this point it is unknown how much impact these new approaches will have on clinical medicine in the future (Figure show1) (Meyer 2009).

The 3D structured scaffolds and hydrogels alone or combined with bioactive molecules or genes and cells are able to guide the development of functional engineered tissues, and provide mechanical support during in vivo implantation. Naturally derived and synthetic polymers, bioresorbable inorganic materials, and respective hybrids, and decellularized tissue have been considered as scaffolding biomaterials, owing to their boosted structural, mechanical, and biological properties (Zielińska, Karczewski et al. 2023).

The newest procedures focusing on the 3D behavior and multi-cellular interactions of native tissues for further use for in vitro model processing are also outlined. Completed and ongoing preclinical research trials for tissue engineering applications using scaffolds and hydrogels, challenges, and future prospects of research in the regenerative medicine field are also presented (Pina, Ribeiro et al. 2019).

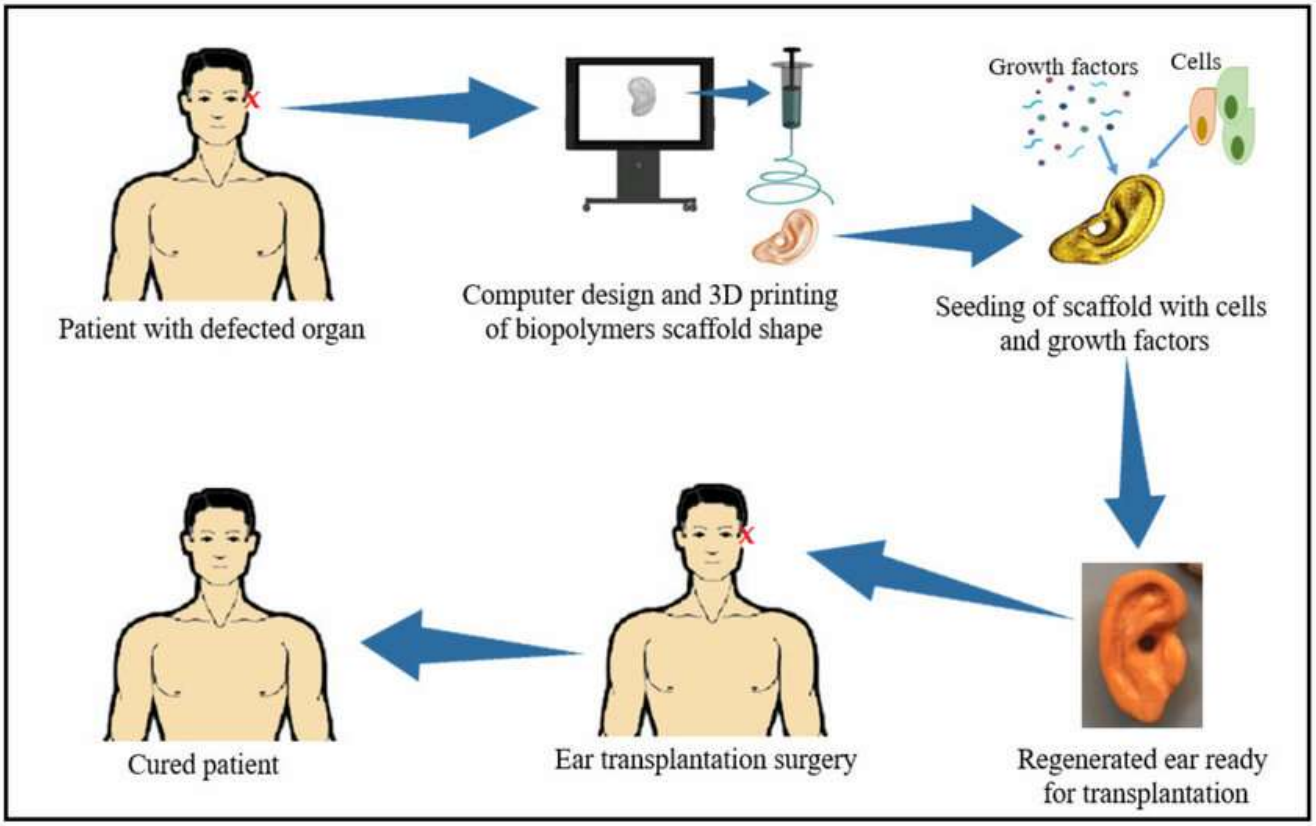

Fig 1: biomedical engineering the contribution of 3D printing to medicine

2. Tissue engineering

Tissue engineering, as a division of regenerative medicine, combines engineering and biological science in order to reproduce tissues and organs that can help to overcome the lack of enough donor organs. Tissue engineering applies cells into desirable biological structures in a defined framework to restore the normal function of tissues. This process includes three cornerstones, namely scaffolds, cells, and signaling factors. As a critical component in tissue engineering, scaffolds provide mechanical stability and structural support for exogenous cell attachment and proliferation and facilitate the delivery of required growth factors for tissue regeneration materials engineering to restore, maintain, and enhance tissue function (Askari, Naniz et al. 2021).

Tissue engineering is the construction, repair or replacement of damaged or missing tissue in humans and other animals. This engineering may take place within the animal body or as tissue constructs to be made in a bioreactor for later grafting into the animal. The minimal set of materials for this are the appropriate types of cells. Usually, however, non-living substrata are used as well. These substrata may be nothing more than materials that bulk up any voids in the damaged tissue and provide the mechanical

strength that has been lost when the tissue is damaged or removed. They may serve a similar pair of functions in the bioreactor. They can do much more in terms of pattern formation. The orientations and morphology of the cells, the arrangement of intercellular material as it is laid down and the relationships between different cell types in the repairing or construct tissue are all of importance, for these should resemble the correct normal tissue as closely as possible. Most of these requirements are ones involving pattern formation (Neishabouri, Soltani Khaboushan et al. 2022).

