Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13102
DOI URL: http://dx.doi.org/10.21474/IJAR01/13102

RESEARCH ARTICLE

HYDRO-GEL POLYMERS AND 3D BIOPRINTING IN SKIN ENGINEERING - A REVIEW

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Manuscript Info

Manuscript History
Received: 05 May 2021
Final Accepted: 10 June 2021
Published: July 2021

Abstract

Tissue engineering is a booming field of science and technology. The sheer potential it emanates is enough to place all attention on it. Tissue engineering is widely researched for the development of skin substitutes to treat patients with infections or disorders. Commercial skin substitutes provide a promising future to medicine. The availability of different biomaterials for the production of artificial skin has posed many questions regarding the feasibility, usability, and practicality of these products. The articles reviewed discuss tissue engineering as a challenging field and how skin substitutes are vital. Biomaterials and hydrogel studies are found to be a game changer for the field. The functional aspects have been discussed with the physical characteristics for better understanding. Different techniques of printing biological structures by using scaffolds are discussed in detail. Scaffolds provide a very efficient structural composition for the bioprinter to manifest the organ to be printed. Bioink composition and preparation with scientific knowledge make it a very important asset in tissue engineering. The future of regenerative medicine and organ transplantation is expected to be safe within the various aspects of tissue engineering. The vast possibilities it offers is hard to not explore further. Even though the research field has come a long way since the beginning of tissue culture in the 1970s, there are still different challenges to be faced and conquered.

Introduction:-

Organ transplantation has become a very common procedure in today's world. Diseases are increasing by the minute. On the brighter side, so is science and medicine. When the only way to save a patient is to transplant his/her organs, it is followed by a million procedures from finding the right donor to successfully harvesting the required cells or tissue from the donor. This process is not as easy as it sounds. A series of procedures exist behind every successful transplant surgery. Merely considering the last two decades, the number of organ transplants has increased substantially (Hans Schmeets, 2021). Lifestyle diseases have taken over like wildfire and it has posed a bit of a problem for doctors and researchers all over the world. Finding the right donor is a vital step in the process. But the screening for donors and finding one may be cumbersome and time-consuming. That kind of time may not be always available for a patient. It is very important to get the materials in a short span of time. This is where the idea of engineering the required tissues arise. Tissue engineering makes the whole process a lot more easier by eliminating the need for a step of selection of donors(Hans Schmeets, 2021). Lack of availability of organs to

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transplant has made organ transplantation limited. Around 2,000 – 3,000 surgeries are done annually to transplant liver, but the number of people who die from liver disease is in the range of 25,000 - 30,000(Langer R. , 1997).

Tissue Engineering

Tissue engineering is defined by The National Institute of health in the United States as "an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionise the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function" (Leong, 2003). As a matter of fact, the field of tissue engineering makes use of engineering techniques to develop living tissue for science and medical purposes. It is a revolutionizing idea that can have an immense impact on fields of biology and medicine. It can take treatments to the most advanced levels and create history. Tissue engineering is a very promising branch with the scope of multitudes. It brings together methodical principles to improve and sustain living tissues.

History of Tissue Engineering

Techniques of tissue engineering date back to the early 1970s. Paediatricorthopaedic surgeons who tried to recreate cartilage tissue from chondrocytes using mice failed but predicted the possibility of regenerating tissues from existing cells(Harada, 1994). They voiced the need for better biomaterials capable of creating artificial tissue using engineering scaffolds. Several years later, the doctor successfully used keratin sheets to regenerate tissues for burn victims. Later on, collagen gels were employed with fibroblasts to regenerate skin.

All this aside, we cannot neglect the fact that skin grafts have been a part of Sanskrit texts from the late 3000 BC. In Sushruta Samhita (600 BC), Sushruta mentioned the use of skin graft to repair irregularities of the nose using inserts from other parts of the body like the forehead. Evidence has been found about the extensive facial surgeries done by physicians from tissue from the buttocks. Over time, these methods have been forgotten (Jerrom, 2015).

