Journal of Science in Medicine and Life

Volume: 2 Issue: 10 Year: 2024
ISSN: 2992-9202 | http://journals.proindex.uz

An Expository Review on 3D Bioprinting Technique with Comparison of Polysaccharide& Graphene Hydrogels as Bioink and Other Properties in Medical Applications

Huda Ali Nafil
University of Thi Qar College of Engineering / Department of Biomedical Engineering

Email: hodahodaali12345@gmail.com

Zahraa Rahman Farhan
University of Thi Qar College of Engineering / Department of Biomedical Engineering
Email: zhrarhman332@gmail.com

Abstract: 3D Bioprinting" or "bioprinting" is a form of additive manufacturing that uses cells and biomaterials instead of traditional metals and plastics to create 3D constructs that are functional 3D tissues. These biomaterials called bioinks, and they mimic the composition of our tissues.

The applications of hydrogels coupled with 3-dimensional (3D) printing technologies represents a modern concept in scaffold development in tissue engineering. Hydrogels based on natural biomaterials extensively used for this purpose. This is mainly due to their excellent biocompatibility, inherent bioactivity, and special microstructure that supports tissue regeneration.

For these characteristics for polysaccharide, advanced researchs have done to find new traits, developing, or enhancing the possible disadvantages. For that, here in this project we focus on graphene hydrogel as a new carbon-based nanomaterial, which has exhibited unique advantages in significantly improving the combination properties of traditional polymer hydrogels. The specific properties of graphene, such as high electrical conductivity, high thermal conductivity and excellent mechanical properties, have made graphene not only a gelator to self-assemble into the graphene-based hydrogels (GBH) with extraordinary electromechanical performance, but also a filler to blend with small molecules and macromolecules for the preparation of multifunctional GBH. According to these, information this review also focus on briefly describe the origins and basic principles of 3D bioprinting using these two types of hydrogel and comparison their properties and applications.

1.1. Background

3D Bioprinting is one of the latest technologies, which is highly used in tissue engineering and regenerative medicine to develop complex tissue structures to mimic native organs and tissues. The bioprinting involves layer by layer deposition of cells- laden biomaterials in a predetermined structural architecture to generate functional tissues or organs. This technique integrates biomaterials, live cells and controlled motor systems for creating complex structures and has shown to have precise control over the developed structures than the other methods which are currently available. Hence, fabrications of very complex structures such as tissue engineering scaffolds with controlled porosity, permeability and mechanical properties, biomedical devices and tissue models are made possible [51]. Such complex 3D tissue structures can be designed and developed in computer-aided design using the complex geometrical data obtained from the medical imaging techniques such as X-ray imaging, magnetic resonance imaging (MRI) and micro-computerized tomography scan (μ-CT-scan). The advantages of using 3D bioprinting in biomedical field include the development of personalized patient-specific designs, high precision, low cost and on-demand creation of complex structures within a short time [52].

Among the currently employed 3D printing technologies like fused deposition modeling (FDM), direct ink writing (DIW), inkjet bioprinting, selective laser sintering (SLS), stereolithography (SLA) and laser-induced forward transfer, the DIW and inkjet bioprinting are frequently preferred for 3D printing of live cells [53]. In DIW, the high viscous solutions or hydrogel or cell suspensions are extruded to obtain 3D structures either with or without a carrier. In case of inkjet bioprinting, low viscous solutions like cell suspensions or colloidal solutions are deposited as droplets at high shear rates (~ 50 μm in diameter). The SLA method is also used in 3D bioprinting, where the curing process takes place without affecting the live cells in the bioink or after printing. In addition, LIFT technology is also preferred in 3D bioprinting in a few cases, where the laser is focused towards a laser absorbing biomaterial layer which helps in developing a local pressure to release ink layer. Also, there are other methods such as acoustic bioprinting, microwave bioprinting, electro-hydrodynamic bioprinting, pneumatic bioprinting, etc. which are currently used for bioprinting of tissues and organs. One of the important components of the 3D bioprinting is the bioink that is used for the printing. This bioink should be highly biocompatible to accommodate live cells, mechanically stable after printing, and it should provide high resolution during printing. Among the different biomaterials, hydrogels are most prominent materials which are used as bioink in the 3D bioprinting. This is mainly due to their ability to hold live cells, modifiable chemical structures, adjustable mechanical and biodegradation properties, and it can yield a good resolution during printing. This review presents the requirements for the selection of bioinks and the properties of the different polymeric biomaterials (natural and synthetic) which are used as bioinks for 3D printing based on their ability to support cell growth, printability, etc. The review covers the different blends and combination of polymeric biomaterials used as bioink [53].

1.2. Introduction

Three-dimensional (3D) printing has attracted increasing interest during the last decades and already used in various industrial sectors for the rapid and easy fabrication of complex structures and materials, exceeding several limitations of conventional manufacturing techniques. 3D printing, also known as additive manufacturing has also led to remarkable advances in the healthcare sector (3D bioprinting), especially in regenerative medicine, as it facilitates on-demand "printing" of cells, tissues and organs. These technological advances have led to the creation of new scientific fields such as "tissue engineering". The most challenging and demanding applications for engineered tissues include the skin, cartilage, hard tissues such as bones. Cardiac tissues. Bio-inks are required for various proper ties because they provide chemically suitable microenvironment in order to cell proliferation, differentiation, and migration, and gives mechanically structural support in 3D printed structures while the cells are growing in the printed structure. Cell-laden hydrogels are used the term bio-inks and they play a crucial role to fabricate three- dimensional structures in 3D bioprinting [54,55,56].

1.3. Graphene hydrogel

is a one-atom-thick layer of carbon atoms arranged in a hexagonal lattice. It is the building-block of Graphite (which is used, among others things, in pencil tips), but graphene is a remarkable substance on its own - with a multitude of astonishing properties which repeatedly earn it the title “wonder material". Graphene's properties is the thinnest material known to man at one atom thick, and also incredibly strong - about 200 times stronger than steel. On top of that, graphene is an excellent conductor of heat and electricity and has interesting light absorption abilities. It is truly a material that could change the world, with unlimited potential for integration in almost any industry[57] .graphene oxide (GO) composites as bioinks for their potential to improve printability, structural stability, and osteogenic activities for osteogenic tissue engineering applications graphene Self-healing materials are one kind of intelligent material capable of feeling external stimulus to repair themselves after damage and restore their intrinsic properties, thus can extend lifespan, improve security, save cost, and achieve sustainable development Conductive hydrogels could be used to control the release of active substances in order to treat certain diseases locally in a more targeted manner. In order to produce electrically conductive hydrogels, conventional hydrogels are usually mixed with current-conducting nanomaterials that are made of metals or carbon, such as gold nanowires, graphene or carbon nanotubes. To achieve a good level of conductivity, a high concentration of nanomaterials is often required [58]. However, this alters the original mechanical properties of the hydrogels, such as their elasticity, and thus affects their interaction with the surrounding cells. and the used in 3d bioprinting, sensors, drug delivery and in tissue engineering.

1.4. polysaccharide hydrogels

Polysaccharides are long-chain polymers of monosaccharide units, joined together by glycosidic linkages. They also known as glycans. They are the most abundant carbohydrates found in food. Carbohydrates are vital macromolecules required for the essential functions of organisms [59]. polysaccharide materials in formulating biocompatible bioinks for extrusion, inkjet, or light projection-based 3D printing techniques [60]. There are diverse forms of polysaccharides. Their structure ranges from simple linear to more complex, highly branched forms. Many of them are heterogenous. Depending on their composition, they may be amorphous or water- insoluble they are classified into the following three classes depending on two criteria, whether they undergo hydrolysis, and if they do, the number of products they form . Natural polysaccharides are ideal materials for the preparation of biomimetic hydrogels because of their good biocompatibility and biodegradability. The self- healing hydrogel can achieve healing without external force and prolong the service life of the hydrogel. Therefore, self-healing hydrogels are of great interest in tissue engineering, wound dressings, drug delivery, and tissue engineering [61].

1.5. Objectives of the project

1. New scope of 3D bioprinting
2. polysaccharide & graphene hydrogels as bioink
3. Comparison the best hydrogels in characteristic & applications
4. Focus on their applications

1.6. Limitations of work

Obstacles to Overcome

Despite these advancements, there are still many challenges to overcome in Iraq, some of which are common to other post - conflict countries and being unique to Iraq:

Low Number of Experts

A huge obstacle in deploying 3D Printing at a larger scale is the need for enough experts and technicians to design models and manufacture different products. The Iraqi 3D Printing community is quite aware of this issue and are trying to overcome it through trainings, however, with a population of over 40 million, there is a lot of ground to cover.

Lack of Legal Framework

As new technology emerges it requires a legal framework to protect providers and users of the technology. One of the hot debates around the world is the 3D printing of copyrighted or patterned objects which presents a challenge to manufacturers requires government intervention. While the lack of government oversight has its own merits (giving the ability to manufacture needed objects without legal restrictions), it still has its downsides. Protection of innovations made by Iraqis is something that must be addressed where the government and community need to cooperate to present a legally binding addressed where the government and community need to cooperate to present a legally binding framework to protect Iraqi innovations and propel R & D in the field.

Iraq's Past

Iraq's past still has its scars on many sectors in the country. Products, technologies and financial services are still restricted and some 3D Printing technologies fall into this category, namely 3D Bioprinters, which can be used to help print tissue and other medical needs that can save lives. A clear solution for this issue is not yet being presented but the persistent efforts by Iraqi entrepreneurs are slowly making a shift in the way Iraq is perceived around the world and may, with government intervention, lead to change.

Future Trends

The future of 3D Printing in Iraq is very promising. The technology is rapidly developing and has a never-ending list of use cases, meaning it is projected to be the leading method of manufacturing in the world. For Iraq, the increasing interest in the sector means it will grow more. There is a rising focus on education as many universities are opening 3D labs or partnering with existing makerspaces.

The business side is also expanding with more players providing 3D printers and printing services to an increasing demand from Iraqi creators. The technology is being used in more industries such as construction, medical and retail giving a country, once scared to move forward from the past, a new and promising future [62].

CHAPTER TWO: GENERAL REVIEW ABOUT 3D BIOPRINTING

2.1. A brief history of 3D bioprinting

Rapid prototyping is not a new technology. Professor Herbert Voelker (then at the University of Rochester, now at Cornell University) began considering ways to take the output from computer designs and use them in some of the automated machinist tools just starting to appear in factories in the late 1960s. His efforts resulted in the first real mathematical models and algorithms for describing three-dimensional parts. His work is the basis forest design tools used today and made rapid prototyping possible. The next step involved a series of tools and machines that tried automate object creation by cutting away at a solid mass of metal or other material based on a preset 3-dimensional design. This method continued to be popular (with mixed results) through the 1970s and early 1980s. Then in the mid-80s. University of Texas researcher Carl Deckard came up with the idea of printing objects layer by layer. The first commercial rapid prototyping machines using this layering technique were produced in 1987 by 3D Systems and unveiled to the public at the auto fact trade show in Detroit in November of that year. This technique is still in use today, although it has been refined considerably [1].

2.3. What Is 3D Bioprinting?

3D Bioprinting is the method of printing biomedical structures with the use of viable cells, biological molecules, and biomaterials. In simple words, 3D bioprinting is the deposition of biological material in a layer-by-layer fashion to create 3D structures like tissues and organs. Bioprinting considered a part of additive manufacturing that involves the formation of materials necessary in industrial applications.

3D bioprinting begins with a suitable microarchitecture, which further stabilized by scaffolds of cells and tissues while considering the effect of manufacturing on cell viability.

The most important motivation behind the development of 3D bioprinting

1. Is the limited availability of biological structures that are required for the rehabilitation of lost organs and tissues.
2. The ultimate aim of the process is to provide an appropriate alternative to tissue implants and animal testing procedures during research on diseases and the development of treatments.

3. Currently, the use of 3D bioprinting is limited to the formation of organs and tissues to estimate the efficiency of drugs, but 3D bioprinting has great scope in its use for replacing lost and failed organs in patients as shown in figure below [2].