2.1 Biomaterials for Tissue Engineering and Regenerative Medicine

2.1.1 Natural Polymers

Natural polymers obtained from renewable resources, such as algae, plant, animal, and microorganisms, are similar to biological macromolecules, and easily recognized by the environment., natural polymers, also known as biopolymers, may also elude chronic inflammation toxicity or immunological reactions, frequently noticed with synthetic polymers. Therefore, these types of polymers are crucial for designing therapeutic systems to be used as bioactive compounds and drug delivery systems for disease treatment,or even to bioengineer functional tissues. Biopolymers that have been clinically used for implant fabrication include proteins Figure2 show (e.g., silk fibroin, collagen, gelatin, keratin, fibrinogen,polysaccharides (e.g., chitosan, alginate, gellan gum, and glycosaminoglycans (Gheorghita, Anchidin-Norocel et al. 2021).

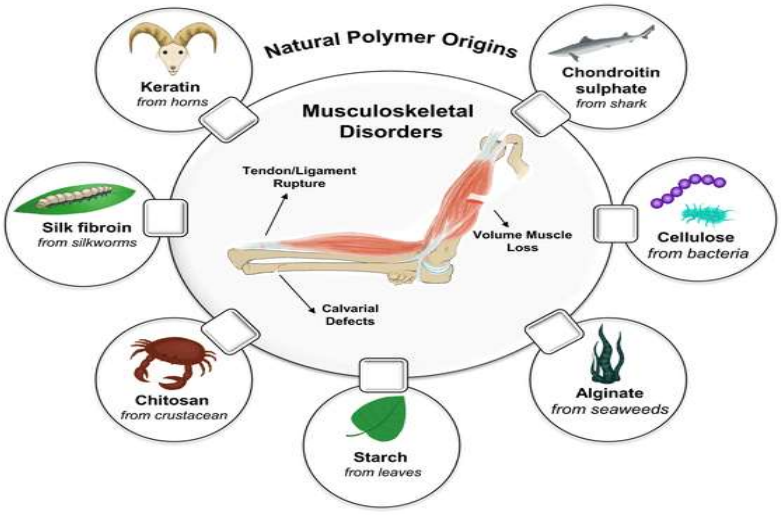

Fig 2:Some biopolymers derived from renewable resources

2.1.2 Synthetic polymers

have excellent processing characteristics in terms of their molecular weight, degradation, and mechanical properties, with the advantage of having tailored property profiles for specific applications. Hydrolytically degradable polymers are mostly chosen as implants due to their minimal site and patient-to-patient variations when compared to enzymatically degradable polymers However, many of these polymers present an immune response or toxicity, particularly when combined with certain polymers and not being capable of being incorporated with host tissues A strategy is to develop hybrid materials by combining them with natural polymers to improve hydrophilicity, cell attachment, and biodegradability (Figure3show).The most-used synthetic polymers in TE are polyglycolide (or poly glycol acid (PGA)), polylactide (or PLA), poly-lactide-co-glycolide (PLGA), poly-D-L-lactic acid) (PDLLA), poly-ethylene-glycol (PEG), and PCL. These polymers can be self-reinforced to enhance their mechanical strength (Suggs, Moore and Mikos 2007).

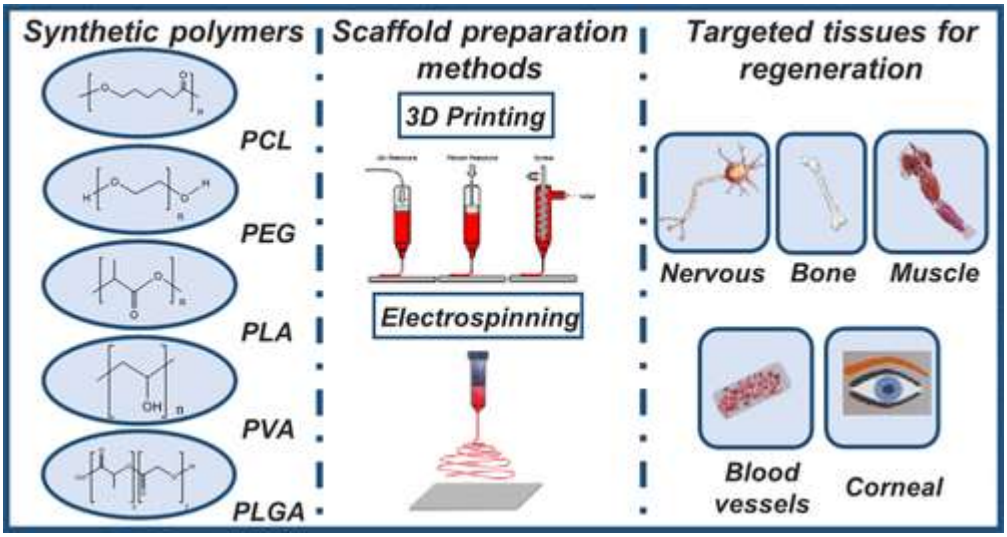

Fig 3: Synthetic Polymers for Tissue Engineering

2.1.3 Inorganic Biomaterials

bioinert, bioactive, or bioresorbable depending on their ability to bond directly with native tissues once implanted. Bioinert materials (e.g., alumina, zirconia, titanium, and its alloys) have no interaction with their adjacent tissue after implantation, typically being applied as structural-support implants, such as bone devices and femoral heads. On the other hand, bioactive materials (e.g., bio glasses and glass-

ceramics) bond directly with living tissues, and have been applied to fill small bone defects and periodontal irregularities (Ben Amara 2023).