Skin and tissue-engineered skin

Being the largest organ of the body, the major function of the skin is protection. It confers temperature regulation and sensitivity as well. The layers of skin perform various functions. Epidermis, the outermost layer makes the skin water-resistant and gives the skin its tones. Sweat glands, follicles and connective tissues form the second or dermal layer. The inner layer is formed by fat and more connective tissue. The epidermis is moderately thin, 0.1–0.2 mm in-depth, with a self-replenishing population of keratinocytes calibrated to be swapped incessantly in a life cycle from basal cells located on the basement membrane (MacNeil, 2008). Keratinocytes ectodermally give rise to approximately 80% of the cells in the epidermis.

The expectation from tissue-engineered skin is that it should possess the property of natural skin such as to deliver a barrier layer of regenerative keratinocytes. The skin needs to be adequately vascularized and elastic. These are relevant features needed in tissue-engineered skin. Vascularization is required for the repair of skin as per needed. Elasticity and support are conferred by extensive cross-linking of fibrous proteins (MacNeil, 2008).

3D Bioprinter

Bioprinting is a scientific technique of combining cells and other growth factors to make tissue-like structures. It can be considered similar to natural tissues. The tissue is created in a layer-by-layer manner by making use of bio-ink. Bio-ink is a material that mostly contains cells and hydrogels that acts as an external carrier to cover the cells. The hydrogels are usually biopolymers. They form the scaffolds in the system. Cells when mixed with the gel adhere better and forms the required structural tissue on bioprinting.

Literature Review:-

Tissue engineering is a vast field and the advances that have been happening is beyond our imaginations. The developments are exceptional. We use the technology to recreate the desired tissue or cells. This process needs different boosters to aid the formation. Growth factors are generally used for this purpose. When vascularization of the tissues is prompted by the presence of growth factors, it creates an adequate stream of oxygen to mature into healthy tissues (Tabata, 2000).

Tissue can be engineered in vitro or in vivo. When using growth factors, in vivo technique exhibit poor stability. This is where the use of drug delivery systems come into the picture. When a part of your body fails, we immediately resort to surgical methods of transplantation. This is effective in many cases. The tissue grafts are

prepared using different biomaterials that can survive inside the body. To regenerate a dead tissue, we need certain conditions and factors. It does not happen overnight. The number of cells must be increased. A more challenging aspect is the need for the increased mass of cells to form the required structure with the extracellular matrix (Tabata, 2000). It is important for the credibility of the cells that the cultured cells need to be able to support the cell structures. There exists few factors that are needed for the proper engineering of tissue. It includes tissue scaffolds, growth factors and the primary cells. Many a time, stem cells are used for the process. For the surgery, it is ideal to get the cells from the patient itself. This eliminates the chances of rejection. But this is no walk in the park. Harvesting cells from a healthy person is difficult, let alone a patient.

Drug Delivery Systems

Tissue engineering comprises mainly two fields of research that are very crucial. They are namely, tissue regeneration and organ substitution. Muscles can be revitalized not only in vivo but also remodelled in vitro.

In vitro tissue engineering is a rather complex process of rebuilding tissues under the comfort of a laboratory. It is an ideal condition for development since all the materials required for tissue development are provided in the right concentrations. The growth environment is tailored to the most convenient way for smooth and stress-free development. This complexity has reduced tissue engineering in vitro to a few examples in real life such as skin restoration, reconstruction of cartilage and arteries.

In vitro tissue engineering is one of the most suitable methods of regenerating tissue. Despite its idealistic features, this method does face many challenges. The primary resource from which the cell is obtained is a real challenge. In vitro techniques involve synthetically creating the skin layers such as the dermis and epidermis. The technique when used correctly help in creating skin equivalents for testing drugs. In vivo tissue engineering do not involve any kind of scaffolds. A healthy extracellular matrix is harvested from the living body itself. The intended cells are mainly the cornea of the eye, retina, and blood cells. A natural application of this technique can be observed when stem cells that are capable of developing into RBCs are injected into a patient suffering from leukaemia, the cells are found to differentiate into functioning cells at the bone marrow region (Tabata, 2000). Stem cells are being used to recreate cells of the cornea and retina when they are damaged beyond repair.