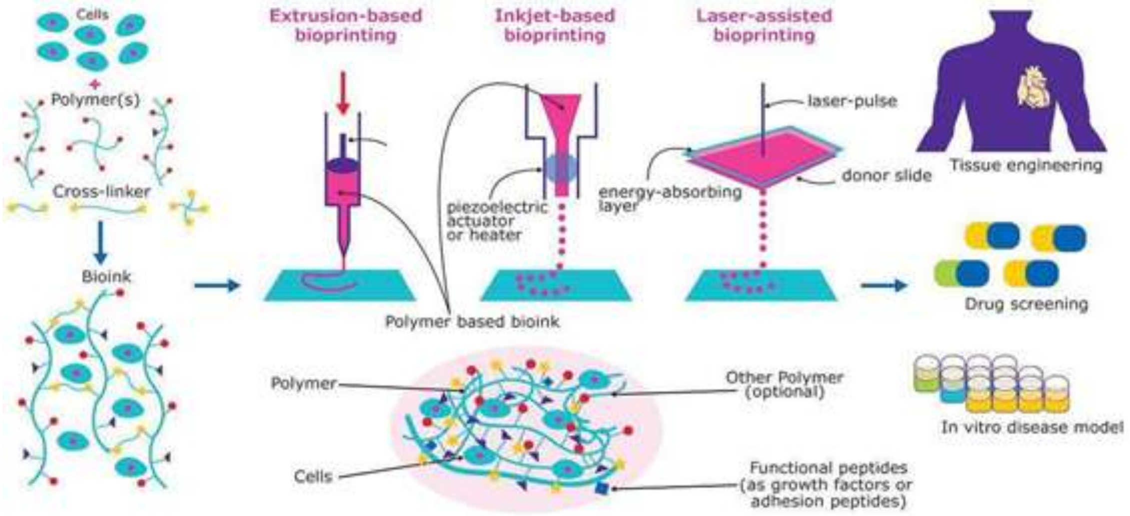


Figure (2- 1) 3D Bioprinting of tissue and organs. Bioink created by combining cultured cells and various biocompatible materials. Bioinks can then be 3D bioprinted into functional tissue constructs for drug screening, disease modeling, and in vitro transplantation [2].

2.3. Basic Principles Of 3D – Bioprinting

3D bioprinting based on the layer - by - layer precise positioning of biological constituents, biochemicals and living cells, by spatial control of the placement of functional constituents of the fabricated 3D structure.

3D bioprinting based on three fundamental approaches:

1. Biomimicry or biomimetics,
2. Autonomous self – assembly.
3. Mini tissue building blocks, as described extensively elsewhere.

The process principally involves preparation, printing, maturation, and application. This can be summarized in the three key steps:

- Pre - bioprinting involves creating the digital model that the printer will produce. The technologies used are Computed Tomography (CT) and magnetic resonance imaging (MRI) scans
- Bioprinting is the actual printing process, where bioink placed in a printer cartridge and deposition takes place based on the digital model
- Post - bioprinting is the mechanical and chemical stimulation of printed parts so as to create stable structures for the biological material [3,4].

2.4. How does bioprinting work?

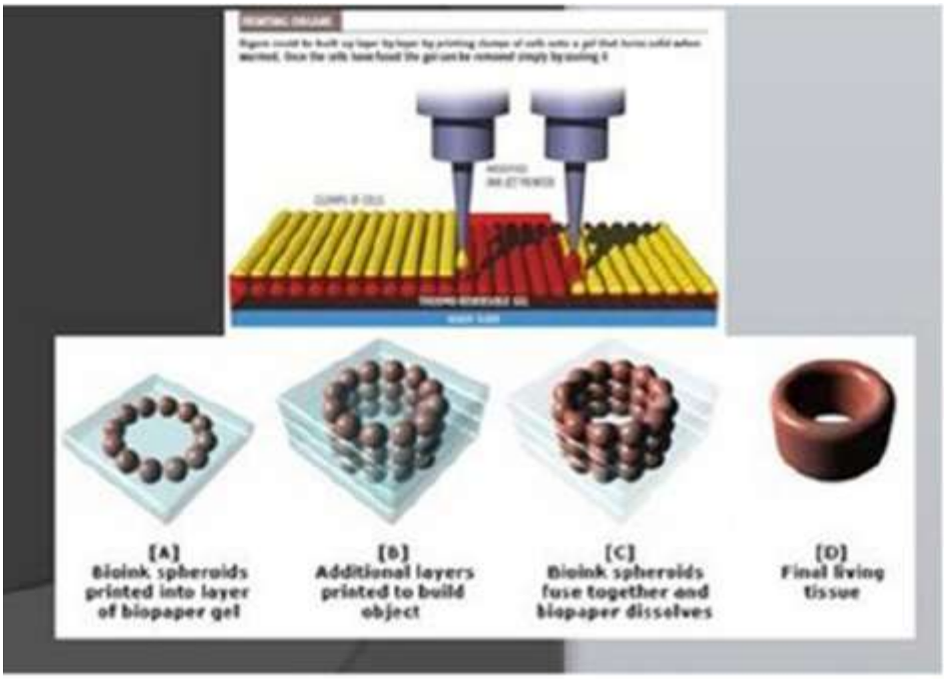


Figure (2- 2) Several bioprinting methods exist, based on either extrusion, ink jet, acoustic, or laser technologies. Despite the various types, a typical bioprinting process has a more - or - fewer standard series of steps [5].

1. **3D Imaging**: To get the exact dimensions of the tissue, a standard CT or MRI scan is used . 3D imaging should provide a perfect fit of the tissue with little or no adjustment required on the part of the surgeon.
2. **3D Modelling**: A blueprint is generated using AutoCAD software. The blueprint also includes layer - by - layer instruction in high detail. Fine adjustments may be made at this stage to avoid the transfer of defects [5,6].
3. **Bio ink Preparation**: Bioink is a combination of living cells and a compatible base, like collagen, gelatine, hyaluronan, silk, alginate or nanocellulose. The latter provides cells with scaffolding to grow on and nutriment to survive on. The complete substance based on the patient and is function specific.
4. **Printing**: The 3D printing process involves depositing the bio ink layer – by – layer, where each layer has a thickness of 0.5 mm or less. The delivery of smaller or larger deposits highly depends on the number of nozzles and the kind of tissue being printed. The mixture comes out of the nozzle as a highly viscous fluid.
5. **Solidification**: As deposition takes place, the layer starts as a viscous liquid and solidifies to hold its shape. This happens as more layers are continuously deposite. The process of blending and solidification is known as crosslinking and may be aided by UV light, specific chemicals, or heat (also typically delivered via a UV light source) [7-9].

2.5. Types of 3D bioprinting

2.5.1. Inkjet-Based Bioprinting

Inkjet Bioprinting allows for the precise positioning of cells, with some studies achieving as few as a singular cell per printed droplet. Cells and biomaterials patterned into a desired pattern using drop lets, ejected via thermal or piezoelectric processes. Inkjet bioprinting is of great interest as it exhibits high resolution and cell viability. With this process, accurate position of multiple cell types is possible. However, the limitations of vertical printing and restricted viscosities may mean that inkjet bioprinting needs to combine with other printing techniques for future developments as shown in figure below [10].

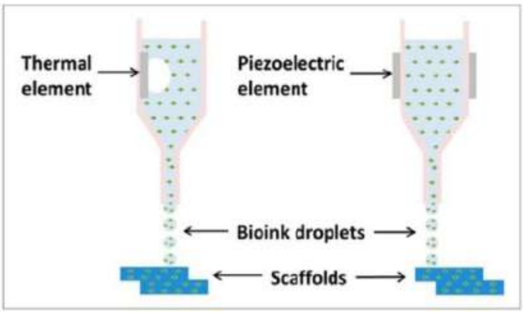


Figure (2- 3) Inkjet-Based Bioprinting [10].

2.5.2. Stereolithography

Stereolithography (SLA) is a technique that uses ultraviolet (UV) or visible light to cure photosensitive polymers in a layer - by - layer fashion. This nozzle - free technique eliminates the negative effects of shear pres sure encountered when using nozzle - based bioprinting. It offers a fast and accurate fabrication, with resolutions ranging between 5-300 μm.

Polymerization occurs at the top of the bio ink vat where the biomate rial is exposed to the light energy as shown in Figure.(2-4) below [11].

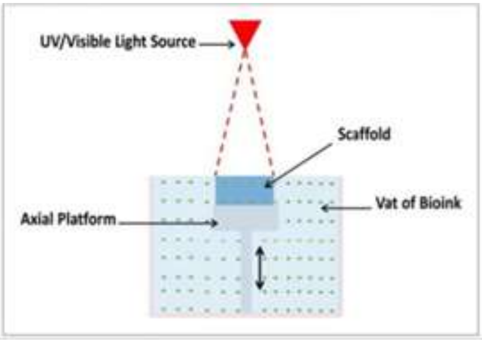


Figure (2- 4) Stereolithography (SLA) [11].

2.5.3. Laser - based bioprinting

Laser - assisted printing initially developed to deposit metals onto receiver sheets. Laser - assisted bioprinting (LAB) consists of three parts: a donor - slide (or ribbon), a laser pulse and a receiver - slide. A rib bon is made of a layer of transparent glass, a thin layer of metal, and a layer of bio ink. The bio ink transferred from the ribbon onto the receiver slide when a laser pulse vaporizes the metal layer under the hydrogel. This scaffold - free technique has very high cell viabilities (> 95) and a resolution between 10-50 μm. Some studies using LAB have demonstrated an accuracy of a singular cell per droplet as shown in Figure. (2- 5) below [11].

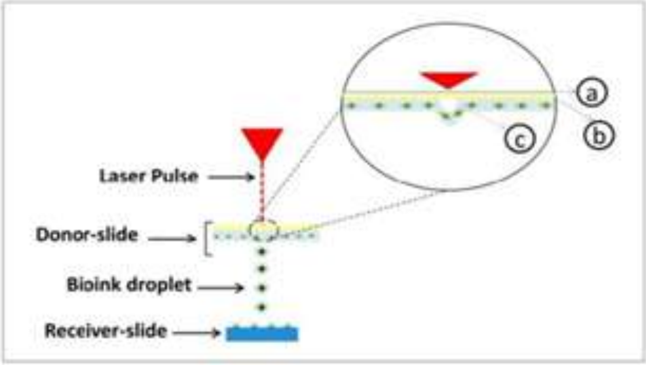


Figure (2- 5) Laser – assisted method [11].

2.5.4. Extrusion - Based Bioprinting

Extrusion - based printing is a pressure - driven technology. The bio ink is extruded through a nozzle, driven either by pneumatic or mechanical pressure, and deposited in a predesigned structure. The main advantage of extrusion bioprinting is the ability to print with very high cell densities. Despite its versatility and benefits, it has some disadvantages when compared to other technologies. The resolution is very limited, as a minimum feature size is generally over 100 µm, which is a poorer resolution than that of other bioprinting techniques as shown in figure (2-6) below [11].

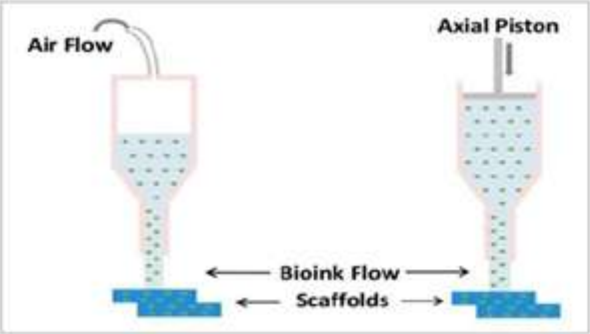


Figure (2- 6) Extrusion method [11].

2.6. What Exactly is Bioink?

- Bioink is the material used to produce engineered (artificial) live tissue using 3D printing technology.
- It can be composed only of cells, but in most cases, an additional carrier material that envelops the cells also added.
- This carrier material is usually a biopolymer gel, which acts as a 3D molecular scaffold. Cells attach to this gel, and this enables them to spread, grow and proliferate.
- Importantly, the gel can also provide protection to the cells during the printing process. Its importance is so high that the term “bioink” often commonly used to describe the carrier material alone, irrespective of the cells that may grow on it as shown figure below [12].

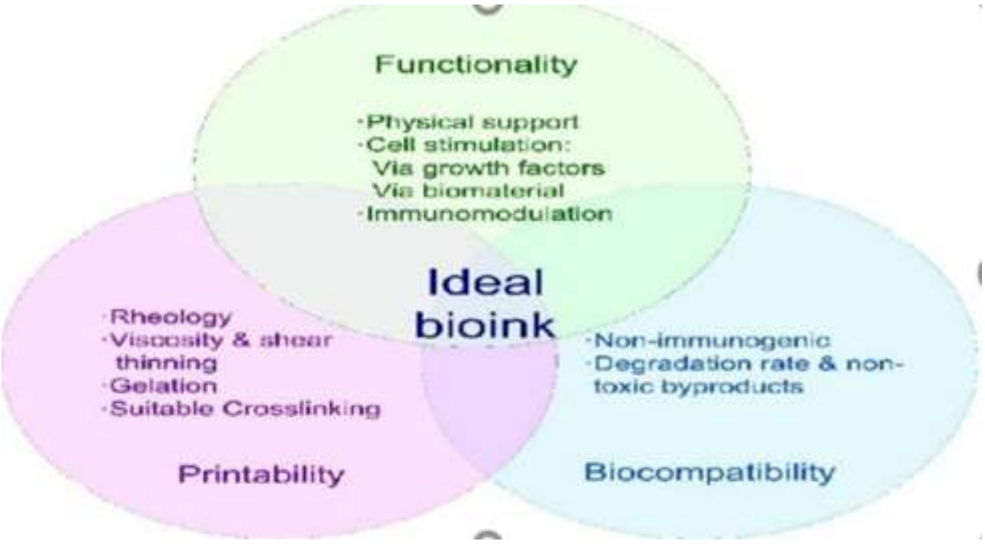


Figure (2- 7) ideal bioink traits [12].