Bioresorbable materials (e.g., CaPs, CPCs, and calcium carbonates or calcium silicates) gradually absorbed in vivo and are replaced by bone over time. Naturally-derived inorganic biomaterials from marine shells, corals, sponges, nacres, and animal (fish and chicken) bones offer an abundant source of calcium compounds e.g- calcium carbonate and calcium phosphate (Bianchi, Vigani et al. 2022).

These biomaterials can be obtained by numerous methods (e.g., aqueous precipitation, hydrolysis, sol-gel synthesis, hydrothermal synthesis, mechanochemical synthesis, microwave processing, and spray drying), resulting in materials with increased crystal size and morphology Among them, the wet precipitation method offers an advantage on the material synthesis, which involves a precise control of the pH, temperature, particle morphologies, and the presence of additives. A number of studies are dedicated to functionalizing bioactive inorganic materials by doping them with ionic elements Figure 4show (e.g., strontium, zinc, magnesium, manganese, silicon) (Pina, Ribeiro et al. 2019).

Fig 4: Inorganic Biomaterials for Tissue Engineering

2.1.4 Organic-Inorganic Hybrid Biomaterials

Hybrid biomaterials formed by combining organic and inorganic compounds result in multifunctional materials with tailored mechanical, thermal, and structural stability properties. Concerning the fabrication of composite scaffolds, it is essential above all to attain a good compatibility between the phases and maintain the porous structure and the mechanical strength of the scaffolds. Furthermore, nanostructured hybrids have also been preferred due to the nanosized features of the fillers (Vach Agocsova, Culenova et al. 2023).

thus enhancing the bonding capacity of the tissue to the organic matrices that the individual materials cannot accomplish The nanoparticles have large surface areas when compared to the micro-sized fillers, thus contributing to upgraded mechanical properties, while retaining the biocompatibility and osteoconductivity, cell adhesion, and proliferation of the fillers Many combinations of polymers and inorganic materials have been proposed to engineer different tissues with enhanced osteoconductivity and mechanical properties, Figure 5show carbon nanotubes and graphene and polylactide (or PLA), organic biomaterials and strontium, zinc, magnesium, manganese, silicon (Pina, Ribeiro et al. 2019).

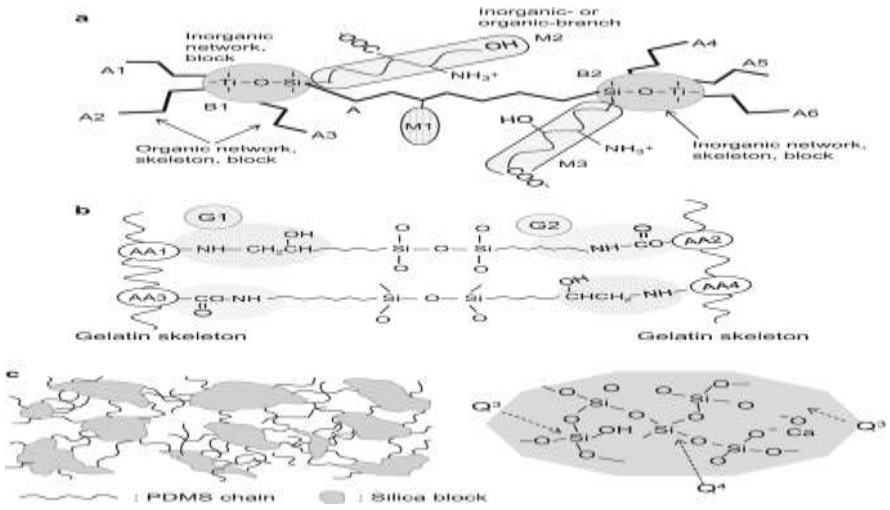

Fig 5: Organic-Inorganic Hybrid Biomaterials

2.2 Type of cells

The cells are generally derived from the patients themselves, from close relatives, or other individuals. For example, autologous chondrocyte transplantation for knee repair is in clinical use. In the case of tissue-engineered skin, neonatal dermal fibroblasts have been used. However, to provide cells for the different applications a source from which a variety of cells could be derived would be useful. One

approach to solve the cell-source difficulty could be the isolation of human stem cells, cells which can be proliferated through multiple generations and made to differentiate into the appropriate cell type. Some studies showed that stem cells derived from human embryonic blastocysts possessed these characteristics. Work on stem cells involved in the creation of cartilage, bone, and muscle has shown encouraging results in tissue-engineering applications(Fredriksson 2008).

Progenitor cells that have been identified can be signalled to turn into cartilage or bone by changing the culturing conditions of the cells before implantation. In addition, small oval cells in the liver have been found, which can turn into either mature liver cells or bile ducts in culture. A critical issue for the future, from a tissue-engineering standpoint, is to learn how to control the permanent differentiation of stem-cell populations into the desired cell types, whether we need cartilage, bone, liver, or some other cell type. There are also a number of technical hurdles such as the need for pure stem-cell preparations those without other cells such as fibroblasts mixed in methods to reduce cell adhesion during culture, and processes to increase the production of the large numbers of cells needed to create tissue. Another solution may be to create cells that could be used as “universal donors”. One approach uses molecules that mask the histocompatibility proteins on the cell surface that normally identify the donor cells as “non-self (Vacanti and Langer 1999).