But when the defects are very large, there is no way to rely completely on the power of stem cells to differentiate. This is where we depend on scaffolds. Cells may or may not be seeded before implanting them. Scaffolds can be used in either case. Collagen sheets can be used to regenerate dermal cells without involving cell seeding. The sheet acts as a scaffold to provide the prospect of fibroblasts to increase their number. Alongside tissue regeneration, the scaffold will be digested by enzymes (Villalona, 2010).

Growth Factors And Release Control

Growth factors are an integral part of tissue regeneration. They are used in various formulations depending on their carriers. It aids in the active functioning of the factors. Due to issues with their protein-polymer denaturation, growth factors are used in combination with hydrogels (Sánchez, 2020). These combinations can be achieved by chemical or physical methods. Protein denaturation always leads to diminished activity. In the human body, there is a natural mechanism for the release of growth factors. The growth factors are immobilized physically by making use of the positively charged site on its surface. When considering their storage arrangements in our body, these factors are stored as complexes paired with the acidic polysaccharides of the extracellular matrix of the cell. They form ionic complexes inside the body. When the body needs its growth factors, it is freed from the ECM complex, atrophied by polysaccharides.

Hydrogels

The human body's natural growth factor releasing mechanism is duplicated by the use of hydrogels. A bioabsorbable polymer is used to create a hydrogel. The hydrogel is negatively charged so that a growth factor can electrostatically form a complex with the hydrogel (Tabata, 2000). The growth factor basically uses the hydrogel as a carrier to immobilize it in the reaction environment to prevent denaturation. Any change in parameters such as pH or ion strength will cause the release of growth factors from the factor-gel complex. The factors are released when the gel deteriorates as well (Sánchez, 2020). Growth factors such as bFGF are incorporated with alginate gel and introduced win mouse to duplicate angiogenesis. It is the formation of new blood vessels (Downs, 1992). When incorporated with collagen, bFGF is exposed in rabbits to recreate cartilage tissues (Harada, 1994). Hydrogel drug delivery systems are foolproof. They provide a perfect stage for biological interactions in a regulated manner, all

credits to their physical properties (Langer R. , 1998). Hydrogels are three-dimensional, cross-linked polymer networks. They are water-soluble and can be developed from practically any water-soluble polymer, comprising a large assortment of chemical structures and bulk physical properties.

Hydrogels are constructed outside the body and infused with medications before positioning them into the body. The cross-linking strategies adopted can be any, such as UV photopolymerization or various other chemical cross-linking techniques (Villalona, 2010). The major hindrance of such tactics is that the material must be established since bulk hydrogels possess defined dimensionality and high elasticity which normally prohibits their bump through a needle. As an alternative, non-cross-linked linear polymers can be used as the drug delivery carrier (Hans Schmeets, 2021). In general, the rate of drug delivery from a linear polymer matrix is inversely proportional to its viscosity. Nevertheless, it may be tricky to dissolve the polymer of interest to a sufficient concentration to monitor the rate of release to the anticipated range. Yet, the yield stress of the resulting material can become so extreme that insertion is impractical. There has been considerable interest in formulations that exhibit the properties of linear polymer solutions outside of the body but gel in situ within the body, providing prolonged drug release profiles. Both physical and chemical cross-linking strategies have been pursued to achieve in situ gelation.

Hydrogels - Pioneering Bio-Inks

Bioink selection is a very important part of bioprinting. All the biological and chemical hints for the growth of cells and their development is offered by the bioink material. Physical properties can also be linked to the bioink material. It mimics the extracellular matrix of biological systems to enrich the regeneration process (Pereira & Bartolo, 2015). Hydrogels can be engineered to mimic cellular properties and act as an effective bioink for 3D bioprinting (Ramiah, C, & Pillay, 2020). Some hydrogels materials have a possibility of limitations and other side effects arising due to polymerization. Residues formed during the reactions may cause cellular damages. To outsmart these limitations, it is necessary to adopt techniques that are capable of reversing polymerisation. So, the biomaterial selected must be in favour of this reaction (Kamata, 2015).

The basic methodology involved in tissue bioprinting can be divided into 3 phases- pre-bioprinting, bioprinting and post-bioprinting. These steps are not simple as it sounds. They involve a lot of sub-steps and processes in between. The cells are treated and cultured in a variety of different ways to make them feasible for printing (Guillemot, 2010).