2.6.1. Materials used to bioinks generally categorized two types :

- Natural derived polymers such as gelatin, collagen, alginate fibrin are studied in tissue engineering and generative medicine and are used for materials of capsulated cells. Natural-de-polymers widely of bioink and isolated animals.

- Meanwhile modified polymers are produced using synthesized or mixed different thissection characteristics of natural polymers and modified polymers summarized. Hydrogels of nature derived materials are employed in the field of tissue engineering and regenerative medicine because natural derived materials are similar to that native tissues or organs in the body.have numerous attractive features for use as tissue scaffolds. For example, they are biocompatible and typically biodegradable, and a majority of them possess specific cell-binding sites that are desirable for cell attachment, spreading, growth, and differentiation [12].

2.7. 3D printing applications and uses.

2.7.1. in the field of medicine

2.7.1.1. Diagnosis

2.7.1.2. Prosthetic devices and prosthetics

2.7.1.3. Learning

2.7.1.4. Medical device industry

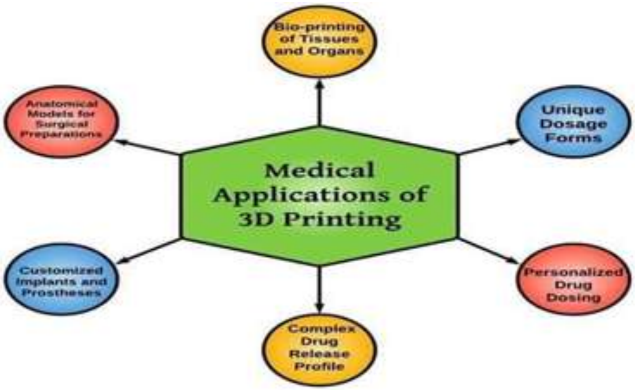


Figure (2- 8) Different medical applications of 3D printing [42].

Figure (2- 9) 3D bioprinting of human embryos [43].

Figure (2- 10) Low-cost prosthetic arm using 3D bioprinting [43].

Figure (2- 11) printed gelatinous, human organs [43].

Figure (2- 12) Implantation of ear using human cells by 3D bioprinting

CHAPTER THREE: POLYSACCHARIDE AND GRAPHENE HYDROGELS REVIEW COMPARISON

3.1. Polysaccharide Structure

Polysaccharides ,or polycarbohydrates, are the most abundant carbohydrates found in food.They are long chain polymeric carbohydrates composed of monosaccharide units bound together by glycosidic linkages. This carbohydrate can react with water (hydrolysis) using amylase enzymes as catalyst, which produces constituent sugars (monosaccharides, or oligosaccharides). They range in structure from linear to highly branched. Examples include storage polysaccharides such as starch, glycogen and galactogen and structural polysaccharides such as cellulose and chitin as shown figure below.

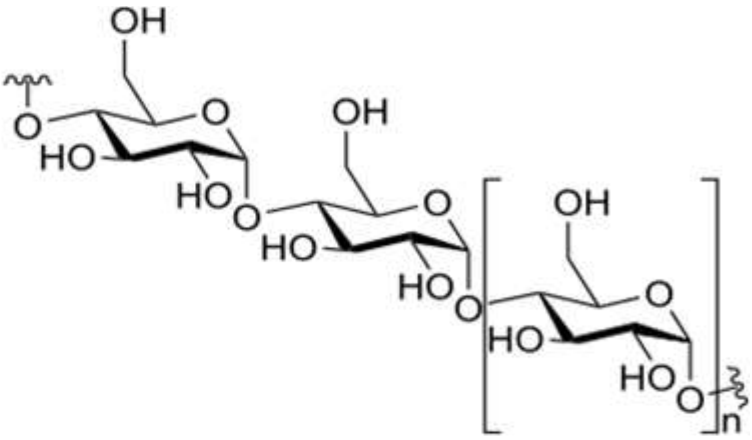


Figure (3- 1) Polysaccharide Structure [13].

Amylose is a linear polymer of glucose mainly linked with α (1→4) bonds. It can be made of several thousands of glucose units. It is one of the two components of starch, the other being amylopectin.

Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. When all the monosaccharides in a polysaccharide are the same type, the polysaccharide is called a homopolysaccharide or homoglycan, but when more than one type of monosaccharide is present, they are called heteropolysaccharides or heteroglycans [13].

3.2. Graphene hydrogel structure

Graphene is a new nanomaterial with strict two-dimensional layers structure .ith excellent mechanical, high electrical and thermal properties, graphene is the ideal filler. for polymer-based nanocomposites. Hydrogel is the moderate crosslinked. and branched polymer with three dimensional network structures. It is widely studied and applied because of the ability to absorb large quantities of water, swell quickly, soft, elastine, and biologic compatibility. Graphene has exhibited unique advantages in significantly improving the combination properties of traditional polymer hydrogels.

Graphene in hydrogels plays two roles: the gelator to self-assemble into the hydrogels, and the filler to blend with small molecules and macromolecules for the preparation of multifunctional hydrogels as shown in figure below [15].

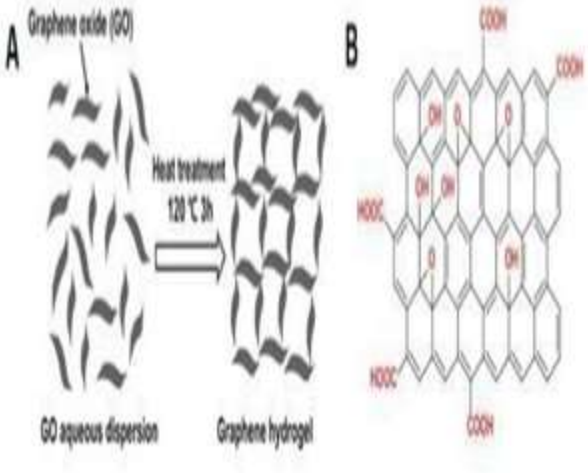


Figure (3- 2) Graphene hydrogel structure [14].

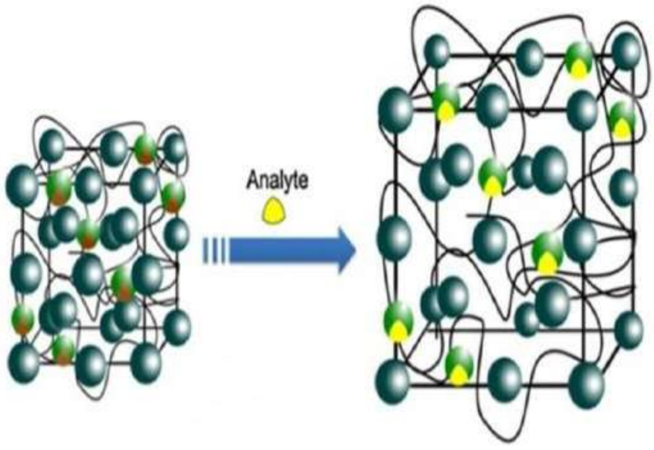


Figure (3- 3) Volume increase of a hydrogel biosensor in response to an analyte [44].

3.3. Polysaccharide and graphene properties

Table 3- 1 Polysaccharide and graphene properties [16,17].

Polysaccharide Characteristics	Graphene interesting properties
a. The formula for polysaccharide are Cx (H2O) y. b. The ration of carbon to hydrogen to oxygen is 1:2:1. c. Polysaccharide do not dissolute in water. d. They are less active and are present in condensed form. e. In taste they aren't pleasing. f. They cannot form crystals g. They are white in color on isolation.	a. High thermal conductivity b. High electrical conductivity c. High elasticity and flexibility d. High hardness e. Highresistance.Graphene is approximately 200 times stronger than steel, similar to diamond resistance, but much lighter. f. Ionizing radiation is not affected.Able to generate electricity by exposure to sunlight transparent material g. High density which doesn't let Helium atoms pass, but it does allow the passage of water, which evaporates at the same speed as if it were in an open container.

3.3.1. Electroconductivity of polysaccharide

In general, electroactive materials with polysaccharide matrices reach conductance levels comparable with synthetic ion conducting. Polysaccharides that show extensive hydrogen bonding appear to be more conductive than those that have few hydrogen bonds EAPs. Water-soluble polysaccharide films are usually prepared by dissolving a small amount (usually 1–5% w/w) into purified water. In some cases, the pH adjusted to facilitate dissolution. The solutions become viscous, are filtered, and then cast onto Teflon or glass surfaces. The self-supporting films are dried or annealed under controlled environmental conditions (temperature, relative humidity). For some applications, electrode surfaces are dip-coated with polysaccharide solutions and dried. Salts dissolved into polysaccharide solutions before casting, to produce ion- conducting films. In some instances, polysaccharide films are soaked in an electrolyte solution to allow diffusion of ions and then dried as shown in figure below [18].

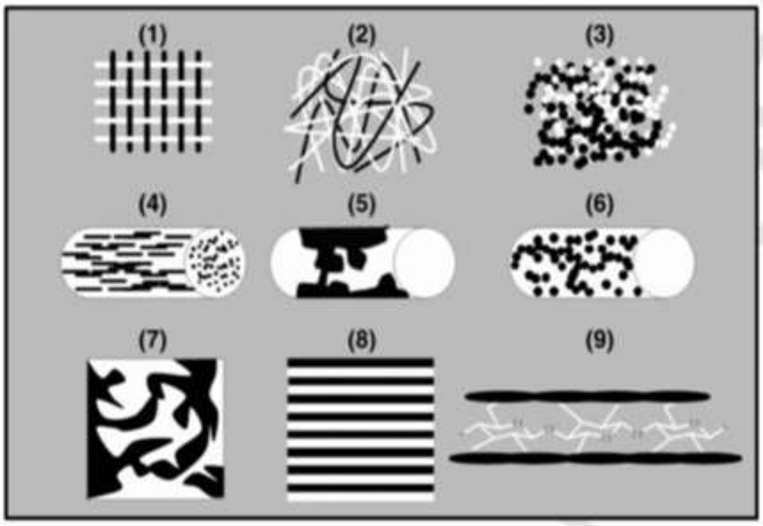


Figure (3- 4) Different general principles for introducing conductivity into polysaccharides [18].

1) textile produced from polysaccharide and conducting filaments
2) random network of polysaccharide and conducting fibers
3) mixture of polysaccharide and conducting particles
4) conducting particles incorporated into a polysaccharide
5) conducting material coated on the surface of a polysaccharide

6) conducting nanoparticles coated onto a polysaccharide matrix
7) blend of polysaccharide and conducting material
8) layer-by-layer deposition of polysaccharide and conducting material
9) chemical derivatization of a polysaccharide (cellulose) with substituents forming an electrically conducting phase.

3.3.2. Extremely conductive graphene hydrogel

Conductive hydrogels could be used to control the release of active substances in order to treat certain diseases locally in a more targeted manner.In order to produce electrically conductive hydrogels, conventional hydrogels are usually mixed with current-conducting nanomaterials that are made of metals or carbon, such as gold nanowires, graphene or carbon nanotubes.

To achieve a good level of conductivity, a high concentration of nanomaterials is often required. However, this alters the original mechanical properties of the hydrogels, such as their elasticity, and thus impacts their interaction with the surrounding cells as shown in figure below [19].