2.2.1 Stem Cell Classification Based on Origin

Stem cells can be grouped into many broad categories based on their origin: ESCs, fetal and adult stem cells, and iPSCs. In general, ESCs and iPSCs are pluripotent, whereas adult stem cells are oligopotent or unipotent (Kolios and Moodley 2012).

2.2.1.1 Embryonic Stem Cells

ESCs are pluripotent, derived from the inner cell mass of the blastocyst, which will form the embryo, and the outer cell mass, called trophoblasts, that will form the placenta. Cells from the inner cell layer are separated from trophoblasts and transferred to a culture dish under very specific conditions to develop ESC lines ESCs are identified by the presence of transcription factors (Tan, Li et al. 2024).

2.2.1.2 Adult Stem Cells

Adult stem cells are derived from adult tissue. Examples include MSCs as well as stem cells derived from placental tissue such as human amnion epithelial cells. These cells have been shown to be anti-inflammatory and augment repair of animal models of injury. They have limited differentiation capacity although these cells have been differentiated into tissue from different germ cell layers in vitro (Deus, Mano and Custodio 2020).

2.2.1.3 Tissue-Resident Stem Cells

The ability of some tissues and organs in the adult to renew and repair following injury is critically dependent on tissue-resident stem cells that generate tissue-specific, terminally differentiated cells. suggest that these cells originate during ontogenesis and remain in a quiescent state till local stimuli activate their proliferation, differentiation or migration (Bhartiya 2021).

2.3 Scaffolds fabrication techniques

The scaffold fabrication techniques coupled with the choice of materials have a vital role in the determining the scaffold properties for the target applications (Mabrouk, Beherei and Das 2020).
tissue engineers are attempting to engineer virtually every human tissue. Potential tissue-engineered products include cartilage, bone, heart valves, nerves, muscle, bladder, liver, etc. Tissue engineering techniques generally require the use of a porous scaffold, which serves as a three-dimensional template for initial cell attachment and subsequent tissue formation both in vitro and in vivo. The scaffold provides the necessary support for cells to attach, proliferate, and maintain their differentiated function. Its architecture defines the ultimate shape of the new grown soft or hard tissue. In the early days of tissue engineering, clinically established materials such as collagen and polyglycolide were primarily considered as the material of choice for scaffolds. The challenge for more advanced scaffold systems is to arrange cells/tissue in an appropriate 3D configuration and present molecular signals in an appropriate spatial and temporal fashion so that the individual cells will grow and form the desired tissue structures and do so in a way that can be carried out reproducibly, economically, and on a large scale (Hutmacher 2001).

3. Three-dimensional Printing (3D printing)

Three-dimensional (3D) bioprinting is a rapidly growing technology that has been widely used in Tissue regeneration disease studies, and drug screening. It provides the unprecedented capacity of depositing various types of biomaterials, cells, and biomolecules in a layer-by-layer fashion, with precisely controlled spatial distribution. This technology is expected to address the organ-shortage issue in the future (Unal and Arora 2021).

3.1 Type of 3Dprinting

The 3D printing technology is being utilized in many specialties of medicine for surgical planning, educational modeling, and the creation of implantable medical devices, to date, no single bioprinting technique enables the production of all scales and complexities of synthetic tissues. The three major bioprinting techniques of inkjet, laserassisted, and extrusion bioprinting each have specific strengths, weaknesses, and limitations (Vijayavenkataraman, Yan et al. 2018).

3.1.1 Inkjet bioprinting

The first bioprinting technology and is very similar to conventional 2D inkjet printing (S A hydrogel pre-polymer solution with encapsulated cells (called a bioink) is stored in the ink cartridge. The cartridge is then connected to a printer head and acts as the bioink source during the electronically controlled printing process. During printing, the printer heads are deformed by a thermal Biotechnology (Figure 6show). the advantages of inkjet printing include: (1) low cost due to similar structure with commercial printers, (2) high printing speed conferred by the ability of the printer heads to support parallel work mode, and (3) relatively high cell viability This process is similar to SLS; instead of fusing the powder bed with laser or electron beam, binding liquid is selectively dropped on to the powdered bed to bind the materials in a layer-by-layer fashion (Iram, a Riaz and Iqbal 2019).

This process is continued until the final object is formed. Thermal and piezoelectric are two types of printing heads used in this technique. In thermal print head systems, an electric heating unit is present inside the deposition head, which vaporizes the binding material to form a vapor bubble. This vapor bubble expands due to pressure, and comes out of the print head as a droplet. Whereas in the piezoelectric print head system, the voltage pulse in the print head induces a volumetric change (changes in pressure and velocity) in the binder liquid, resulting in the formation of a droplet. These printers are known for their precise deposition of the binder liquid with speed and accuracy (Tappa and Jammalamadaka 2018).

Water, phosphoric acid, citric acid, PVA, poly-DL-lactide (PDLLA) are some of the commonly used binding materials for inkjet 3D printing. A wide range of powdered substances, including polymers and composites, are used for medical and tissue engineering applications. Finished 3D printed objects are often post-processed to enhance the mechanical properties, have used phosphoric acid and PVA as binding liquids to bind HA/β-TCP powders for bone tissue regeneration applications (Sandler, Määttänen et al. 2011).