Pre-Bioprinting

Pre-bioprinting is the first phase of the tissue bioprinting method. It involves many smaller steps like selecting the desired tissue, isolating, and then cultivating it. The tissue is selected based on the research requirement and application. The desired tissue is dissected from the patient or donor. When the extraction is from blood or fluid tissues, we can adopt centrifugation and apheresis. Apheresis is the process when we drain the blood from a patient's body to separate individual components needed (Rock & Sarode, 2020). The required components are collected, and the remaining fluid is re-introduced into the patient’s body. But when the tissue needed is solid, there are a lot of steps involved in the extraction.

Solid tissue is cut into really small pieces. The extracellular matrix is removed by treated the minced pieces with collagenase or trypsin(Hans Schmeets, 2021). Trypsin and collagenase are proteolytic enzymes. They have the capability to dissociate cells. Their mechanism of action involves breaking down the proteins present in the cell. The cell membranes are destroyed along with proteins and the cell components freely float in the solution. Collagenases are used when muscle tissues are involved. It can destroy peptide bonds of muscle tissues. Trypsinization is a very commonly used method. After the enzyme treatment, the free-floating cells are separated by using centrifugation. Non-invasive techniques of tissue extraction are becoming very popular currently.

The extracted cell is then isolated and cultivated. The primary cells that are collected from the donor or patient itself are cultured, used as a scaffold to 3D-print artificial skin and then implanted into the person. Before implantation, the artificial structure for tissue formation is seeded with the original cells. This process is also complex and important.

Bioprinting

Bioprinting is the actual technique of printing artificial tissues by using a bioprinter. This is the phase where the bioink is positioned in the printer. It gives a 3D structure. This digital model is used as a foundation to print out the required tissue. The cells, growth factors and the skin substitute biomaterial is combined together to form the

hydrogel which behaves as the bioink(Pereira & Bartolo, 2015). The bioink need to be banked on the scaffold structure layer by layer. This ensures the proper formation of the tissue or organ. The cells need to be differentiated as well. The layer-by-layer approach takes care of all this (Rock & Sarode, 2020). Bioprinting uses many techniques like inkjet bioprinting, laser-assisted bioprinting, extrusion bioprinting etc.

Inkjet bioprinting

Inkjet bioprinting is a widely used method of bioprinting. The bioink is plunged onto the preferred platform in really small quantities, usually as little as 1 to 100 picolitres. Small drops are formed via the piezoelectric effect. They have ejected afterwards; thermal inkjet printers make use of heat to vaporize small volumes of bioink as required(Pereira & Bartolo, 2015). Apart from thermal, there are other types of inkjets available such as piezoelectric. Spraying of the bioink drops is achieved by heat or other forces. The survival rate of cells is found to be significantly better by the use of a piezoelectric inkjet printer (Nie, Wang, Deng, & Shavandi, 2020). The heating component employed in thermal printers retains the cells at higher temperatures leading to pore development on their surfaces(Pereira & Bartolo, 2015). The benefits of inkjet bioprinting are their low cost owing to its comparable construction to commercial printers, great printing speed owing to the skill to support parallel operation means, and reasonably high unit survival rate established by many investigational findings(Nie, Wang, Deng, & Shavandi, 2020). Nevertheless, the dangers of cells and materials subjected to thermal and mechanical pressures, low droplet directionality, lopsided droplet range, recurrent nozzle plugging, and erroneous cell encapsulation have fetched substantial limits to its functional aspects in tissue engineering.

Laser-assisted bioprinting

The characteristic laser-aided bioprinter incorporates laser beams, systems for focus, and donor bands that retort to laser spurs, involving glass covered with laser energy absorbing layers, and biomaterial layers composing of hydrogels and cells. The principle of laser-assisted bioprinting is to employ a high-energy pulse laser to the donor colour band encrusted with bioink.The optical assets of the bioink or the wavelength of the laser oversee bioink ejection (Guillemot, 2010). The exceptional range and output of the printer make LAB an appropriate addition to tissue engineering labs.

Post - Bioprinting

Structures formed by the crosslinks and scaffolds depend upon their stability. Crosslinking is achieved by the use of ionic solutions or even ultraviolet light. Post bioprinting involves time for the tissue to mature. These conditions are provided inside the comfort of a bioreactor(Fuentes-Mera, 2019). Cells interact between themselves, and their kinetic properties vary with the conditions provided inside the bioreactors. This environment is flexible to alterations and can be optimized per requirement.