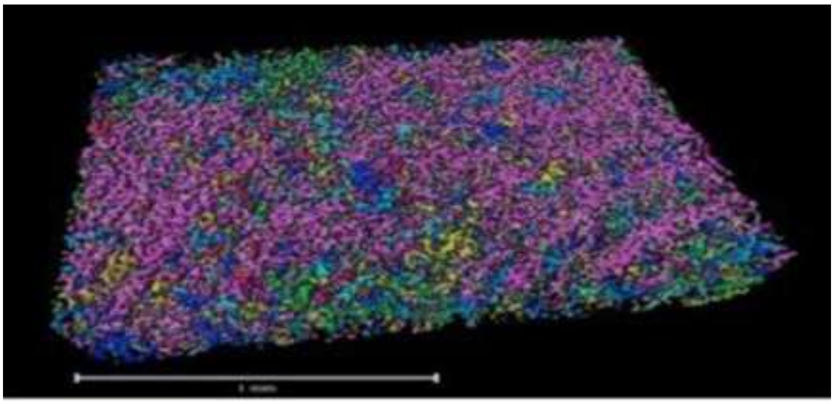
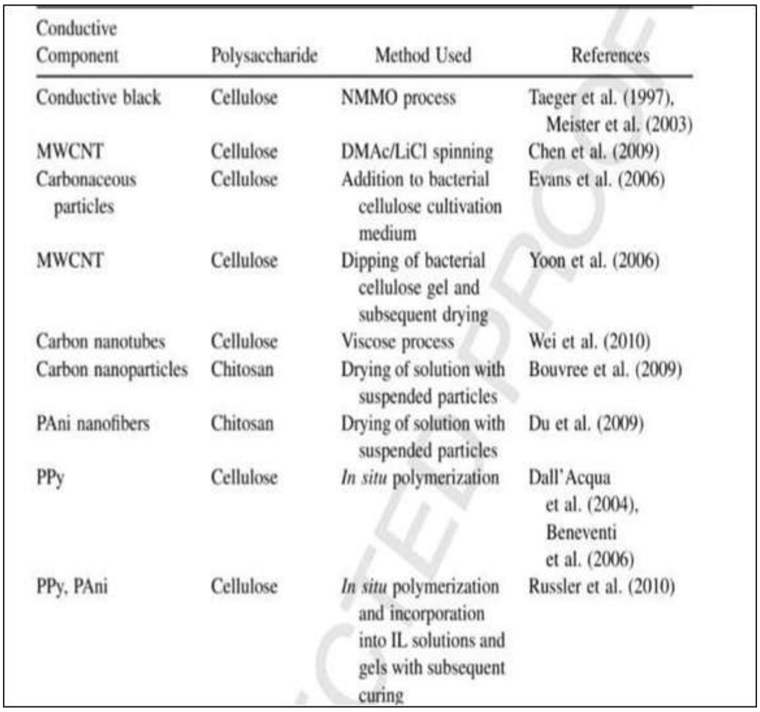


Figure (3- 5) shown each colour indicates a connected microchannel: the microcomputer tomography image clearly shows how interlinked the individual channels and thus how reliably electrical signals through the entire material [10].

Table 3- 2 Overview of the Approaches Presented for "Incorporation" [18].



Conductive Component	Polysaccharide	Method Used	References
Conductive black	Cellulose	NMMO process	Taeger et al. (1997), Meister et al. (2003)
MWCNT	Cellulose	DMAc/LiCl spinning	Chen et al. (2009)
Carbonaceous particles	Cellulose	Addition to bacterial cellulose cultivation medium	Evans et al. (2006)
MWCNT	Cellulose	Dipping of bacterial cellulose gel and subsequent drying	Yoon et al. (2006)
Carbon nanotubes	Cellulose	Viscose process	Wei et al. (2010)
Carbon nanoparticles	Chitosan	Drying of solution with suspended particles	Bouvree et al. (2009)
PAni nanofibers	Chitosan	Drying of solution with suspended particles	Du et al. (2009)
PPy	Cellulose	*In situ* polymerization	Dall'Acqua et al. (2004), Beneventi et al. (2006)
PPy, PAni	Cellulose	*In situ* polymerization and incorporation into IL solutions and gels with subsequent curing	Russler et al. (2010)

3.4. Hydrogels applications in 3d bioprinting

3.4.1. Applications of polysaccharide in bioprinting Polysaccharide - Based Bioink.

Limitations in wound management have prompted scientists to introduce bioprinting techniques for creating constructs that can address clinical problems. The bioprinting approach is renowned for its ability to spatially control the three- dimensional (3D) placement of cells, molecules, and biomaterials. These features provide new possibilities to enhance homology to native skin and improve functional outcomes. However, for the clinical value, the development of hydrogel bioink with refined printability and bioactive properties is needed. In this study, we combined the outstanding viscoelastic behavior of nanofibrillated cellulose (NFC) with the fast cross-linking ability of alginate (ALG), carboxymethyl cellulose (CMC), and encapsulated human-derived skin fibroblasts (hSF) to create a bioink for the 3D bioprinting of a dermis layer. The shear thinning behavior of hSF-laden bioink enables construction of 3D scaffolds with high cell density and homogeneous cell distribution. The obtained results demonstrated that hSF-laden bioink supports cellular activity of hSF (up to 29 days) while offering proper printability in a biologically relevant 3D environment, making it a promising tool for skin tissue engineering and drug testing applications [20].

3.4.2. Graphene hydrogel as bioink in 3d bio printing

3D bioprinting is known as an additive manufacturing technology that builds customized structures from cells and supporting biocompatible materials for the repair of damaged tissues or organs. In this study, we prepared water-dispersible graphene and graphene oxide, which are 2D nanomaterials with high conductivity and potential applications in neural tissue engineering. Moreover, we synthesized a new biodegradable waterborne polyurethane with soft segments that mostly contained poly(ε-caprolactone) (2 kDa) and 20 mol% of shorter (1.5 kDa) poly(D,L-lactide) chains. This polyurethane dispersion at a solid content of 25% in a cell culture medium underwent a sol–gel transition near human body temperature with a suitable gel modulus. After this, we mixed graphene or graphene oxide with polyurethane to prepare a graphene-based nanocomposite hydrogel for neural stem cell (NSC) printing. The rheological properties of the graphene-based nanocomposite hydrogel were suitable for the printing and survival of NSCs. Furthermore, the addition of a very low content (25 ppm) of graphene nanomaterials to the hydrogel significantly enhanced the oxygen metabolism (2- to 4-fold increase) as well as the neural differentiation of NSCs. In summary, the graphene–polyurethane nanocomposite hydrogel may be a possible bioink for printing 3D cell-laden tissue constructs for neural tissue engineering as shown in figure below [21].

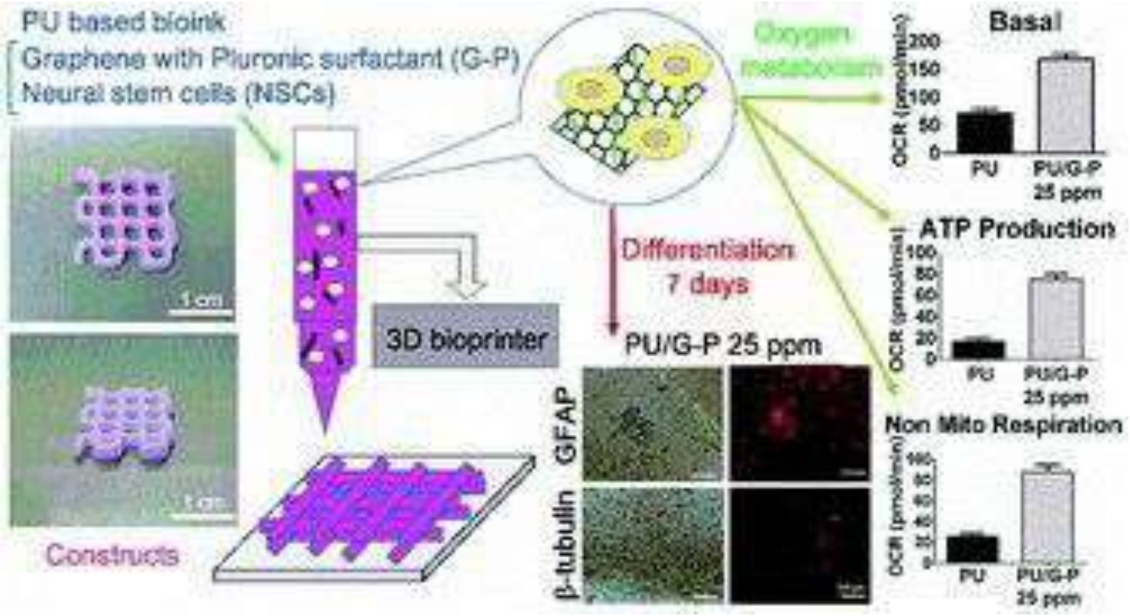


Figure (3- 6) hydrogel as a potential bioink for 3D bioprinting [21].

3.5. Different applications of hydrogels.

3.5.1. Applications of polysaccharide

3.5.1.1. Self-healing hydrogels

Hydrogels with controlled structure and mechanical performances have attracted tremendous interests in the field of self-healing materials. With the self- healable properties, polysaccharide-based hydrogels should be capable of reconstructing their structure and functionality from damage, which could facilitate the optimization of integrated functions of hydrogels with good safety, reliability, and durability. Self-healing hydrogels could be divided into external stimulus self-healing and autonomous self-healing depending on the healing behaviors. The former strategy needs stimuli such as heat, UV light, and other self-healing agents to trigger the healing process. While the later one is based on the dynamic covalent bonds or noncovalent interactions (e.g., hydrogen bonding, ionic interaction, host–guest interactions, and hydrophobic interactions) to repeatable self-heal the materials.The noncovalent bonds not only serve as recombination moieties for self-healing but also act as sacrificial bonds to increase the toughness. Polysaccharides (i.e., cellulose, alginate, and chitosan) have abundant hydroxyl groups on backbones, which could form hydrogen bonding with other polymer such as PVA to form self-healing composite hydrogels.When TA was employed to modify the CNCs, mussel-inspired hydrogels with high mechanical self-healing properties via extensive multihydrogen bonding were achieved as shown in figure below [22,23].

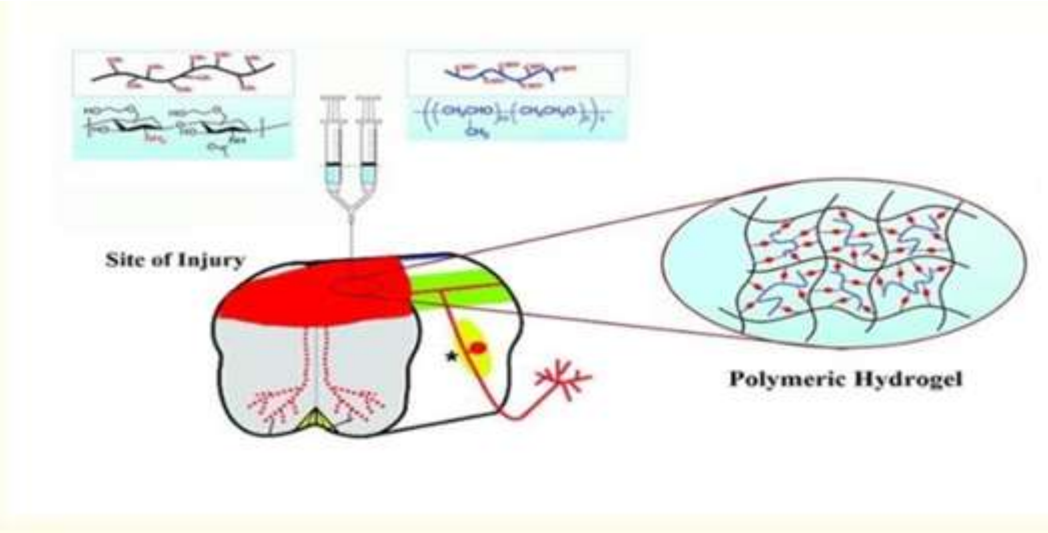


Figure (3- 7) Injection of liquid hydrogel into the site of injury [45].

3.5.1.2. Sensors

Hydrogels could alter their volume significantly in response to certain alterations or stimuli. This behavior can transform into different output signals including chemical, physical, and electrical properties, which makes them promising candidates for sensors (such as pH sensors, chemical sensors, strain sensors, and pressure sensors).The sensitivity of the sensor is dominated by the quantity changes from input to output. The sensitivity of the sensor is dominated by quantitative changes from input to output. To have a good sensor, it must be sensitive to objective characteristics and insensitive to other performance.When suitable functional groups or conductive fillers are introduced into the polymer networks, the hydrogels will exhibit an ability to respond to stimuli Manufacture of responsive stimuli. Polysaccharide-based hydrogels associated with non-covalent interactions have been studied to prepare self-healing materials, so those hydrogels should also inherit the advantages of non-covalent interactions to respond to stimuli as shown in figure below [24].