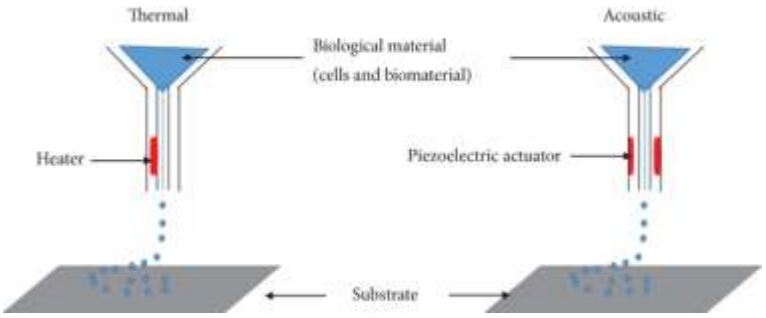

Fig 6: Inkjet bioprinting components. Thermal inkjet printers heat the print head electrically to produce pressure pulses that force droplets of biological material through a nozzle. Acoustic inkjet printers use pulses generated by piezoelectric pressure to break liquids into droplets.

3.1.2 Laser-assisted printing

Laser-assisted bioprinting originated from laser-induced transfer technology It is a modified version of inkjet bioprinting that overcomes clogging and compatibility issues. A typical laser-assisted bioprinting setup involves three components (Figure7 show) an energy-absorbing donor layer that responds to laser stimulation, a bioink layer underneath the donor layer, and a collecting layer to form tissue constructs During bioprinting, a laser pulse is focused on a small area of the top donor layer. Upon energy absorption, this small area in the donor layer vaporizes and creates a high-pressure air bubble at the

interface between the donor and bioink layers. The air bubble propels the suspended bioink to form a droplet that is eventually received by the bottom collecting layer (Chang and Sun 2023).

A tissue construct is thereby formed in a droplet-by-droplet manner. Laser-assisted bioprinting is compatible with highly viscous materials and high cell density. In addition, it has been reported that cells maintain high cell viability, over 95%, due to the short period of the laser pulse. However, the generation of pulse laser and the fabrication of a non-reusable donor layer increase costs relative to inkjet bioprinting. Consequently, only a few prototypes of laser-assisted bioprinters exist today. Another significant limitation of laser-assisted bioprinting is the unresolved challenge of building large-scale 3D structures using a drop-by-drop approach (Loai, Kingston et al. 2019).

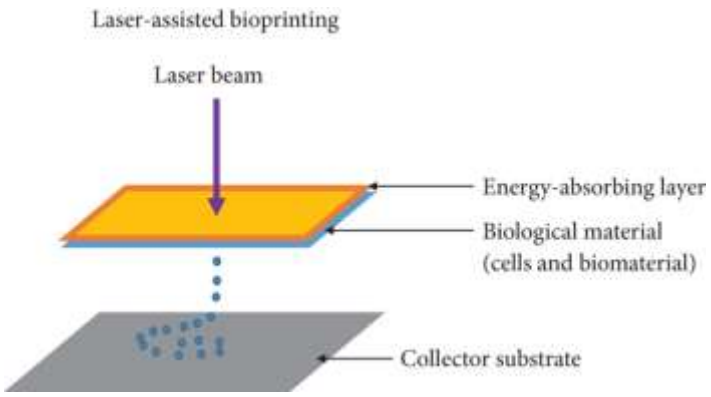

Fig7: Laser-assisted printers are made up of a pulse laser beam which is focused on an absorbing substrate resulting in the generation of a pressure bubble that forces biological material onto the collector substrate.

3.1.3 Extrusion printing

Extrusion printing is a modification of inkjet printing. In order to print to print the viscous materials inkjet printers cannot deposit, extrusion printing uses either an air-force pump or a mechanical screw plunger to dispense bioinks (shown in Figure 8) by applying a continuous force, extrusion printing can print uninterrupted cylindrical lines rather than a single bioink droplet. Almost all types of hydrogel pre-polymer solutions of varying viscosity as well as aggregates with high cell density can be printed with extrusion bioprinters. While extrusion bioprinters can print a wider range of materials, they also expose

the encapsulated cells to larger mechanical stresses that are thought to reduced cell viability (Iram, a Riaz and Iqbal 2019).

Extrusion bioprinting provides good compatibility with photo, chemical and thermal crosslinkable hydrogels of very different viscosities at a reasonable cost. a typical extrusion printer, the Multiread tissue/organ building system the substrate plate contains heating and cooling functions to control thermally sensitive hydrogels. Pneumatic micro nozzles powered by compressed gases support a wider range of viscosity, Screw-based nozzles can print without inlet air and are much cheaper, but they experience problems in high viscosity dispensing (Chen, Anvari-Yazdi et al. 2023).