Tissue can be artificially engineered using the above-discussed methods. The tissue obtained is similar to that of the original tissue in terms of appearance and functionality. Tissue engineering has been proven an effective method for developing usable flat organs like skin and cartilage. Skin, being a protective organ is prone to accidents and damages more often (Tissue Engineering for Artificial Organs: Volume 1. , 2017). Severe burns from fire or acid can be fatal enough for the skin to not regenerate quickly. These accidents pose a need for artificial tissue that can be transplanted to the patient.

Tissue regeneration has a variety of applications in the field of science and medicine. Any organ that needs replacement can be achieved with this technique. Patients who require emergency transplants for organs such as heart or liver usually depend on a perfectly matching donor. Not everyone is lucky enough to get a match either. Artificially creating organs can help every patient receive the organ they require in the perfect conditions. There will be minimized risks associated.

Having said all the advantages of artificial tissue and skin, they are not above limitations. The skin substitutes that are available commercially often show irregular vascularization (Fuentes-Mera, 2019). Many instances of inadequate mechanical integrity have been found. Sometimes the substitutes fail to integrate with the other body cells (Groeber, Holeiter, Hampel, Hinderer, & Katja Schenke-Layland, 2011). Even though artificial skin can avoid immune rejection, it is possible only to an extent. Occurrences of immune rejection have been observed in the case of commercial skin substitutes as well. Reduced vascular tissues can often lead to the frequent death of cells. Apart from this, artificially culturing tissue is a tedious process. The basic culturing process requires multiple weeks, maybe up to 3 weeks (Harada, 1994). This is followed by grafting where a number of technological advances are

involved in the conventions. Even then the cells may show many issues regarding their growth and development. Many shortcomings are bound to occur. Overcoming the concerning matters require attention and time. The available skin substitutes composed of fibroblasts and keratinocytes are not capable of forming differentiated glands. This raises the need for the extra set of endothelial cells to be engineered into the tissue. even though it sounds simple, this process follows complex protocols. the high cost of these protocols makes them less desirable as well. When the demand for skin substitutes are increasing every day, the need for a better and larger scale of production is inevitable. This can help manage the cost of production (Jerrom, 2015). Otherwise, tissue engineering is going to remain an expensive medical procedure affordable to only the rich, like many other existing techniques.

Conclusions:-

Tissue engineering is an extremely promising area of research. It is highly impactful in numerous fields. The success of tissue engineering is owed to the totipotency of our cells. Their capacity to dedifferentiate and re-differentiate is impeccable. Exploiting these powers has been proven useful for humankind in a number of instances. Organ transplantation is a very significant example. The number of people who received successful organ transplants is huge enough to conduct extensive research in the field. The promise of tissue regeneration and wound healing makes it the key to future medicine. The need for improvements is crucial. Better vascularization in skin substitutes and more evolved integrity can improve the artificial tissue. Using bioreactors in order to achieve mechanical stimulation for the complete development and maturation of young blood vessels is rings us one step closer to the perfectly engineered tissue with immaculate vasculature. Standardizing the production protocols and reducing the cost of production will be the next few goals to be achieved by the industrial aspects of tissue engineering. Minimizing the risk of rejection is another important goal(Downs, 1992). Eradicating the practical and therapeutic shortcomings of skin substitutes plays a major role in future prospects. The current skin substitutes formed from the primary culture of donor cells can be improved further with the use of combinations of cells(Fuentes-Mera, 2019). Stem cells, fibroblast, and endothelial cells along with biomaterials are used in combinations in the hope of creating better and more realistic tissues. Treating sick and flawed organs is one of the noble objectives of tissue engineering. Astonishing breakthroughs and upgrades in the previous decades pledge more sustainable headway in the near future. Short-term and long-term goals define this coveted research area. 2D and 3D techniques of bioprinting require revival for novel systems. Augmenting existing monitoring and ethical reflections would make way for the simpler and benign establishment of tissue engineering and regenerative medicine solutions. This is what we hope to achieve in the future.

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