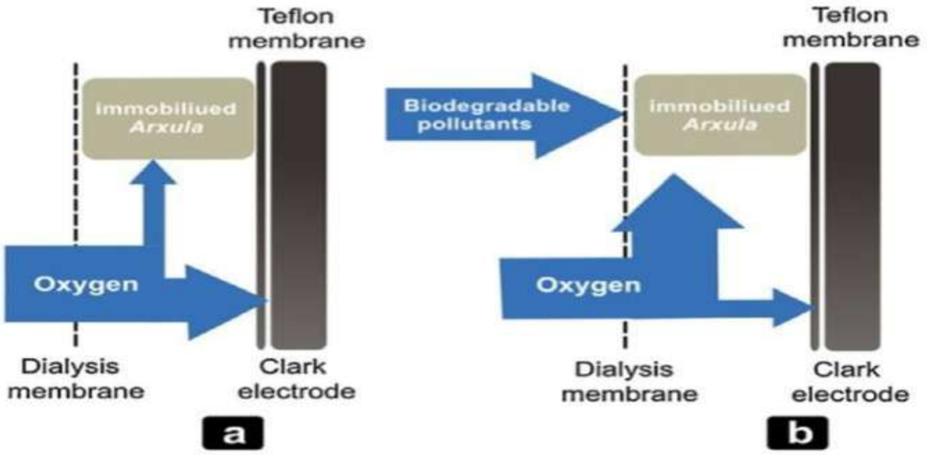


Figure (3- 8) shown Schematic diagram of the A. adeninivorans LS3 microbial sensor illustrates the microbial consumption of dissolved oxygen (a) before and (b) after the addition of biodegradable pollutant [46].

3.5.1.3. Drug Delivery

Polysaccharides based biomaterials for drug delivery

With the advancement in the area of drug delivery systems, numerous delivery strategies and materials have been utilized in order to attain the control release of drugs to improve their therapeutic index along with enhanced targeting efficiency . A broad range of biomaterials have been reported as carrier vehicles for diverse class of therapeutics. Among all, polysaccharides have been of interest for many researcher groups for being a potent drug delivery system for delivering therapeutics. Properties like non-toxicity, biodegradability, biocompatibility, low immunogenicity are some of the key parameters that mark the success of polysaccharides as an efficient candidate in drug delivery applications as shown in figure below [25].

1. Physical encapsulation of drugs
2. Chemical coupling of drugs
3. Active and passive targeting

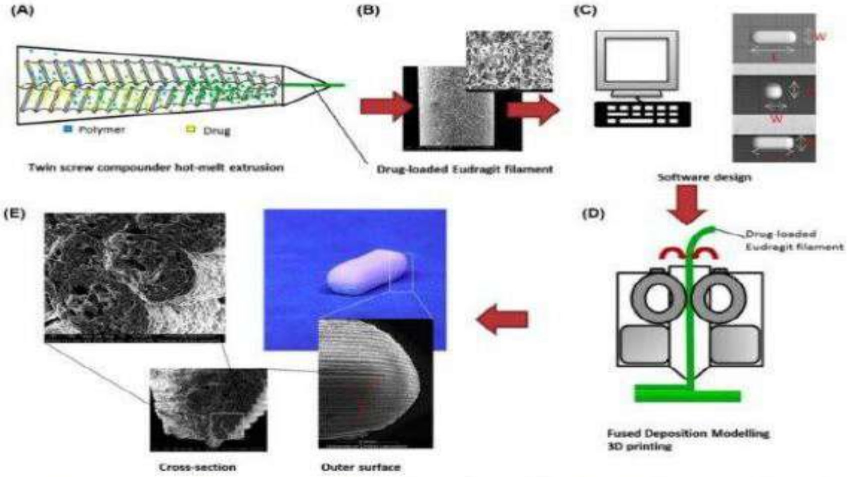


Figure (3- 9) Fabrication of 3D-printed controlled-release theophylline tablet. (A) Schematic illustration of hot-melt extrusion to produce theophylline loaded filaments.

(B) Drug-loaded filament. (C) Design of the capsule-shaped tablet. (D) Printing by fused deposition modelling. (E) 3D printed Eudragit tablets with a 200-um layer thickness as shown in scanning electron [25].

3.5.1.4. Polysaccharides based biomaterials for tissue engineering

Tissue engineering involves damaged tissue regeneration or repairing of the malfunctioning tissue by providing suitable suiTable 3D microenvironment for the cell attachment, differentiation and proliferation. To achieve the required 3D microenvironment several advanced materials based 3D scaffolds are being developed which can mimic the naturally occurring extracellular matrix (ECM) and provide a support for desired cell growth. In this regard, numerous synthetic and natural polymers have been used individually or in combination for fabricating tissue engineering scaffolds. Here, natural polymers like polysaccharides provide extra advantage of being biocompatible and mimicking ECM. Hence, polysaccharides based biomaterials have emerged as a capable platform for tissue engineering applications as shown in figure below.

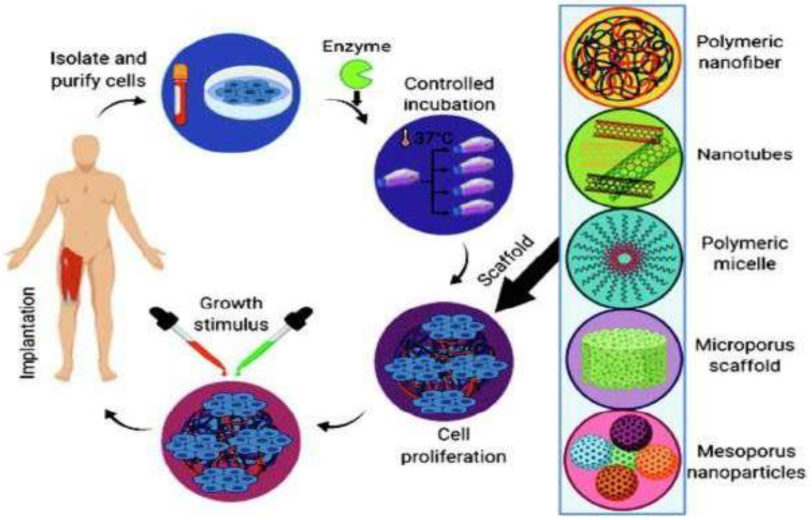


Figure (3- 10) Schematic representation of the different processes involved in the field of tissue engineering to develop a scaffold ready for implantation [47].

Chitosan is categorized as natural polycationic polysaccharides and itcan show electrostatic interactions that can be used for the production of biomaterials. It can be an interesting choice of material in a physiological environment, where most biomolecules are anionic. This, in combination with properties such as its biodegradability, biocompatibility, increased cell adhesion and antimicrobial properties marks the success of chitosan as a potent material in the field of tissue engineering [25].

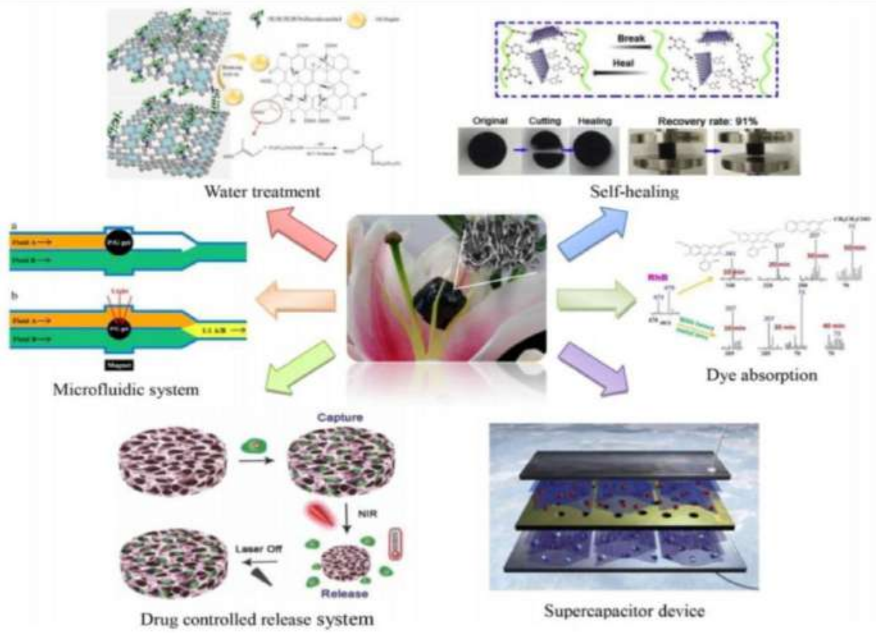


Figure (3- 11) Figure (3-11) applications of graphene [48].

3.5.2. Applications of graphene

3.5.2.1. Self-Healing Mechanism for graphene hydrogel

Various mechanisms have been followed to obtain self-healing hydrogels. The heal- ing mechanism is broadly classified as-covalent and non-covalent bonding . Dynamic covalent bonding includes imine bonds, boronate bonds, Diels-Alder reaction, acylhydrazone bonds, oxime bonds, and disulfide bonds whereas non- covalent interaction includes hydrogen bonds, ionic interaction, host guest interaction, and hydrophobic inter- action. The hydrogels obtained from non-covalent interactions are generally highly flexible and self-heal because of their ability to easily break and reconstruct crosslinks, whereas those obtained from covalent bonding are highly stable as shown in figure below [26,27].

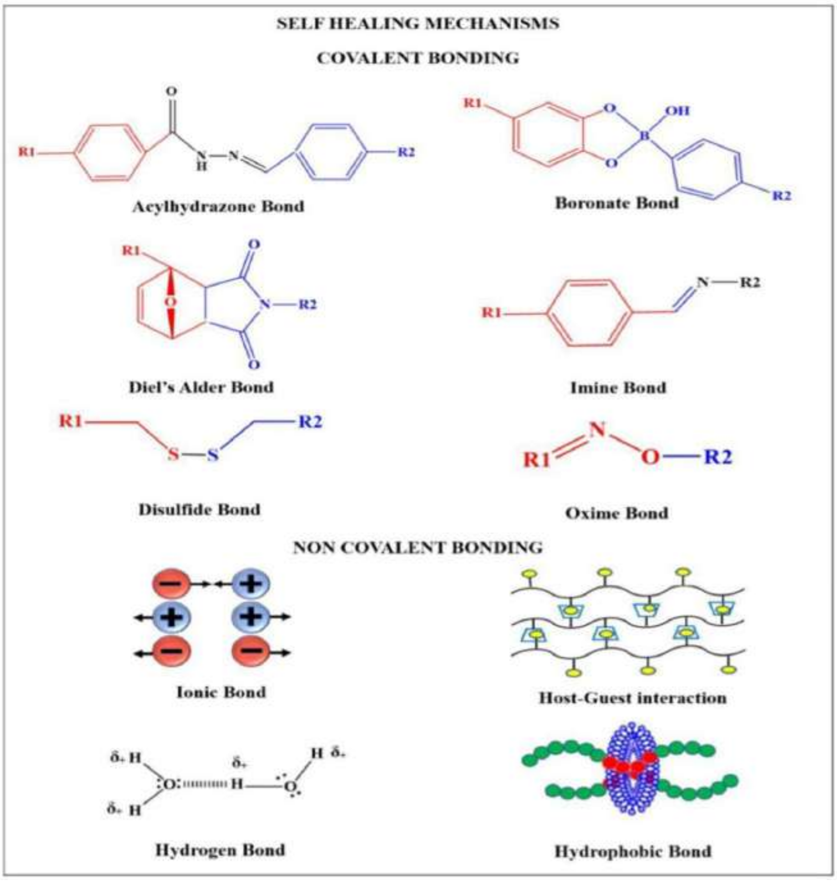


Figure (3- 12) Bonds involved in self-healing mechanisms [26].

3.5.2.2. Biosensors

Are important devices in the medical field for diagnostic and therapeutic applications. Biosensor systems are based on the detection of biomarkers such as tissues, enzymes, nucleic acids, antibodies, and microorganisms. As technology evolves and the understanding of biological systems improves the need for accurate, sensitive, and selective biosensors also gains importance. Graphene-based materials have shown great potential for the development of next- generation biosensors due to their excellent electrical and thermal conductivity, high surface area, two-dimensional structure, and durability. In biosensor applications, graphene is utilized as transducing agent.,GO, rGO, and GODS are commonly utilized graphene materials in biosensor applications [28].

3.5.2.3. Drug Delivery

Medical drugs have been the backbone of medical practices for a very long time. However, conventional drugs have some crucial drawbacks including non- specific targeting, short blood circulation time, and burst release, resulting in low availability, poor therapeutic efficiency, and side effects. Hence, the new generation of medical applications has focused on targeted drug delivery systems to overcome these drawbacks of conventional drugs. Targeted drug delivery systems offer selectivity, controlled release of drugs, easier, accurate and less frequent dosing, decrease in required drug concentration, and toxic effects on the human body [29].