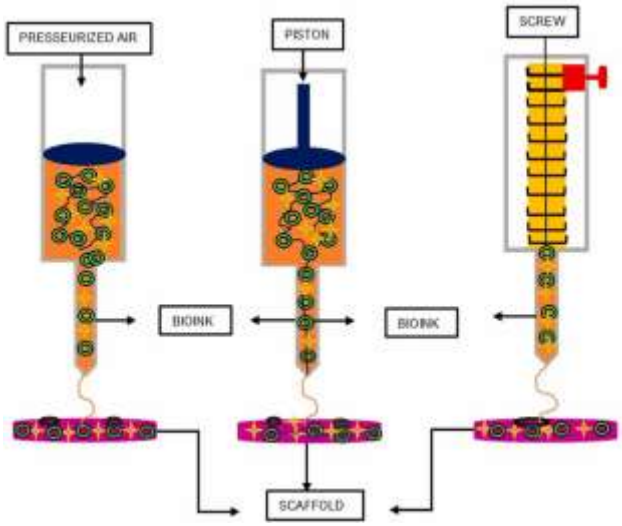

Fig 8: shows a schematic of Extrusion printing

3.1.4 Stereolithography printing

Also been modified for bioprinting purposes Like laser-assisted printing, stereolithography bioprinters (Mandrycky, Wang et al. 2016).In addition to the "traditional" bioprinting techniques described, many newer techniques have emerged within the past five years. The most representative is stereolithographic bioprinting, a light-based printing technique that is compatible only with photosensitive bioinks. During stereolithographic bioprinting (Figure 9) a patterned binary image from a projector is used to cure a layer of photo-curable bioink. Only the areas exposed to high-intensity white light receive sufficient energy to cure. In this way, a layer of solid tissue construct is formed. Stereolithographic bioprinting offers several advantages over previous techniques: no matter how complex the pattern is in one layer the printing time remains constant because the entire pattern is projected over the printing plane (Li, Chen et al. 2016).

As a result, this technique is faster than extrusion or other point-based bioprinting systems Stereolithographic bioprinting also provides the highest spatial resolution of all existing bioprinting methods, because the printing resolution is defined by the pixel size of the projector, which is often less than 50 µm. Even higher resolution has been achieved through variations on standard stereolithographic bioprinting. Direct laser bioprinting, for example, which replaces the projector with a high-density laser permits ultrafast patterning (under 10 min) of tissue constructs with high resolution around 30 µm.Despite these advantages, one significant challenge of stereolithographic bioprinting is a limited ability to print multiple materials simultaneously (Li, Wang et al. 2023).

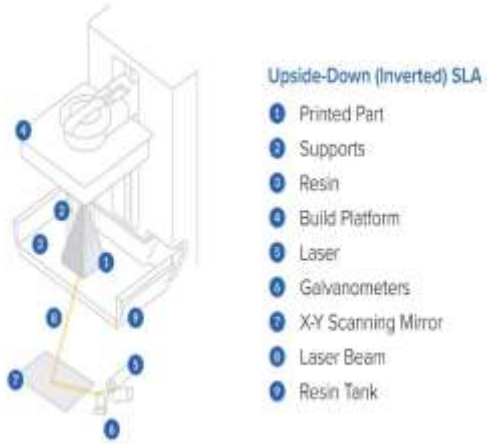

Fig 9: shows a schematic of Stereolithography printing

3.1.5 Fused Deposition Modeling (FDM)

The continuous layer-by-layer extrusion of a thermoplastic polymer filament characterizes fused deposition modeling (FDM), a revolutionary scaffold-building technology. The absence of an organic solvent, the quick solidification of the extruded polymer, and the structural integrity of the resulting three-dimensional matrix are all advantages of FDM. The FDM bio-printer's goal is to fabricate configurable, reproducible scaffolds that facilitate.due to its potential to generate regenerative tissues and organs, this technique has paved the way for the development of artificial multicellular tissues and organs (Kantaros 2022).

Three-dimensional bioprinting now uses a wide range of biomaterials and different methods developed by researchers. In this method (Figure10show) the material is either melted by the heated nozzle to form

a layer on the build platform or it is fed into the extrusion nozzle as a liquid with a predetermined viscosity. The necessary ink is created as a solid filament, which is subsequently heated throughout the extrusion process to a semi-molten condition. The filament material is then oozed out through a temperature-controlled nozzle. Layer by layer, the extruded material that was forced out is deposited onto a platform. The platform is further lowered after one layer is finished, and the subsequent layer is then deposited. Layer thickness or height, printing speed, infill rate, nozzle temperature, retraction, shell thickness (Wu and Hsu 2015).

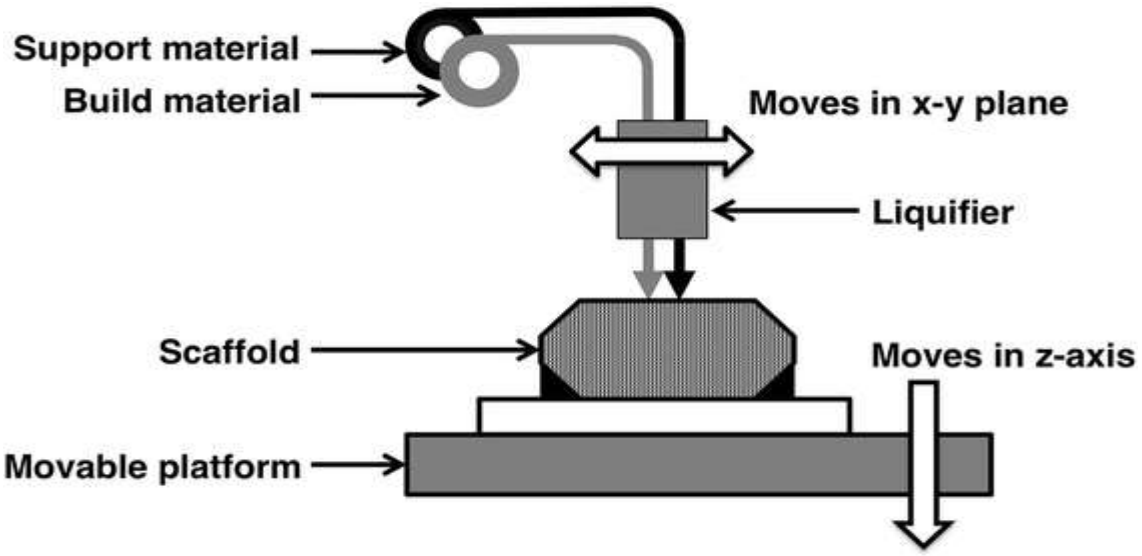

Fig 10:Scheme of fused deposition manufacturing (FDM). Melted polymer is extruded from nozzle to build scaffold

3.1.6 Direct Ink Writing (DIW)

The extrusion-based 3D-printing technique known as DIW uses a nozzle to extrude materials (Figure 11) onto a build platform layer-by-layer, similarly to the FDM method. By using this method, it is possible to control the deposition of raw materials in a highly viscous liquid condition, allowing them to keep their shape during the deposition stage. Due to the fact that it can use a wide range of materials, including ceramics, hydrogels, plastic, food, and even living cells, DIW can be seen as being more versatile than FDM. Nozzle size, material viscosity and density, printing speed, and thickness maintained between layers are the main factors that determine the final properties of the fabricated item. Similarly to FDM and SLA, support structures must be used in DIW when complicated geometric shapes with overhangs and steep deposition angles are present. However, the use of dissolvable materials as supports helps to

overcome this problem because they can be quickly removed after the printing process is complete(Kantaros 2022).

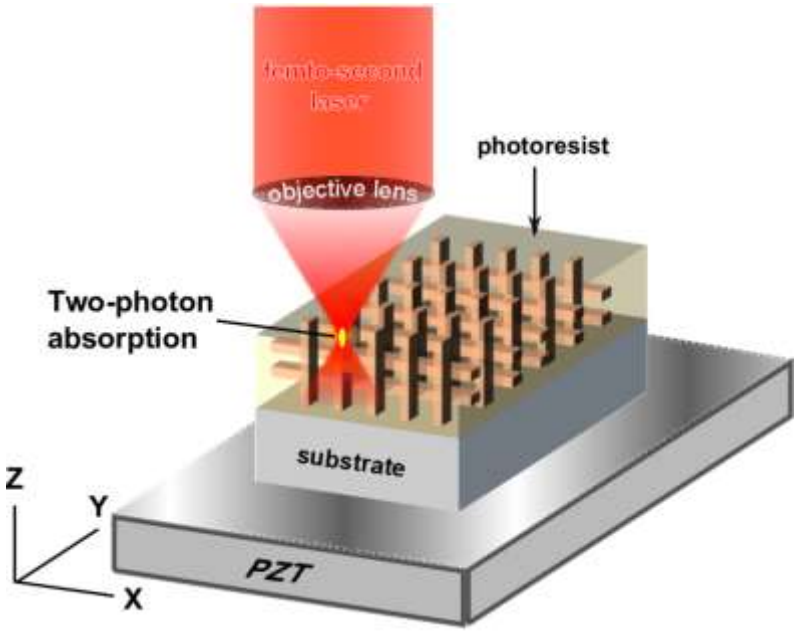

Fig 11: Schematic illustration of a direct laser writing system

3.2 Applications of 3D Printing

There has been much progress in the realm of 3D bioprinting in the past decade, allowing for future applications within many areas of clinical medicine and, potentially, every major system in the body. Due to the inability of certain tissues to regenerate naturally, surgical repair or artificial restoration are the mainstays of treatment Consequently, bioprinting has shown vast success in cases where organ transplant is difficult or not a viable option. Major body tissues such as the heart, blood vessels, and skin have seen success with 3D-bioprinted tissue implantation (Saini, Segaran et al. 2021).

3D printing techniques have been widely applied in tissue engineering and regenerative medicine, such as in neural, hair follicle, bone, cartilage, and canine models The scaffolds of hybrid constructs using various polymers and combinations of various 3D printing technologies are essential for fabricating tissues or organs for tissue regeneration (Kim, Kim and Kwon 2023).

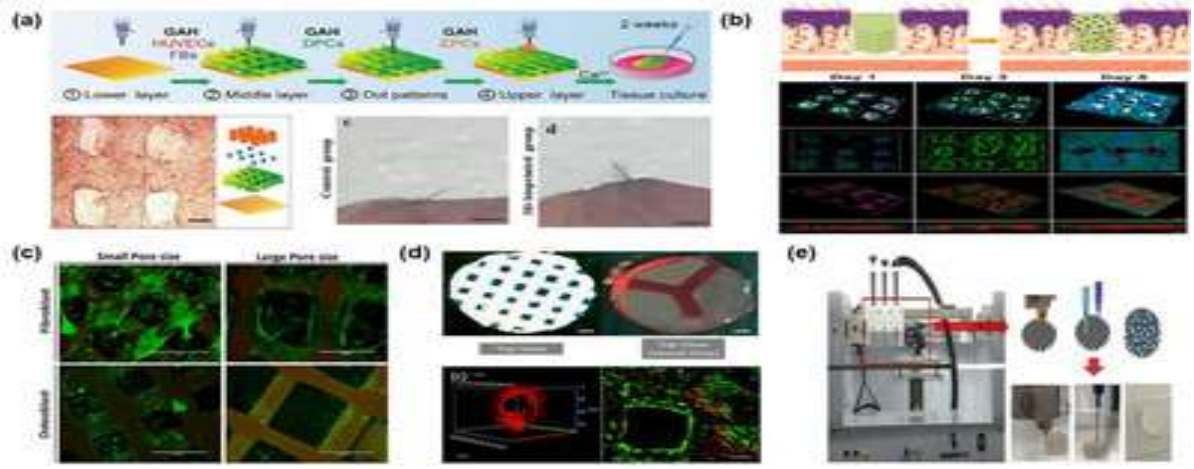

Fig12: Tissue regeneration using 3D bioprinting technology. (a) 3D-bioprinted multilayer composite scaffolds and hair follicle-like structures in vitro and in vivo. (b) Bioprinted skin implanted into a skin defect area(c) Images of 3D-printed PCL membranes with different porosities and aminolyzed 3D-printed membranes after gelatin attachment(d) Image of a fabricated 3D construct and vessel layer. Confocal microscopic fluorescence images of HUVECs in a blood vessel and hMSCs in bone. (e) Scheme of a 3D bioprinting process in articular cartilage engineering.