3.5.2.4. Tissue Engineering

Tissue engineering is an up-and-coming interdisciplinary field that requires the input of biological engineering and material sciences. Tissue engineering applications focus on the development of biological substitutes that can repair, maintain, or improve tissue's function. The practice of tissue engineering is mainly based on the development of degradable scaffolds with biocompatible chemistry to provide cellular attachment, proliferation, differentiation, and support new tissue formation as well as the mechanical strength to support and mimic the tissue. Furthermore, these scaffolds must be biodegradable to avoid the need for surgery for removal, highly porous to facilitate cell adhesion and diffusion, have compatible pore size and pore interconnectivity to favor tissue integration and vascularisation. Tissue engineering scaffolds are often composites of polymers and bioactive materials. Biocompatible polymers that can be natural or synthetic are used to obtain biodegradable scaffolds as shown in figure below [30].

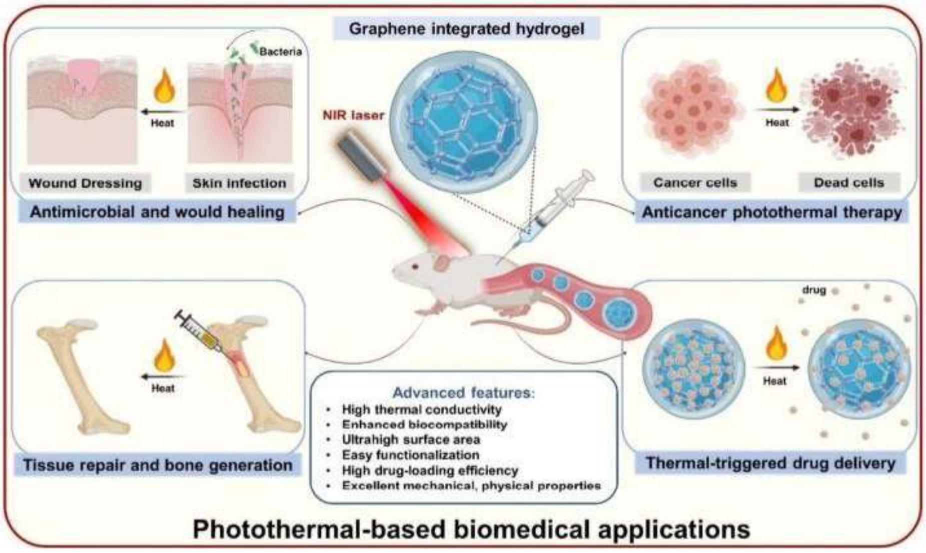


Figure (3- 13) Graphene integrated hydrogel [49].

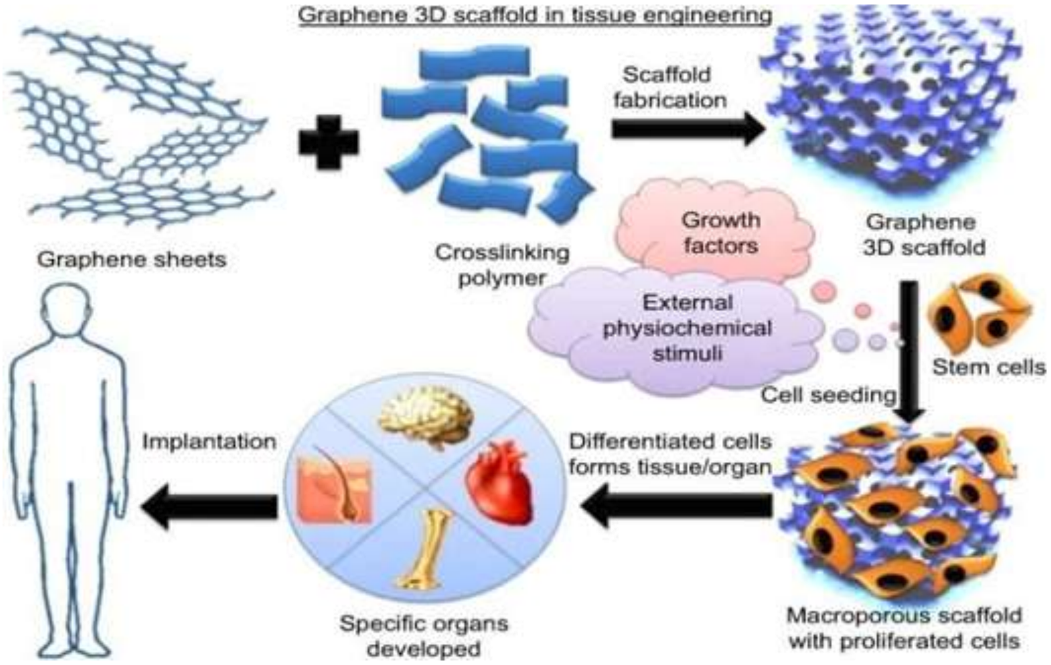


Figure (3- 14) Graphene 3D scaffold in tissue engineering [50].

3.6. Conclusion

A hydrogel is a three-dimensional (3D) network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains. Hydrogels were first reported by Wichterle and Lím (1960). By definition, water must constitute at least 10% of the total weight (or volume) for a material to be a hydrogel. Hydrogels also possess a degree of flexibility very similar to natural tissue due to their significant water content. The hydrophilicity of the network is due to the presence of hydrophilic groups such as -NH2, -COOH, -OH, -CONH2, - CONH -, and -SO3H. Hydrogels undergo a significant volume phase transition or gel-sol phase transition in response to certain physical and chemical stimuli. The physical stimuli include temperature, electric and magnetic fields, solvent composition, light intensity, and pressure, while the chemical or biochemical stimuli include pH, ions, and specific chemical compositions. However, in most cases such conformational transitions are reversible; therefore, the hydrogels are capable of returning to their initial state after a reaction as soon as the trigger is removed. The response of hydrogels to external stimuli is mainly determined by the nature of the monomer, charge density, pendant chains, and the degree of cross-linkage. The magnitude of response is also directly proportional to the applied external stimulus.

There are numerous original papers, reviews, and monographs focused on the synthesis, properties, and applications of hydrogels. Here in our study, we chose two types of hydrogels (graphene and polysaccharide) and doing comparison in their properties and some applications

The specific properties of graphene, such as high electrical conductivity, high thermal conductivity, and excellent mechanical properties, have made graphene not only a gelator to self-assemble into the GBH with extraordinary electromechanical performance, but also a filler to blend with small molecules and macromolecules for the preparation of multifunctional GBH. It fully exploits the practical applications of traditional hydrogels. In view of the developing trend of hydrogel in recent years, theoretical researches are relatively high. Researchers are very interested in the application prospect of hydrogels for biomedical, tissue engineering, super capacitor, water treatment, dye absorption, catalyst carrier, and intelligent response for microfluidic system. However, compared to the practical applications, the actual operation research is very weak. Polysaccharide hydrogels including alginate, agarose, hyaluronic acid and chitosan have been widely used as scaffolds in 3D bio- printing field. They exhibit excellent properties of water solubility, biocompatibility and biodegradability. The complex hydrogels possessed the shear thinning character at high shear rate, which are appropriate for the 3D-printing technique. More importantly, the addition some materials to hydrogel not only improved the mechanical strength and bio-printability of composite hydrogels, but also significantly enhanced the electrical conductivities, which satisfies the needs of cells for micro- current stimulation to proliferation and differentiation. This lead to conclude that the electroactive hydrogels have the potential applications in tissue regeneration such as muscle and cardiac nerve tissue repair.

CHAPTER 4: RESULTS AND DISCUSSION

Analyse of study review

Hydrogels have existed for more than half a century, and today they have many applications in various processes ranging from industrial to biological. There are numerous original papers, reviews, and monographs focused on the synthesis, properties, and applications of hydrogels. Therefore, this study focused on doing a relative comparison of two types of hydrogels known in different fields in some properties. Our goal is to find which one is better in some applications according to their properties, as well as to find out which one of properties need improvement for better performance in the future by analyse the results of some researches by obtained the average percentages of their results regards to each property. So according to their results we did an analysis of the following properties and some applications

1. Electroconductivity
2. Biocompatibility

3. Swelling& toxicity
4. Bioink
5. Self healing

4.1. According to some properties

4.1.1. The electroconductivity

The researches that in our hand which related to electroconductivity showed that graphene was the best in ability to conductivity in 75% compared to 40% for polysaccharide but after adding materials and minerals that help to improve this characteristic, although the same minerals were also used for polysaccharide but the susceptibility of graphene is more due to its low water content and denser texture [33,31,32] as shown in figure (4-1).

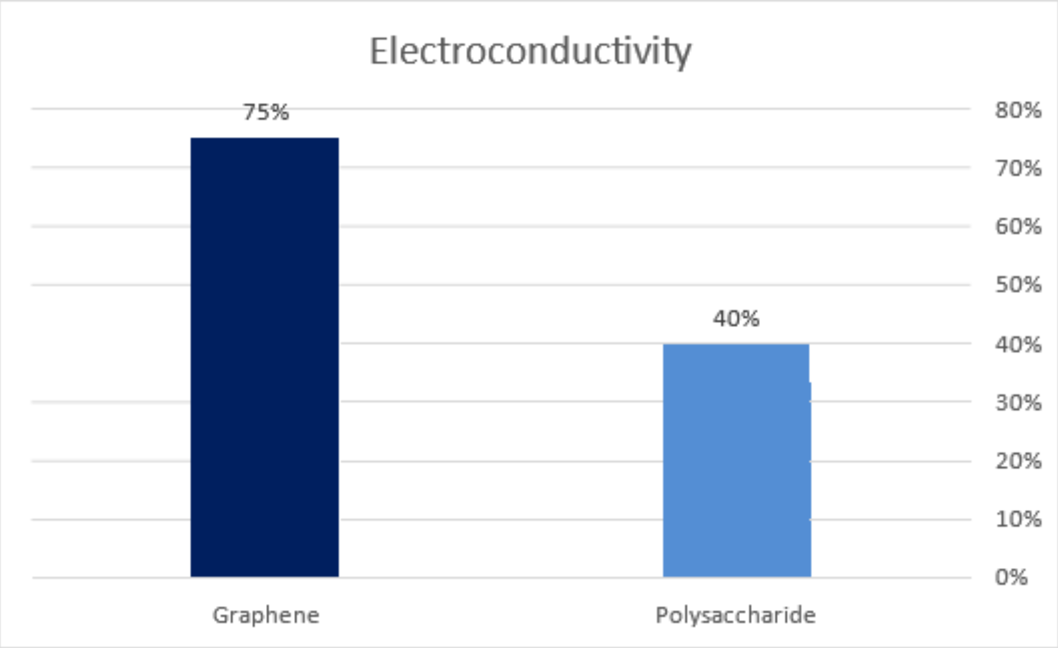


Figure (4- 1) Electroconductivity percentage for both hydrogels.

4.1.2. Biocompatibility

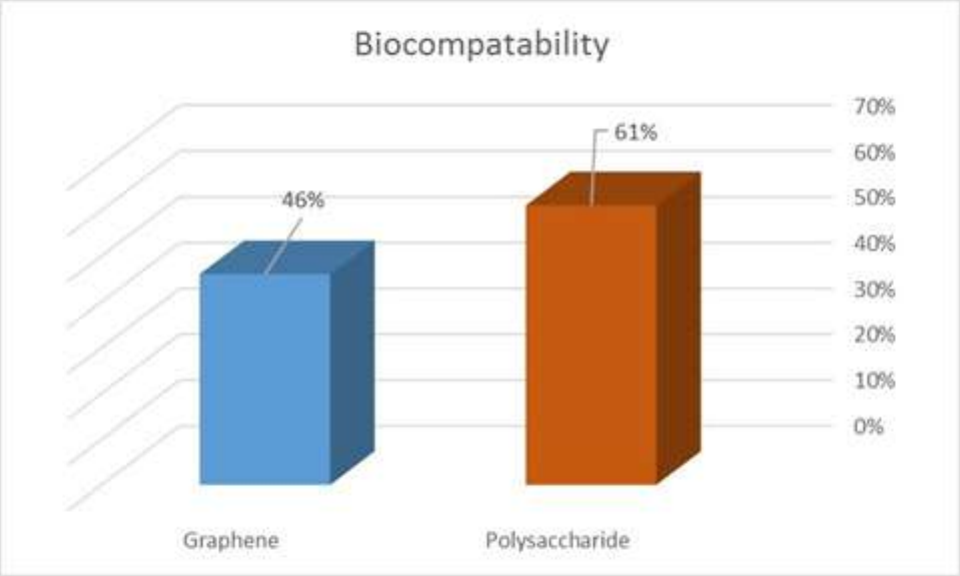


Figure (4- 2) Biocompatibility percentage for both hydrogels.