4. Discussion

Regenerative medicine technologies aim to repair and regenerate poorly functioning organs. One goal is to achieve an immunosuppression-free state to improve quality of life, reduce complications and toxicities, and eliminate the cost of lifelong antirejection therapy. regenerative medicine has recently shown the potential to meet the two major needs of transplantation, namely the identification of a new, theoretically inexhaustible source of organs and clinical operational tolerance(COT),Vessels, bladders, segments of upper airways, and urethras have been bioengineered from autologous cell(Orlando, Wood et al. 2011).

3D bioprinting represents a new advanced technology within the realm of additive biomanufacturing, allowing the fabrication of on-demand, patient-specific structures for regeneration of tissues (McMillan, McMillan et al. 2023).

3D bioprinting approach is gaining more and more interest for its ability to produce cellularized 3D constructs with a well-defined architecture closely replicating tissue organization in a quick and highly reproducible manner, regardless of the printing system, this target often proves challenging. Indeed, the technical requirements of 3D printing combined with the necessity for a biocompatible cellular scaffold force researchers to find a compromise in formulating bioink between what is convenient for the machine (Fornetti, De Paolis et al. 2023).

Innovative strategies include decellularization to fabricate acellular scaffolds that will be used as a template for organ manufacturing, three-dimensional printing and interspecies blastocyst complementation. Induced pluripotent stem cells are an innovation in stem cell technology which mitigate both the ethical concerns associated with embryonic stem cells and the limitation of other progenitor cells, which lack pluripotency (Jung, Bhuiyan and Ogle 2016).

Regenerative medicine technologies hold promises in a wide array of fields and applications, such as promoting regeneration of native cell lines, growth of new tissue or organs, modelling of disease states, and augmenting the viability of existing ex vivo transplanted organ (Edgar, Pu et al. 2020).

3D bioprinting is an emerging technology in the field of tissue engineering and regenerative medicine (Aljohani, Ullah et al. 2018).methods have also made great and rapid advancements and are now utilized to produce structures with complex geometries used in a wide variety of fields (Ramos and Moroni 2020).

pertaining to eleven organ systems of human body including skeletal, muscular, there has been a commendable and substantial progress in the bioprinting (Vijayavenkataraman, Yan et al. 2018).dimensional (3D) bioprinting is an emerging manufacturing technology that layers living cells and biocompatible natural or synthetic materials to build complex, This technology holds tremendous promise across a plethora of applications as diverse as regenerative medicine, pathophysiological studies, and drug testing. (skin, bone/cartilage, cardiovascular, central/peripheral nervous systems, skeletal muscle, kidney, and liver (Loai, Kingston et al. 2019).

Regenerative medicine is an emerging field that centers on the restoration and regeneration of functional components of damaged tissue. Tissue engineering is an application of regenerative medicine and seeks to create functional tissue components and whole organs (Saini, Segaran et al. 2021).

(3D) printing have increased feasibility towards the synthesis of living tissues. Known as 3D bioprinting, this technology involves the precise layering of cells, biologic scaffolds, and growth factors (Bishop, Mostafa et al. 2017).the goals of tissue-specific bioprinting and then summarizes the major techniques and identification of particular material development (Wang, Kapadia et al. 2021).

Tissue engineering and/or regenerative medicine are fields of life science employing both engineering and biological principles to create new tissues and organs and to promote the regeneration of damaged or diseased tissues and organs (Dzobo, Thomford et al. 2018).

3D bioprinting is a relatively new aspect to tissue engineering and has opened the possibility of creating an unprecedented biomimicry, which could ultimately replace the current gold standard of autografts. Biomimicry, in form and function, has great significance in regenerative medicine, drug screening and understanding pathology ,In vitro applications have been used to assess pathological and toxicological conditions, as well as implant integration, and offers a methodology with a high-throughput Biomimetic microfluidic chips have great potential in replacing animal studies for drug and material screening.Each bioprinting technique has different requirements for the bioink that can create diverse effects on the encapsulated cells (Zielińska, Karczewski et al. 2023).

5. Conclusion

Three-dimensional printing technology has advanced significantly over the last decade, with many new processes being developed in a number of disciplines and industries. Reduced fabrication times are now available, as are newly developed materials with a variety of characteristics. The development of 3D bioprinters that use biomaterials compatible with the human body allows for the creation of highly regulated porous, interconnected structures that serve as biological substrates for human cells to proliferate and form tissues. These structures must have a number of properties, including biocompatibility, bioresorbability, and the desired mechanical behavior. In this context, sophisticated biomaterials, such as bio-inks used as raw materials in 3D bio-printers, may now create high viability cells, tissues, and can even directly create DNA. The careful adjustment of process parameters in 3D bio-printer settings, as well as the continuous introduction of novel biomaterials, provide the only feasible method to fully exploit the capabilities of this technology.

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