As for the biocompatibility, the polysaccharide hydrogel showed a greater conformity rate 61% with a little graphene hydrogel 46% in relation to its carbohydrate nature, especially the natural source of polysaccharids polymers.

polysaccharides are extremely advantageous which these are easily procured from plants and more often being produced by recombinant DNA techniques. Polysaccharides are usually non-toxic, biocompatible and show a number of peculiar physico-chemical properties that make them suitable for different applications [40,41].

4.1.3. Toxcicity & swelling

The Toxicity Graphene-family nanomaterials (GFNs) are widely used in many fields, especially in biomedical applications. Currently, many studies have investigated the ocompatibility and toxicity of GFNs in vivo and in intro. Generally, GFNs may exert different grees of toxicity in animals or cells due to its unique structural specific surface area and mechanical characteristics. This is what we also found through research analysis in 71%. Compared to 33% for polysac.

While most reports showed less toxicity towrds poly saccharide this due to thier molecular structure that can be linear or highly branched, composed by the same (homopolysaccharide) and easily geting them from natural resources [34, 35].

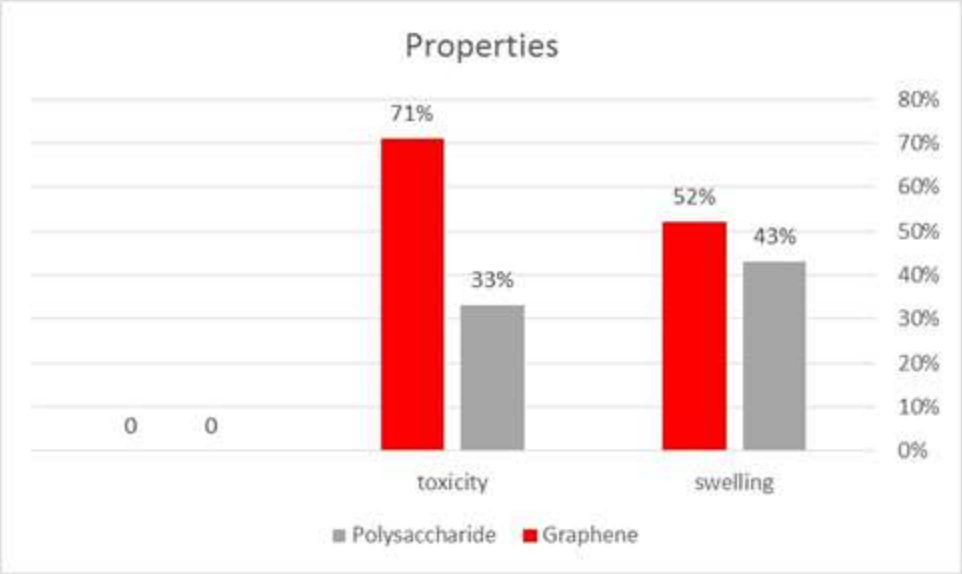


Figure (4- 3) Toxicity & swelling percentage for the both hydrogels.

As for swelling,

The opposite appeared, which is the superiority of graphene 52% over polysaccharides 43% with a clear difference becuase it have reported that most graphite oxide (GtO) and graphene oxide (GO) multilayered laminates are hydrophilic materials easily intercalated by water and other polar solvents while the polysaccharide showed less ability becuase most hydrogels require a long time to reach equilibrium swelling due to the slow absorption of water by diffusion due to presence of multiple hydrogen bonds the water cannot invade the molecules making them hydrophobic [35,29].

4.2. According to some applications

4.2.1. Bioink application

Through research and reports, it has been shown that graphene is very iedial as a bioink at 52% of researcher's results because of its three-dimensional nature, as well as the possibility of forming it according to the mold, in addition to the success of the scaffold that is included in its composition which it is offers several unique advantages, including electrical conductivities significantly higher than previously reported 3D-printed carbon materials. This conductive bioink can be deposited into user-defined structures and handled immediately after being bioprinted. As for polysaccharide, it is not very different from graphene as its percentage 49% that it is a good source of bioink, but it varies according to the fabric to be printed.**[36,37].**

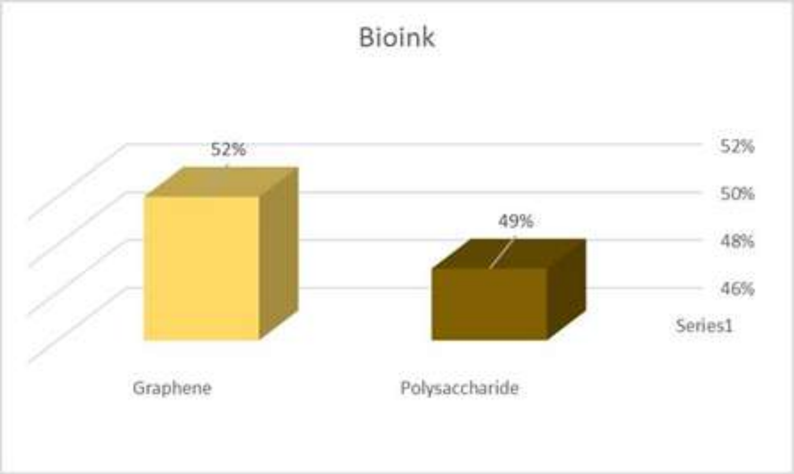


Figure (4- 4) Percentage of bioink applications for both hydrogels.

4.2.2. Self-healing in tissue engineering

Self-healing materials are one kind of intelligent material capable of feeling external stimulus to repair themselves after damage and restore their intrinsic properties, thus can extend lifespan, improve security, save cost, and achieve sustainable development. However, their mechanical property, energy absorption and conversion efficiency, and self-healing property still need to be further enhanced in scientific research and practical application, graphene is one of these materials a two- dimensional material with outstanding mechanical, electrical, and thermal properties, as well as excellent energy absorption and conversion characteristics which showed it is ideal in healing the hard tissue and organs such as bones , cartilage and joints in 47%. As for polysaccharides, the natural polysaccharides are ideal materials for the preparation of biomimetic hydrogels in 65% because of their good biocompatibility and biodegradability. The self-healing hydrogel can achieve healing without external force and prolong the service life of the hydrogel. Therefore, self-healing hydrogels are of great interest in tissue engineering, wound dressings, drug delivery, and tissue engineering specialy for soft tissues [37,38,39].

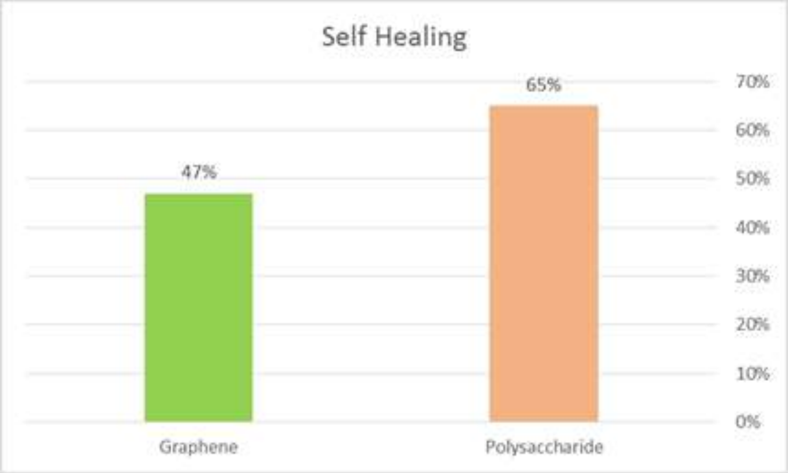


Figure (4- 5) Self healing application percentage for both hydrogels

Table 4- 1 summery of comparsion for some properties of hydrogels

Hydrogels	Electro conductivity	Biocompatibility	Swelling	Toxicity
Graphene	75%	46%	52%	71%
Polysacc.	40%	61%	43%	33%

The basic structure of graphene oxide (GO) exhibits it's preferable than polyzoic. Whereas it is spontaneously translated into a stable 3D graphene structure under the interaction of non-covalent p-p bond. It considered an effective approach for fabricating graphene hydrogels and they can obtain some unique structures and characteristics, such as porous network structure, ultralow density, excellent thermal properties, and thermal stability. The electrical conductivity of the graphene hydrogels and the storage modulus make it greater several orders than the traditional hydrogels. The composite material added displayed more excellent swelling characteristics and electrical response [31]. While the decrease in conductivity of polyzoic. is then a reflection of the decrease in free ions within the polymer matrix [32]. Different polysaccharides show different swelling capacity depending upon. The formation of hydrogel, film, beads etc. for various applications.[35]. Polysaccharide-based hydrogels are derived from living tissues that either are components of or have macromolecular properties similar to the natural ECM. Therefore, they are inherently biodegradable and biocompatible and less toxicity [29].

Table 4- 2 *Summary of comparision for some applications of hydrogel*

	Applications	
Hydrogels	**Bioink**	**Self-healing**
Graphene	52%	47%
Polysacc.	49%	65%

GO is suitable for biomedical applications such as drug delivery, gene therapy, biomedical imaging, combined cancer therapy, antibacterial agents, as biosensors. However, the actual application of any nanomaterial in biology and medicine is decided critically by its biocompatibility. Biocompatibility, responsiveness to various stimuli and biomimicry of extracellular matrices for soft tissues. One emerging strategy for creating engineered tissue constructs is 3D bioprinting technique, with lots of promises in the field of tissue engineering and regenerative medicine due to its high precision, controllability, its ability to allow cells to be directly embedded or distributed inside the scaffold and repeatability for complex structures. The material employed as a Bioink is a fundamental factor in 3D bioprinting. Hydrogels are ideal candidates to be employed as bioinks. Therefore, self-healing hydrogels are of great interest in tissue engineering, wound dressings, drug delivery, and tissue engineering specialy for soft tissues [39].

4.3. CONCLUSION

With the development of modern science, 3D printing technology has become an indispensable medical solution for many diseases or correction of impairments, and there must be bioink that has different biological characteristics such as biocompatibility, biodegradibility and non-toxic in order to produce the target organ or tissue.

Here appears, the role of hydrogels as one of the appropriate options as a biological ink. However, these compounds or molecules are certainly not devoid of some undesirable characteristics. Therefore, many studies have been working to improve these properties in order to obtain an ideal biological ink for printing.

Through our study of many of the referencecs mentioned here and others not, we conclude that

1. The most important characteristic that should be improved is the elimination of toxicity of graphene
2. The frothiness of the bio-compatibility characteristic is for graphene

3. the characteristic of the lack of swelling and electrical conductivity in polysaccharides needs further studies and practical work through modification approachs on structure level or by adding different materials to enhance the desireble property in order to get the ideal bioink.

4. Graphene has the ability to self-heal when the fracture surfaces have maintained contact at low temperature or even room temperature for short periods. The recovery rate of the hydrogel can reach up to 88% at a prolonged healing time.

5. Graphene hydrogels properties like integrates mechanical strength, electrical conductivity, adsorption, hygroscopicity, water retention, controlled-release and biocompatibility together, gave it broad application prospect in biomedical, supercapacitor, water treatment, dye absorption, catalyst carrier and intelligent response for microfluidic system and are attracted much attention in tissue engineering.

6. Electroactive polysaccharide (EAPs), a new class of materials, have the potential to be used for applications like biosensors, environmentally sensitive membranes, artificial muscles, actuators, corrosion protection, electronic shielding, visual displays, solar materials, and components in high-energy batteries.

7. There is current progress of incorporating polysaccharides as matrices for doped, blended, and grafted electroactive materials.

8. However, during fabrication, GBNs usually undergo several chemical treatment processes for functionalization, including doping with metals, oxidation, which introduces functional groups, and a material reduction. This indicates that some of the graphene derivatives considered for bio applications contain metals and/or impurities other than carbon.

4.4. RECOMMENDATIONS

1. Conducting linkage studies between polysaccharide and graphene together and forming a hybrid and studying it at the biological levels by applying it to invivo laboratory animals, or invitro studies on human or animals cell lines in the future.
2. Modify the polymeric structure to obtain desired functionality, the areas of applications are rapidly expanding. They can be designed in such a way that they can respond to a specific stimulus including pH, temperature, light, etc. at a predefined level and thus be stimuli responsive
3. The mechanical properties of the hydrogels (i.e., viscosity and shear thinning behavior) could meet the requirements of the applied technology, ensuring biocompatibility and cell survival during the printing process
4. Recommend defining the following parameters in all studies. Concentration of materials and protocols to prepare hydrogels should be fully detailed and could be complemented with its viscosity
5. Cellular tests must include the identification of cell line and assay-kit information with quantifications at different time points (0, 1, 3, 7, and 21-days)
6. The use of renewable resources to produce nontraditional biobased products will affect the worldwide dependence on petroleum-based and synthetic products and feedstock.
7. Among their amazing characteristics, the biocompatibility and biodegradability make them a powerful candidate to use in biological and environmental applications as implants or materials for removal of toxic pollutants. In addition, conducting hydrogels are often a good choice in designing and fabrication of supercapacitor, which promise the most rapid developments in electronics.
8. Sustainability will be achieved by the tailored biosynthesis of conductive biopolymers, using plants and microbes. Biosensors and actuators will use plant or bacterial biopolymer matrices for EAPs that convert energy into either mechanical force or chemical charge or both.

4.5. Future Work

Nowadays, bioprinting is rapidly evolving and hydrogels are a key component or its success. In this sense, synthesis of hydrogels, as well as bioprinting process, and cross-linking bioink represent different challenges for the scientific community. However, the actual application of any nanomaterial in biology and medicine is decided critically by its biocompatibility. To date, none of the grapheme oxide (GO) and polysacc. applications have been approved for clinical trials.

1. Some issues must token related to toxicity and biosafety became pertinent during preliminary biological applications of GO**.**

2. Graphene materials consist of solely carbon. However, it is a matter of serious concern to understand how carbon derivatives like GO behave in a biological system and how long it takes to excrete from the human body need more study

3. It is known from the information on structural properties of GBNs that graphene is a hydrophobic material, so it requires modification of. functional groups to make it a biomedical material. This modification may include covalent and non-covalent functionalization.

4. There are major opportunities for the development of polysaccharide biotechnology using integrated biological, chemical, and engineering approaches, with bioreactors, fermentations, and organisms specifically designed to produce electroactive biopolymers.

5. Electroactive biopolymers have better biocompatibility and are more environmentally friendly than synthetic EAPs.

6. Grafting natural polysaccharides with ICPs in a continuous process will make the production of EAPs more economically feasible.

7. Focus on comparing results of commercial vs. homemade bioprinters (cell viability, mechanical behavior), analyzing other rheological properties (swelling ratio, surface tension), printability vs. precision, or degradation speed for different hydrogels

References

1. https://www.smithsonianmag.com/science-nature/what-lies-ahead-for-3-d- printing-37498558/
2. December 16, 2020 by Anupama Sapkota
3. Elomaa L , Yang YP . Additive manufacturing of vascular grafts and vascularized tissue constructs . Tissue Eng Part B Rev. 2017 ;23 : 436 -450. [PMC free article] [PubMed] [Google Scholar]
4. Kesari P , Xu T , Boland T. Layer – by – layer printing of cells and its application to tissue engineering . Mater Res Soc Symp Proc . 2005 ;845 : 111-117 . [Google Scholar]
5. Nishiyama Y , Nakamura M , Henmi C , et al . Development of a three – dimensional bioprinter : construction of cell supporting structures using hydrogel and state – of – the – art inkjet technology . J Bio mech Eng . 2009 ; 131 : 035001 . [PubMed] [Google Scholar]
6. Norotte C , Marga FS , Niklason LE , Forgacs G. Scaffold – free vascular tissue engineering using bioprinting . Biomaterials . 2009 ; 30 : 5910 5917. [PMC free article] [PubMed] [Google Scholar]
7. Chua CK , Yeing WY . Bioprinting . Principles and Applications . Singa pore : World Scientific ; 2015. [Google Scholar]
8. Centola M , Rainer A , Spadaccio C , et al . Combining electrospinning and fused deposition modeling for the fabrication of a hybrid vascu lar graft . Biofabrication . 2010 ; 2 : 014102 . [PubMed] [Google Scholar]
9. Skardal A , Zhang J , Prestwich GD . Bioprinting vessel – like constructs using hyaluronan hydrogels crosslinked with tetrahedral poly ethylene glycol tetracrylates . Biomaterials . 2010 ; 31 : 6173-6181 . [PubMed] [Google Scholar

10. Itoh M , Nakayama K , Noguchi R , et al . Scaffold – free tubular tissues created by a bio – 3d printer undergo remodeling and endotheliali zation when implanted in rat aortae . PloS One . 2015 ; 10 : e0136681 . [PMC free article] [PubMed] [Google Scholar]
11. Pinnock CB , Xu Z , Lam MT . Scaling of engineered vascular grafts using 3d printed guides and the ring stacking method . J Vis Exp . 2017 : 121 . [PMC free article] [PubMed] [Google Scholar]
12. By Anne Marie Helmenstine, Ph.D.Updated on January 12, 2020
13. IUPAC , Compendium of Chemical Terminology , 2nd ed . (the " Gold Book ") (1997) . Online corrected version : (2006-) " heteropolysaccharide (heteroglycan) " . doi : 10.1351 / goldbook.H02812
14. https://www.chegg.com/homework-help/questions-and-answers/4-5- points-graphene-hydrogel-formed-hydrothermal-reduction-graphene-oxide-go- aqueous-solut-q49817280
15. (Geim, 2009; Shi et al.,2018).
16. https://www.atriainnovation.com/en/graphene-characteristics-and- applications/
17. https://researchtweet.com/polysaccharide-definition-structure- examples/
18. 2012 John Wiley & Sons, Inc. Published 2012 by John Wiley & Sons, Inc.
19. https://www.nanowerk.com/nanotechnology-news2/newsid=57553.php
20. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7221685/
21. https://pubs.rsc.org/en/content/articlelanding/2017/tb/c7tb01594a
22. C. H. Li, J. L. Zuo, Adv. Mater. 2019, 1903762
23. S. Wang, M. W. Urban, Nat. Rev. Mater. 2020, 5, 562
24. https://onlinelibrary.wiley.com/doi/full/10.1002/agt2.21
25. https://scholar.google.com/scholar?hl=en&as_sdt=0,5&as_vis=1&qsp =15&q=tissue+engineering+polysaccharide+hydrogels&qst=bh#d=gs_qabs&t =1651861360398&u=%23p%3DgNgpXIx8ZPcJ
26. Jeon, I.; Cui, J.; Illeperuma, W.R.K.; Aizenberg, J.; Vlassak, J.J. Extremely Stretchable and Fast Self-Healing Hydrogels. Adv. Mater.2016, 28, 4678–4683. [CrossRef]
27. Saunders, L.; Ma, P.X. Self-Healing Supramolecular Hydrogels for Tissue Engineering Applications. Macromol. Biosci. 2019, 19,1–11. [CrossRef]
28. Palmieri, V., Papi, M., Conti, C., Ciasca, G., Maulucci, G., & De Spirito, M. (2016). The future development of bacteria fighting medical devices: the role of graphene oxide. Expert review of medical devices, 13(11), 1013-1019.
29. Tonelli, F. M., Goulart, V. A., Gomes, K. N., Ladeira, M. S., Santos, A. K., Lorençon, E., … & Resende, R. R. (2015). Graphene-based nanomaterials: biological and medical applications and toxicity. Nanomedicine, 10(15), 2423-2450.
30. Cardenas, L., MacLeod, J., Lipton-Duffin, J., Seifu, D. G., Popescu, F., Siaj, M., … & Rosei, F. (2014). Reduced graphene oxide growth on 316L stainless steel for medical applications. Nanoscale, 6(15), 8664-8670.
31. Victoria Lynne Finkenstadt.Natural polysaccharides as electroactive polymers.Applied Microbiology and Biotechnology ·Published: July 2005.
32. Axel rußler and thomas rosenau.electrical conductivity and polysaccharides. Published: 2012.

33. Thabitha P. Dasari Shareena . Danielle McShan . Asok K. Dasmahapatra . Paul B. Tchounwou.A Review on Graphene-Based Nanomaterials in Biomedical Applications and Risks in Environment and Health.Published: 2018.
34. Lingling Ou " , Bin Song , Huimin Liang ' , Na Liu , Xiaoli Feng , Bin Derig , Ting Sun and Longquan Shao.Toxicity of graphene - family nanoparticles : a general review of the origins and mechanisms.Published: 31 October 2016.
35. Jia Kuang , Kun Young Yuk , Kang Moo Huh.Carbohydrate Polymers Volume 83 , Issue 1 , 1 January 2011 , Pages 284-290 Polysaccharide - based superporous hydrogels with fast swelling and superabsorbent properties.
36. Tanja Zidarič , Marko Milojević , [… .] , and Tina Maver.Polysaccharide - Based Bioink Formulation for 3D Bioprinting of an In Vitro Model of the Human Dermis
37. Jianhua Zhang … Ralph Müller.3D bioprinting of graphene oxide incorporated cell – laden bone mimicking scaffolds for promoting scaffold fidelity , osteogenic differentiation and mineralization.Volume 121 , February 2021 , Pages 637-652
38. Yongyan Yang et al . Carbohydr Polym . Recent advances in polysaccharide – based self – healing hydrogels for biomedical applications.2022.
39. Guanghao Li a ... Yi Huang.Graphene based self - healing materials.Volume 146 , May 2019 , Pages 371-387
40. Stefanie A. Sydlik , Siddharth Jhunjhunwala , […] , and Robert Langer.In Vivo Compatibility Of Graphene Oxide With Differing Oxidation States.
41. Yu.A.Ant Onov ... V.B. Tolstoguzov.Thermodynamic compatibility of polysaccharides in aqueous media .Volume 29 , Issue 12 , 1987 , Pages 2729- 2735
42. https://www.rs-online.com/designspark/what-is-3d-printing
43. https://sites.google.com/site/3dddpriter/home/use
44. https://www.intechopen.com/chapters/51535
45. H. Nomura , Y. Katayama , M.S. Shoichet , C.H. Tator , Complete Spinal Cord Transection Treated by Implantation of a Reinforced Synthetic Hydrogel Channel Results in Syringomyelia and CaudalMigration of the Rostral Stump , Neurosurgery . 2006 ; 59 (1) : 183-192 , DOI : 10.1227 01.NEU.0000219859.35349.EF
46. T. Renneberg , R.C.H. Kwan , C. Chan , G. Kunze , R. Renneberg , A Salt - tolerant Yeast based Microbial Sensor for 24 Hour Community Wastewater Monitoring in Coastal Regions , Microchim . Acta . 2004 ; 148 (3

) : 235-240 , DOI : 10.1007 / s00604-004-0266-7
47. https://www.sciencedirect.com/science/article/pii/S2666893921000359
48. https://www.frontiersin.org/articles/10.3389/fchem.2018.00450/full
49. https://www.mdpi.com/2079-4991/11/4/906
50. Marx V. Tissue engineering: organs from the lab. Nature.

    2015;522(7556):373–377.
51. Gu BK, Choi DJ, Park SJ, Kim MS, Kang CM, Kim CH. 3- dimensional bioprinting for tissue engineering applications. Biomater. Res. 2016;20(1):12.
52. Guvendiren M, Molde J, Soares RM, Kohn J. Designing biomaterials for 3D printing. ACS Biomater Sci Eng. 2016;2(10):1679–93.
53. Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet- based bioprinting: past, present and future. Biomaterials. 2016;102:20–42.

54. Li J , Chen M , Fan X , Zhou H. Recent advances in bioprinting techniques : approaches . applications and future prospects . J Transl Med . 2016 ; 14 : 271 . [PMC free article] [PubMed] [Google Scholar]
55. Cubo N , Garcia M , Del Canizo JF, et al . 3D bioprinting of functional human skin : production and in vivo analysis . Biofabrication . 2016 ; 9 : 015006 . [PubMed] [Google Scholar]
56. Vijayavenkataraman S , Lu WF , Fuh JY . 3D bioprinting of skin : a state - of - the - art review on modelling , materials , and processes . Biofabrication . 2016 ; 8 : 032001 . [PubMed] [Google Scholar]
57. https://www.grapheneinfo.com/graphene-introduction?amp
58. https://healthcare-in-europe.com/en/news/conductive-hydrogel-could- replace-brain-tissue.html
59. https://conductscience.com/polysaccharides-properties-functions-and- applications/
60. https://www.researchgate.net/publication/352148310_Polysaccharide-based_3D_bioprinter_inks_for_tissue_engineering
61. https://pubmed.ncbi.nlm.nih.gov/35153030/
62. https://iraqtech.io/the-future-of-3d-printing-in-i